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Inside the magnetic spiral

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Uncovering elements of risk

A newly identified set of genomic loci appears to be selectively associated with prostate cancer in East Asian men

Prostate cancer represents a serious threat to men all over the world, especially those over the age of 65, and is the second leading cause of cancer death among males in both the United States and United Kingdom.

Until recently, the risk level for men living in East Asia was lower than virtually any other region of the world. This is now changing, however, as a result of both lifestyle and demographic factors. "This increased risk is probably due to the shift to a westernized lifestyle, including food, and the rapid increase in the aging population," explains Hidewaki Nakagawa of the RIKEN Center for Genomic Medicine in Yokohama. Indeed, estimates from the United Nations suggest that the percentage of the population of East Asia that are over the age of 65 will more than double between 2009 and 2050, and Japan in particular is projected to have by far the greatest proportion of elderly citizens[1] (Fig. 1).

In addition to these environmental factors, researchers have identified dozens of genetic changes that appear to represent potential risk factors for prostate cancer. All of these were identified based on screens performed on individuals of European ancestry, but a new genomic screen performed by Nakagawa and collaborators from throughout Japan has now identified several novel genetic variants that may prove valuable in diagnosing and treating the growing pool of at-risk Asian men[2].

Figure 1: The rapid growth of an aging population in East Asia will likely contribute to increasing numbers of men diagnosed with prostate cancer.

Taking it to the bank

Nakagawa's team has routinely partnered with scientists from BioBank Japan, an initiative launched in 2003 at The University of Tokyo in order to help scientists identify the bases for diverse medical conditions. "This project was started with the goal of collecting samples from a total of 300,000 individuals who have had at least one of 47 diseases, from a collaborative network of 66 hospitals located throughout Japan," he explains. For this particular study, the researchers obtained DNA from1,583 prostate cancer patients and 3,386 cancer-free control subjects.

Nakagawa and his colleagues used these samples to perform what is known as a genome-wide association study (GWAS). Any given human genome is littered with large numbers of individual nucleotide variations, also known as single-nucleotide polymorphisms (SNPs), which reside both within and in-between genes. From a GWAS, researchers aim to identify SNPs that are significantly more likely to appear in affected individuals than in controls; a SNP with very strong disease association might either represent an actual sequence variation in a relevant gene or provide a useful physical marker for identifying neighboring candidate genes within the same chromosomal region.

The team's initial analysis of more than half a million different SNPs revealed 37 significantly associated variants at eight different genomic loci, two of which had not been previously linked with prostate cancer. A subsequent replication study, performed with an independent set of 3,001 affected and 5,415 control subjects, enabled the investigators to identify three additional loci, for a total of five novel SNPs (Fig. 2). Interestingly, although many of the cancer-associated SNPs that had been previously identified in European populations also exhibited significant linkage among Japanese subjects, more than one-third (12 out of 31) did not. On

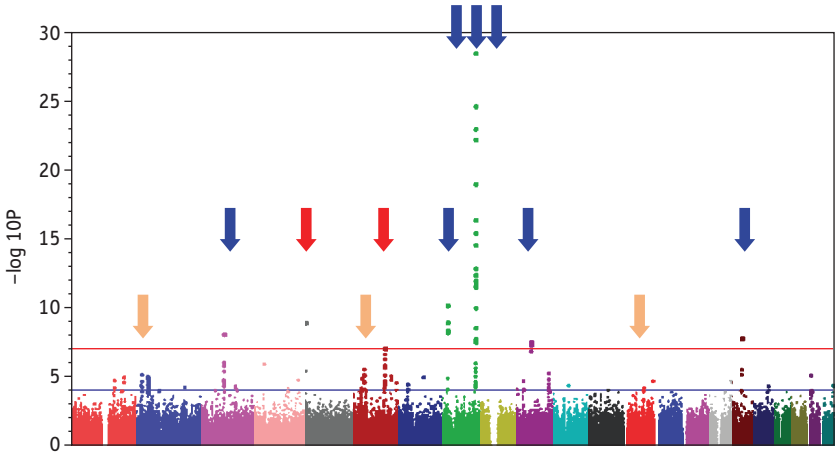

Figure 2: Results from the prostate cancer GWAS of Japanese individuals. Each SNP is plotted horizontally based on chromosomal location and vertically based on the statistical significance of their association with prostate cancer. This study revealed novel associations for five SNPs (red and orange arrows) and confirmed several previously identified associations (blue arrows).

the other hand, several recent large-scale genomic studies conducted using similar analytical methods but focused primarily on subjects of Northern European ancestry failed to find a significant association for any of the five SNPs identified by Nakagawa and colleagues.

Getting to know the candidates

Beyond the strong evidence supporting their apparent association with disease risk, the majority of these novel SNPs proved to be highly enigmatic. Two of them, rs12653946 and rs9600079, reside within stretches of DNA ranging in length from 20–40,000 bases that contain no known genes. Another two were situated within non-protein-coding regions of a pair of genes; rs13385191 is found in *C2orf43*, which produces a protein of unknown function, while rs1983891 is located within the gene encoding the so-called 'forkhead box P4' (FOXP4) transcription factor. Although it belongs to a family of proteins that have been associated with cell cycle regulation and tumorigenesis, the function of FOXP4 has not yet been characterized.

The final SNP appears to be the most intriguing candidate, as it occurs within a stretch of DNA containing the gene for G protein-coupled receptor C6A (GPRC6A), a protein normally expressed by testosterone-producing Leydig cells. "The *GPRC6A* gene is likely to be associated with sex hormone production, as shown [by experiments] in knockout mice," says Nakagawa, "and male hormone levels are one of the most important factors in prostate carcinogenesis." However, further analysis will be needed to confirm that this is indeed the gene being flagged by rs339331.

Although much work remains to be done in characterizing how the genomic regions identified here contribute to prostate cancer risk, they nevertheless represent important additions to an already large pool of SNPs with potential diagnostic or prognostic value. "This is a much bigger number than exists for other cancers," says Nakagawa.

Nakagawa also points out that these five SNPs appear to represent loci that could possibly be used for the selective characterization of prostate cancer predisposition within specific ethnic groups, and that these may represent the first set of Asian-specific cancer risk biomarkers. "We are now dedicated to trying to establish a risk estimation system for prostate cancer among Japanese and other Asians by combining many SNPs and other risk factors of prostate cancer," he says. ■

1. United Nations. *World Population Ageing 2009*. Economic and Social Affairs, Population Division, ESA/P/WP/212 December 2009, New York.
2. Takata, R., Akamatsu, S., Kubo, M., Takahashi, A., Hosono, N., Kawaguchi, T., Tsunoda, T., Inazawa, J., Kamatani, N., Ogawa, O., *et al.* Genome-wide association study identifies five new susceptivility loci for prostate cancer in the Japanese population. *Nature Genetics* **42**, 751–754 (2010).

About the researcher

Hidewaki Nakagawa was born in Osaka, Japan, in 1966. After graduating from the Osaka University School of Medicine in 1991, he embarked on a career in clinical oncology, specifically gastrointestinal and breast cancer, and critical care medicine as a surgeon. He obtained his PhD in 2000 from Osaka University for research on hereditary colorectal cancer. He spent three-and-a-half years as a postdoctoral researcher in cancer genetics with the Human Cancer Genetic Program at Ohio State University, USA, then returned to Japan where he joined The University of Tokyo as an assistant professor in the Institute of Medical Science studying molecular therapeutic targets for prostate and pancreatic cancer. In 2008, he joined the RIKEN Center for Genomic Medicine as team leader of the Laboratory for Biomarker Development. His current research focuses on biomarker development for prostate and gastrointestinal cancer, and cancer genomics based on whole genome sequencing. He is also a participant in the International Cancer Genome Consortium project.

A lack of order

A comparative study of two closely related organic insulators highlights the unusual properties of quantum spin liquids

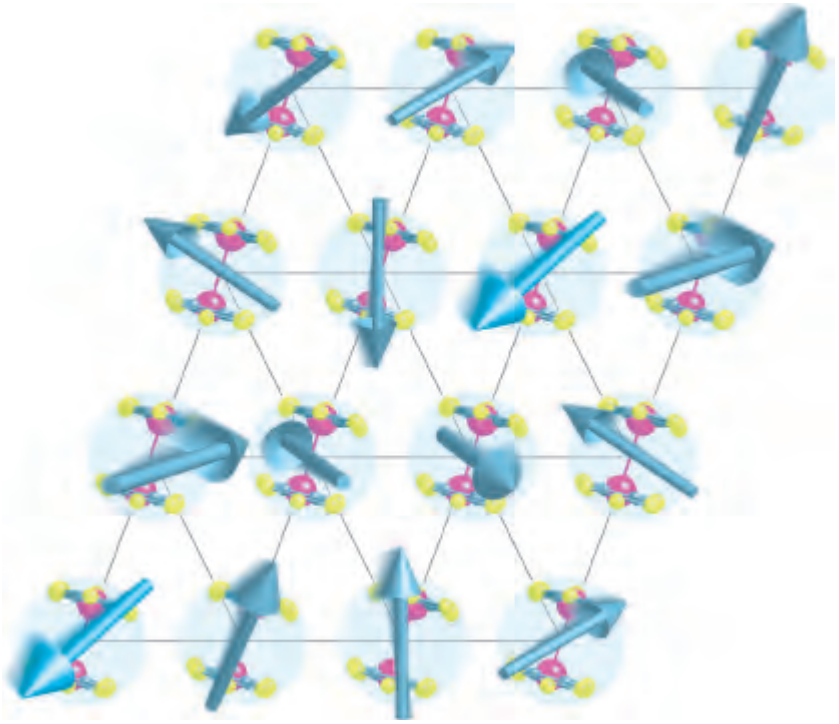

Figure 1: A schematic diagram of a quantum spin liquid. The electron spins, indicated by blue arrows, show no long-range ordering even at low temperatures.

A growing body of experimental evidence is lending support to the theory that an exotic state of matter called a quantum spin liquid actually exists. In a quantum spin liquid, the way electrons spin on their axes lacks any sense of organization throughout the material—even at temperatures approaching absolute zero, where order tends to reign supreme. However, definitive proof has proved elusive, particularly in two-dimensional systems.

Signatures of a quantum spin liquid have now been observed in an organic insulator by Reizo Kato at the RIKEN Advanced Science Institute, Wako, working in collaboration with researchers from Kyoto University and the Japan Science and Technology Agency[1].

In a quantum spin liquid, the magnetic arrangement of the material is incompatible with the underlying crystal geometry, thus preventing the spin from showing any order (Fig. 1). "This leads to liquid-like properties among the spins, even at absolute zero temperature," explains Kato. In contrast, molecules in ice arrange into a crystalline lattice—a pattern maintained throughout the material.

The team compared two closely related organic insulators $EtMe_3Sb[Pd(dmit)_2]_2$ (abbreviated as dmit-131) and $Et_2Me_2Sb[Pd(dmit)_2]_2$ (abbreviated as dmit-221). Scientists previously proposed that dmit-131 may show quantum-spin-liquid state properties. Indeed, using nuclear magnetic resonance measurements, scientists have never identified any long-range magnetic order at temperatures as low as 19 millikelvin. The reason for this remains unclear. The crystal structure of dmit-221 is very similar; however, it exhibits a charge-ordered state. Kato and colleagues therefore thought that a comparison between the two should reveal any properties particular to quantum spin liquids.

The researchers measured the thermal conductivity at temperatures between 10 and 0.1 kelvin, since one of the most important experimental parameters is the thermal conductivity divided by the temperature. In dmit-221, this parameter approaches zero as the temperature gets closer to absolute zero. "This is typical behavior of insulators where lattice vibrations carry thermal energy," says Kato. In dmit-331, however, they extrapolated the parameter to be nonzero at 0 kelvin. "This is more akin to metallic behavior where free electrons carry the thermal energy." This indicates the presence of so-called 'gapless excitations', meaning that there is no energy gap between the ground state and excited states. However, there is also some evidence for spin-gap-like excitations.

These results indicate that this system is a quantum spin liquid with a dual nature. "The next step is to address the fundamental question of whether a quantum spin liquid undergoes instabilities other than classical ordering," Kato notes. ■

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Magnets with a twist

The first direct observation of an unusual magnetic structure could lead to novel electronic and magnetic memory devices

In conventional ferromagnets, the individual magnetic moments of the atoms that together comprise the magnetism of the material are all aligned parallel, pointing in a common direction. In some magnets, quantum-mechanical interactions between the electrons of a material or the presence of internal electric fields, for example, mean that the magnetic arrangements are more complex. Now, a rare arrangement of magnetic moments, so-called skyrmions, has been directly imaged by a team led by Yoshinori Tokura of the RIKEN Advanced Science Institute, Wako. Tokura and his colleagues from RIKEN and other research institutes in Japan and Korea confirmed that skyrmions are very stable and that their manipulation could form the basis for novel magnetic memories or electronic devices[1].

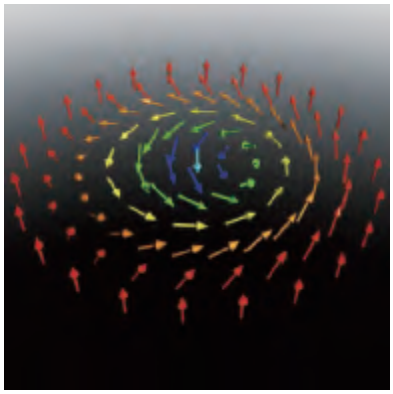

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Figure 1: The structure of a skyrmion. Its atomic magnetic moments start to point inwards under an externally applied magnetic field.

A skyrmion can be envisaged as a vortex-like arrangement of magnetic moments that, towards the center of the structure, increasingly twist and bend in downwards direction (Fig. 1). In earlier experiments by other research groups, the existence of skyrmions had been inferred indirectly but efforts to image them, and to confirm their structure, failed owing to their small size with diameters of around 90 nanometers.

Tokura and his team accomplished their direct observation of skyrmions by using a Lorentz transmission electron microscope, which is suited to image magnetic structures at very high resolution. Previously, physicists considered this type of experiment impossible because observing skyrmions would require the application of external magnetic fields that they thought would disturb the imaging process of the microscope. The team realized, however, that this problem could be overcome by applying the external magnetic fields perpendicular to the imaging lens of the microscope. Tokura says that this led to the breakthrough that allowed them to show the emergence of skyrmions unambiguously (Fig. 2).

In addition to observing the expected periodic arrangement of many skyrmions, the researchers were able to observe isolated skyrmions and establish that they are also stable entities. The manipulation of individual skyrmions could find application in novel magnetic memories or in electronic devices, Tokura notes.

Realization of such applications, however, still requires substantial work.

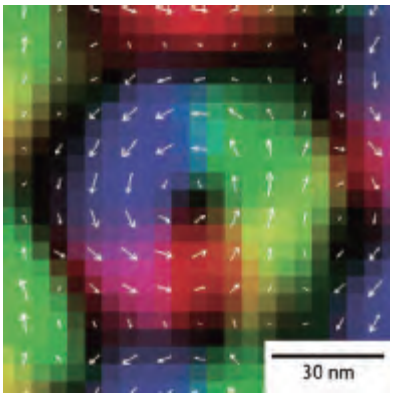

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Figure 2: An overhead view of skyrmions observed directly using a Lorentz transmission electron microscope.

Thus far, skyrmions have been observed only at temperatures of around 40 kelvin. "In future, we not only need to find new materials where skyrmions are stable at room temperature, but also find ways to manipulate their motion through electromagnetic effects," explains Tokura. He says that a number of known oxide magnetic materials could fulfill these criteria and may eventually lead to skyrmion-based devices. ■

1. Yu, X. Z., Onose, Y., Kanazawa, N., Park, J, H., Han, J. H., Matsui, Y., Nagaosa, N. & Tokura, Y. Real-space observation of a two-dimensional skyrmion crystal. *Nature* **465**, 901-904 (2010).

Lasers in a flash

Producing isolated laser pulses in just attoseconds made easier using a two-color laser field

Ultrafast time-resolved laser spectroscopy is a technique that uses the interaction of light with matter to study the properties of physical systems. Researchers can generate laser pulses lasting mere attoseconds—quintillionths of seconds—to examine the nuclear dynamics in different states of matter, including single atoms.

Generating isolated attosecond pulses reliably is challenging. Commonly, physicists use few-cycle laser pulses with a near infrared wavelength as a pump to temporarily ionize specific atoms, typically those of a noble gas. When an electron re-collides with a nucleus from which it has been pulled away, it emits light with a much higher frequency than the one in the pump laser. This so-called 'high-order harmonic generation' usually in the extreme ultraviolet region can create an attosecond pulse.

Eiji Takahashi and his colleagues at the RIKEN Advanced Science Institute in Wako, in collaboration with scientists at the Vienna University of Technology, Austria, have now reported a way to easily produce isolated attosecond pulses, which surpasses all previous attempts for simplicity and reliability[1].

A number of research groups have recently generated isolated laser pulses as short as 80 attoseconds. However, their energy is still too low be used in practice, since the energy of the pump pulses is limited. High pump energy would induce high gas ionization such that the atoms hit by the pump pulses would be highly ionized, but this would prevent the whole process of re-collision. In addition, to guarantee reliable production of isolated attosecond pulses, the phase of the carrier envelope wave connected to the pump pulse needs to be stabilized, which requires an expensive and complicated process.

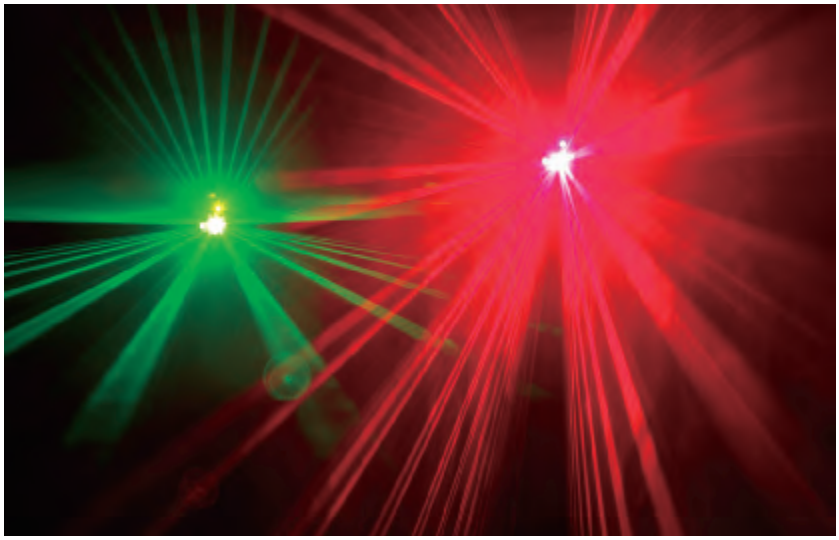

Figure 1: An artistic representation of light from two lasers. Conventional, two-color infrared laser beams can be used simply and reliably in systems designed to produce attosecond laser pulses.

To circumvent these limitations, Takahashi and colleagues used a two-color laser field: a pump laser with an 800-nanometer wavelength superimposed on one of 1,300 nanometers. The combination of the two lasers allowed the generation of a higher harmonic spectrum without needing to stabilize the carrier envelope phase.

Crucially, they used conventional lasers that are readily available and inexpensive (Fig. 1). "This novel two-color laser scheme also enables one to markedly suppress the detrimental gas target ionization," notes Takahashi. "Consequently, not only the most appropriate phase-matching technique, but also an energy-scaling scheme, can be applied to produce intense isolated attosecond pulses."

Takahashi also says that this method has the potential to produce isolated, attosecond, extreme-ultraviolet x-ray pulses with microjoule energy from a table-top system. He believes this would open the door to the realm of strongly nonlinear attosecond science. ■

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Taking aim at a slippery target

Powerful synchrotron light captures never-before-seen electronic interactions of molecules dissolved in liquids

When molecules take the plunge into a liquid solvent, they undergo constant twists and turns as they spread out and interact within their new environment. Such haphazard movements in solvents make it difficult for scientists to measure specific reactivity changes. "Especially for liquids and solutions, it is not known how intermolecular interactions affect the electronic structure of the reactants, despite this being one of the key physical processes of chemistry," says Takashi Tokushima from the RIKEN SPring-8 Center in Harima.

Now, Tokushima and colleagues from RIKEN and Hiroshima University have used high-energy synchrotron light to capture the signals of molecular orbitals (MOs)—quantized spatial distributions of electrons that determine chemical reactivity—from acetic acid molecules in solution[1] (Fig. 1). This approach enables the measurement of solvation effects with atom-by-atom precision, which is crucial information for understanding essential reactions such as enzyme-based catalysis.

The researchers achieved their result by smashing accelerated photons into an acetic acid solution, setting off an x-ray emission signal from the valence, or bonding, MOs of the target molecule. By observing the difference in x-ray signals when the incoming photons were polarized horizontally or vertically, the team hoped to find the spatial symmetry of the emitting MO—a parameter that can identify solvent-induced changes to acetic acid's electronic structure.

However, detecting symmetry changes in liquids is difficult because the differences between polarized signals are quite small.

Figure 1: Acetic acid molecules, best known as the main component of vinegar, can change electronically when mixed into a solvent.

According to lead author Yuka Horikawa, the team overcame this problem by using a solvent called acetonitrile (CH_3CN) that does not interfere with the oxygen x-ray emissions of acetic acid. When the incident x-ray energy was tuned to the oxygen signal, a nitrogen emission from the acetonitrile solvent appeared that was proportional to the incident light intensity, no matter the polarization direction. This nitrogen signal was used to normalize the polarized acetic acid spectra, allowing the solvated symmetry changes to be revealed.

In contrast to expectations, the acetic acid emissions showed pronounced polarization dependence, indicating that the MOs retained the same symmetry as a molecule without solvent. While this result shows that acetonitrile had little effect on most of the compound, one particular MO—corresponding to a lone pair of electrons on the acetic acid oxygen atom—showed a pronounced change. The researchers propose that this change in MO symmetry arises from solvent effects. The new-found ability to precisely pinpoint activation sites has the potential to unlock the secrets of many solvent-based reactions, say the researchers. ■

1. Horikawa, Y., Tokushima, T., Hiraya, A. & Shin, S. Pronounced polarization anistropy in resonant X-ray emission from acetic acid molecules in solution. *Physical Chemistry Chemical Physics* **12**, 9165 –9168 (2010).

A signal change for antifungal agents

Chemical-genomic profiling of bioactive therapeutic compounds reveals therapeutically exploitable signaling activity at fungal cell membranes

While some fungal species, such as *shiitake* and *enoki*, are edible, some microscopic species can trigger numerous infections in the human body. Antifungal drugs are the best means to fight these parasitic species; however, because mammalian and fungal cells share many similarities, such as lipid-based membranes, these medications can produce serious side-effects.

Now, researchers led by Minoru Yoshida from the RIKEN Advanced Science Institute in Wako have characterized the biological properties of theonellamide (TNM), an antifungal natural product isolated from marine sponges[1]. They found that, unlike typical modes of action, TNM specifically targets ergosterol lipid molecules in fungal cell membranes, not proteins. This bonding interaction rapidly activates a protein called Rho1 to over-produce 1,3-β-D-glucan sugar chain molecules—a process that forms an aberrant fungal cell wall. This unique mechanism promises to spur development of innovative antifungal agents. "We believe that TNM is the first compound that activates membrane signaling molecules by binding to a lipid," says Yoshida.

Despite previous efforts to identify TNM's specific biological actions, its sub-cellular targets were unknown until now. Yoshida and colleagues used a yeast complex to generate nearly 5,000 'open reading frames' (ORFs), which are long strands of DNA that can encode proteins. This was to screen for sequences with altered susceptibility to TNM—so-called 'hit genes'. Extensive bioinformatic analysis of the chemical-genomic profiles showed that the hit genes showed traits related to sterol binding, Rho-type protein activation or inhibition, and 1,3-β-D-glucan synthesis. However, none of the hit genes showed any physical interaction with TNM, demonstrating that proteins were not the primary target of this molecule.

By synthesizing fluorescently labeled TNM derivatives and comparing their *in vivo* localization to filipin molecules—known membrane-binding compounds—the team discovered that TNM directly targets ergosterol and related sterols in fungal cell membranes (Fig. 1). Attachment of TNM to these lipid molecules enhanced 1,3-β-D-glucan synthesis—but only in the presence of Rho1, confirming the unprecedented signaling behavior. Further experiments on Rho1 mutants determined that TNM can independently lower membrane integrity, gradually inducing lesions into the cellular structure.

The researchers' next task—unraveling the complex mechanisms of TNM-induced membrane signaling—may throw light on how to avoid unwanted side-effects in humans during antifungal treatments. "TNM binds to not only ergosterol but also cholesterol, a mammalian counterpart," explains Yoshida. "Our preliminary findings show that mammalian cells rapidly and transiently change morphology upon TNM treatment—making this compound a fabulous tool to dissect the function of membrane sterols in general." ■

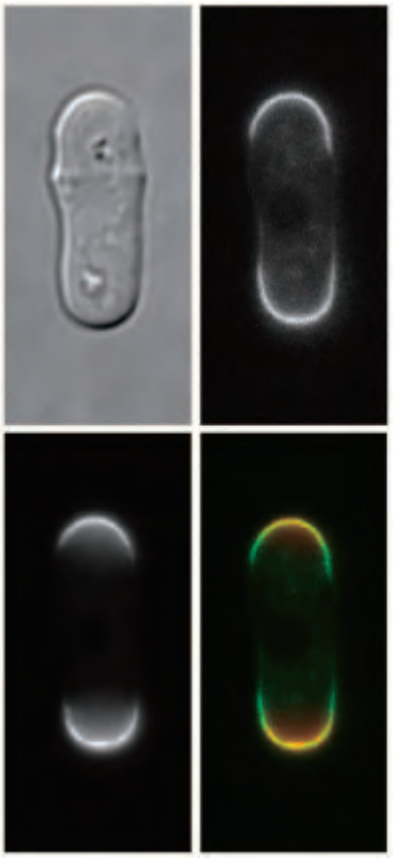

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Figure 1: ***In vivo*** **imaging reveals the binding sites of theonellamide (TNM), a novel antifungal agent. The upper panels show a differential interference contrast micrograph of a yeast cell (left) and a fluorescent image of the cell stained with the membrane probe filipin (right), where the brightness indicates filipin binding at the cell membrane. The lower panels show a fluorescent image of a cell stained with TNM (left) and a merged image (right) of the cell labeled with TNM (red) and stained with filipin (green).**

1. Nishimura, S., Arita, Y., Honda, M., Iwamoto, K., Matsuyama, A., Shirai, A., Kawasaki, H., Kakeya, H., Kobayashi, T., Matsunaga, S. & Yoshida, M. Marine antifungal theonellamides target 3 β -hydroxysterol to activate Rho1 signalling. *Nature Chemical Biology* **6**, 519–526 (2010).

Releasing the brakes

Two regulators of protein filament assembly use dramatically different—and competing—methods to inhibit a common target

Actin-based protein filaments participate in biological activities ranging from cell migration to muscle contraction. These filaments can be highly dynamic, with individual actin molecules spontaneously attaching to or dissociating from the ends of the fiber. Typically, however, such activity is closely regulated by factors like actin capping protein (CP).

Filaments exhibit physical polarity, with extension specifically occurring at the 'barbed' end, and CP inhibits addition of new actin molecules by firmly seating itself at this end. CP is widely conserved in species ranging from yeast to humans and acts a crucial regulator for a variety of actin-mediated cellular functions.

Accordingly, cells also produce factors that help remove CP from filament ends, such as the V-1 and CARMIL proteins. Yasushi Nitanai at the RIKEN SPring-8 Center in Harima recently partnered with Nagoya University researchers Shuichi Takeda and Yuichiro Maeda to characterize the mechanisms employed by these two CP regulators via structural analysis[1].

CP is composed of an α and a β subunit, each of which has a projecting 'tentacle' domain. Previous work from Takeda and Maeda showed that CP relies on the α tentacle to latch onto actin while the β tentacle stabilizes the complex[2]. Their work with Nitanai has now demonstrated that V-1 acts as a direct counter to this process, binding the same portions of the α tentacle that mediate actin binding and thereby physically preventing them from associating with the filament.

Takeda and colleagues identified a markedly different mechanism for CARMIL, based on data that revealed a surprisingly dynamic structure for CP. "We had believed that CP was a rigid molecule, and never imagined that it was an intrinsically flexible molecule, continuously undergoing twisting motions," says Takeda. CARMIL appears to actively exploit this flexibility, interacting with CP via a relatively unstructured domain. This association does not physically obstruct actin binding (Fig. 1), but instead constrains CP into an arrangement that reduces its affinity for both the barbed end of actin filaments and the V-1 inhibitor.

The team's results are in keeping with previous findings indicating that CARMIL can bind to CP that is already bound to filament ends and triggers its rapid dissociation. "We were impressed with the way that CARMIL utilizes the intrinsic fluctuation of CP to suppress capping activity," says Takeda. In future studies, he and his colleagues hope to apply alternative structural biology techniques, such as nuclear magnetic resonance, to better capture the subtle details of the dynamic interactions between CARMIL, V-1 and CP. ■

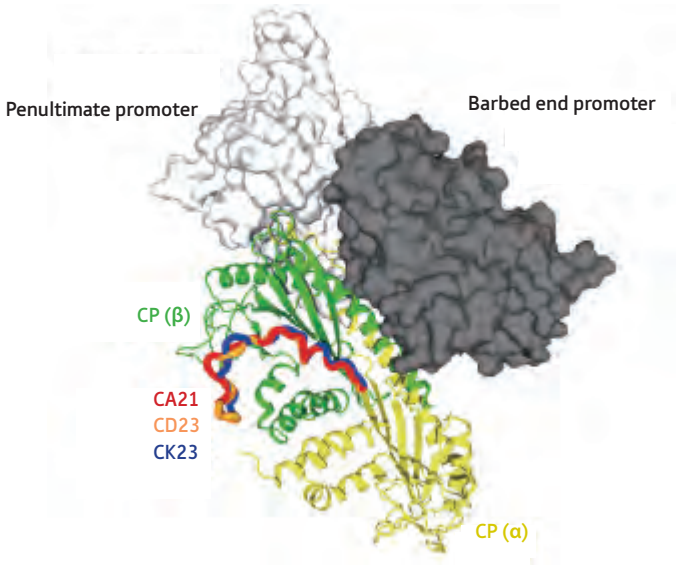

Figure 1: The various CARMIL proteins (CA21, CD23 and CK23) interact with CP (green and yellow) via a relatively linear and unstructured domain. By binding to a site that is distinct from where CP interacts with actin (grey), CARMIL can force stably bound CP to dissociate from the 'barbed end' of the filament.

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Boon to plant science

RIKEN's release of an online database pertaining to the regulatory mechanisms of plants offers new research and engineering opportunities

Figure 1: Comparisons of phosphorylation features between evolutionary distant plant species, monocot rice (left) and dicot *Arabidopsis* (right), show that targets of phosphorylation are often conserved among highly diversified plant species such as these.

In both plant and animal cells, protein activity is often regulated by phosphorylation, by which a phosphate group is added to one or more sites on a protein. A team led by Ken Shirasu of RIKEN Plant Science Center, Yokohama, has found very similar patterns of protein phosphorylation even in distantly related plant species[1], a discovery that should advance plant engineering. The data is now freely available online from RIKEN's new Plant Phosphoproteome Database[2].

Just as a 'genome' includes all of the hereditary information of an organism encoded in its DNA, the entire suite of phosphorylated proteins and their phosphorylation sites found within an organism constitutes its 'phosphoproteome'. "Elucidating and comparing the phosphoproteomes of different species is key to understanding diverse biological phenomena, including growth and development," explains Shirasu.

Until now, the only plants whose phosphoproteomes have been studied in any detail are model species such as *Arabidopsis thaliana*, which belongs to a group of flowering plants known as the dicots. To broaden knowledge of plant phosphoproteomes, Shirasu and his colleagues embarked on the first large-scale protein phosphorylation screen in rice (*Oryza sativa*). Unlike *Arabidopsis*, whose phosphoproteome they have also updated, rice is a monocot.

"Rice is an economically important crop species providing the staple diet of millions of people, and since its genome has been sequenced it has also become an important model plant species," says Shirasu. "Moreover, because rice is a monocot, it was interesting for us to compare its phosphoproteome with that of *Arabidopsis*, which being a dicot is only distantly related to rice in evolutionary terms."

The researchers identified over 5,000 unique phosphorylation sites on a total of 3,393 proteins expressed in cultured rice cells. Of these, more than half are also phosphorylated in *Arabidopsis*, often sharing the same phosphorylation sites—despite their distant evolutionary relationship. Thus, the two species showed considerable overlap in the composition of phosphorylated proteins (Fig. 1). The researchers also found significant similarities between the recently characterized phosphoproteome of *Medicago truncatula*, a small clover-like plant, with those of rice and *Arabidopsis*.

"What our findings show is that phosphorylation patterns are remarkably well conserved among plant species, meaning that what we learn from one species can be applied to another with relative ease," says Shirasu.

The researchers hope that their findings will facilitate further comparative studies of plant phosphoproteomes, leading to better understanding of core regulatory mechanisms in plants, and the engineering of agronomically important plant species. ■

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2. RIKEN Plant Phosphoproteome Database https://database.riken.jp/sw/links/en/ria102i/

Crossing the line

Understanding of blood cell lineages advances with the discovery of a transcription factor crucial to T cell differentiation

A master gene that underpins the development of specific blood cell lineages has been identified by a research team led by Hiroshi Kawamoto at the RIKEN Research Center for Allergy and Immunology in Yokohama. The team has published its findings in the journal *Science*[1].

Precursor cells in the immune system, known as hematopoietic progenitor cells, can give rise to multiple immune cell types. Kawamoto and his team cultured multipotent progenitor cells from mice that could become T cells that shape the immune response, B cells that generate antibodies, or myeloid cells that can engulf pathogens. Their special culture system could stimulate the Notch signaling pathway, which is required for progenitor cell renewal, and included immune system regulators such as interleukin-7 (IL-7).

The researchers found they could induce the immune progenitor cells to lose their ability to become B cells under these conditions. However, this halted development of the cells past this stage, as the progenitors were unable to cease proliferating and mature into either T cells or myeloid cells.

Kawamoto and colleagues then observed that removing IL-7 from the cell culture medium was sufficient to drive the progenitors to mature into T cells. They found that withdrawing IL-7 induced the expression of the transcription factor Bcl11b, which is known to be expressed in T cells. Interestingly, even when IL-7 was present in the cell culture medium, they could push immune progenitor cells into becoming T cells by forcing Bcl11b to be expressed in the cells. This suggested to the researchers that this transcription factor drives this step in the commitment of these immune progenitor cells to the T cell lineage.

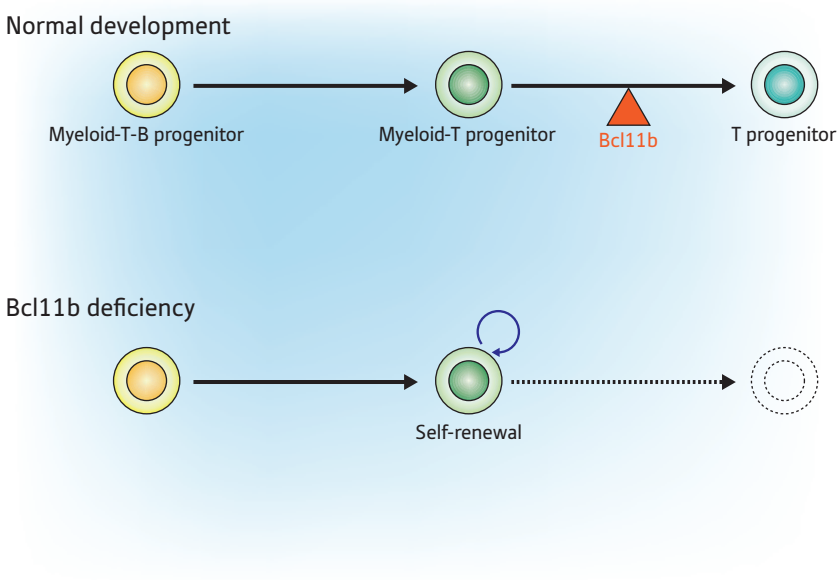

Figure 1:The transcription factor Bcl11b regulates the formation of T cells from immune progenitor cells.

The team also showed that progenitor cells lacking the *Bcl11b* gene were unable to mature into T cells, and could continue to proliferate (Fig. 1). This is consistent with previous findings by other research teams that disruption of the function of *Bcl11b* is linked to leukemia and lymphoma, which may be caused by the inability of the progenitor cells to mature properly into T cells, and to instead continue to proliferate. Kawamoto and his colleagues think that Bcl11b may drive progenitor cells to take on the T cell fate by suppressing the genes that characterize the myeloid cell lineage.

"Our findings may facilitate the study of the molecular mechanisms of T cell lineage commitment by elucidating the exact timing for this commitment," explains Kawamoto, "and by identifying a master gene for the establishment of T cell lineage." ■

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Ready and waiting

A subset of immune cells remain ideally positioned to respond quickly to the reappearance of previously encountered pathogens

Like a burglar tripping an alarm, infectious threats within the body set off a chain reaction of signaling events that enable the immune system to mount a proper defensive response. Once the crisis is averted, populations of target-specific 'memory' B cells ensure that any return visit by the same pathogen will be dealt with promptly and harshly.

Memory B cells initially arise within germinal centers in spleen lymph nodes, but it has proven challenging to determine whether they continue to reside there or circulate throughout the body. By applying sophisticated cellular imaging techniques, a team led by Tomohiro Kurosaki of the RIKEN Center for Allergy and Immunology in Yokohama has now resolved this question for at least one major subset of these cells[1].

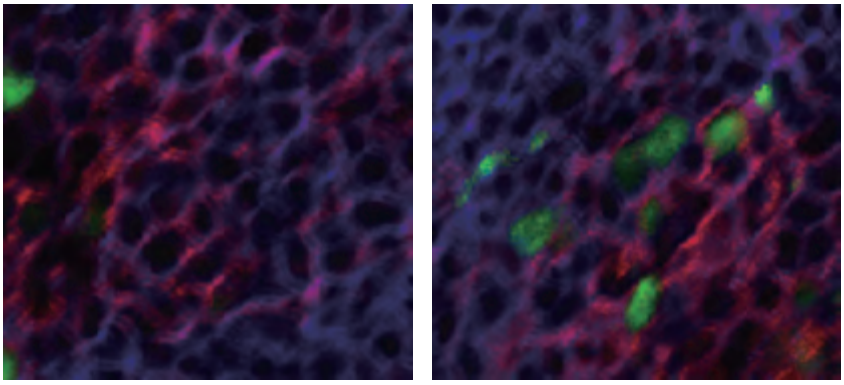

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Figure 1: A fluorescent indicator of cell division (green) reveals that IgG memory B cells (purple) are largely quiescent during an initial antigen challenge (left), but undergo activation and proliferation in response to a secondary challenge (right).

B cells are primarily categorized based on the immunoglobulin protein chains they incorporate into their antibodies, and Kurosaki's team primarily focused their attention on immunoglobulin G-expressing (IgG^+) cells. Using a variety of fluorescent labeling strategies, they were able to determine that IgG^+ memory cells remain clustered close to the germinal centers long after the initial immune response in mice injected with the immunostimulatory molecule nitrophenol. By comparison, immunoglobulin M-expressing (IgM^+) memory cells are found scattered at discrete sites throughout the spleen.

Subsequent experiments with a fluorescent indicator of cell division showed that IgG^+ cells replicate rapidly in response to a secondary challenge with nitrophenol (Fig. 1), and that this process is dependent on direct interaction with helper T cells, which are also located in close proximity to germinal centers. The communication between these two cell types appears to directly contribute to elevated production of antigen-specific antibodies. "Although preliminary, our data suggest that IgG^+ memory B cells are more prone to differentiate into antibody-producing plasma cells than IgM^+ memory B cells, which may contribute to regeneration of the memory pool after a secondary antigen challenge," says Kurosaki.

The memory cell-mediated immune response is generally faster and more robust with regard to target recognition than the 'first encounter' with a given pathogen, and Kurosaki believes that these findings represent an important step toward understanding the efficiency of the memory cell response. "Before our study, people believed that memory B cells leave the germinal centers and are recirculated all over the body by the lymphatic system and blood," he says. "However, our study clearly demonstrates that some IgG—but not IgM—memory B cells reside continuously near germinal centers and thus [enable] rapid activation after antigen re-challenging." ■

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Intercepted messages reveal cells' inner workings

A pair of molecular biology techniques enables detailed characterization of the gene expression of small numbers of cells while preserving initial cell function

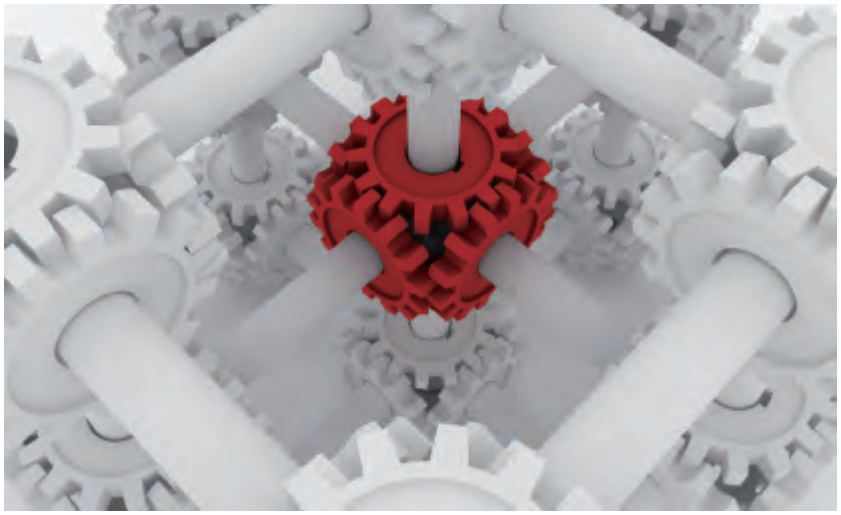

Figure 1: Proper operation and regulation of the cellular machinery relies on messages produced from a diverse array of genes.

A cell's RNA content provides a complete snapshot of its gene expression activity so can yield a bonanza of information not only about how that cell functions, but also the disruptions that result from disease or environmental changes (Fig. 1).

The cap-analysis of gene expression (CAGE) technique developed by Piero Carninci and his colleagues at the RIKEN Omics Science Center in Yokohama has provided an invaluable tool for such profiling, enabling researchers to compile libraries of partial sequences from a large percentage of cellular RNAs[1]. However, CAGE requires large quantities of RNA material, limiting its usefulness for more focused cellular analyses. "We have been working with neurons, but there are so many types," says Carninci. "If we isolate specific populations of fluorescently labeled transgenic neurons, we may obtain no more than several thousand cells."

Working with an international team of collaborators, Carninci's group has now developed two CAGE variants that bring such analyses within reach[2]. The first, nanoCAGE, can be applied to as little as ten nanograms of RNA—5,000-fold less than is needed for standard CAGE. Using nanoCAGE, the investigators could even selectively characterize RNAs from the nucleus, nucleolus and other cellular compartments.

Thanks to cellular splicing mechanisms, a single gene can yield multiple, functionally diverse gene products. However, CAGE and nanoCAGE only characterize the beginning of each RNA molecule, making it hard to identify splice variants. The second technique, CAGEscan, has therefore been adapted to yield sequence data from both ends, and initial demonstrations of this method on cultured liver cells enabled a detailed analysis of architecture and revealed a startling diversity of novel RNA molecules. Many of these arise from within non-protein-coding segments of known genes, and potentially exert yet-unknown regulatory functions.

These two techniques should enable a diverse array of genetics and cell biology studies that were not possible with traditional CAGE or other technologies. "Analyzing isolated, homogeneous neuron populations from mice and rats is a high priority for us," says Carninci. "These technologies could also be used on biopsies or samples, where the amount of RNA is generally limited or to look for diagnostic markers in the blood."

Carninci and his colleagues are also thinking smaller, and attempting to focus their method all the way down to the single-cell level. "Scaling down the technology to a single cell will help solve the issue of how many cell types we have in the body," he says, "and particularly in the brain, where this issue is especially debated." ■

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Synthetic biology — creating biological resources from information resources

Tetsuro Toyoda

Director
RIKEN Bioinformatics And Systems Engineering Division (BASE)

Databases are becoming increasingly important in the life sciences as a key tool for deriving results. The Bioinformatics And Systems Engineering Division (BASE) is drawing worldwide attention for its SciNetS information infrastructure for handling the vast amounts of data generated through routine research in the life sciences. "A database is not merely a container for data, it is also a place where even life can evolve," says Tetsuro Toyoda, director of the BASE. "We can create useful biological resources from information resources by selecting useful genes from databases, designing new genomes, and returning them to the world of living organisms. What databases are needed to realize rational organism design? That is the question we attempt to answer." To inspire creative use of databases for genome design, the BASE is holding its first International Rational Genome Design Contest.

Toward an age of genome design

Toyoda believes that in the future, our key source of resources will shift from oilfields to genomes. For this reason, he calls genomes the "second oilfields". In the 1990s, Toyoda worked for a private research institute to develop anti-malarial drugs. "In drug development, the key is how to design compound shapes so that the compound can combine perfectly with disease-related proteins and control their functions. The structures of the proteins, however, are so complex that trial-and-error-based design approaches often end in failure. 'Rational' design is therefore needed, which involves creating logical programs and designing drugs on the basis of the computed three-dimensional structure of the relevant proteins." However, even if a compound is designed perfectly, the compound may still not be creatable using the techniques currently available in organic chemistry. This is one of the technological barriers Toyoda has encountered.

In the 2000s, scientists embarked on the sequencing of genomes from a range of organisms, including humans. "The genome carries genetic information in the arrangement of four bases in the gene region, and proteins are produced according to this arrangement. If the arrangement can be 'designed', we could be able to design organisms with new functions more reliably. The information resources necessary for such an exercise are now becoming available," says Toyoda.

The concept of rational design has also recently begun to draw attention in the field of medicine, where medical scientists are working toward a personalized medicine approach in which drugs and treatments are designed according to the genetics of the patient. Database-supported rational approaches to design are therefore finding applications in various fields and beginning to displace the previous 'blind' approaches.

Believing that the age of rational design would soon come to genomics, Toyoda initiated the Genomic Knowledge Base Research Team in the RIKEN Genomic Sciences Center (GSC) in 2001. Around that time, the GSC was working on ambitious genomics projects under the leadership of Akiyoshi Wada, the first director of the GSC. The projects were generating vast amounts of data for

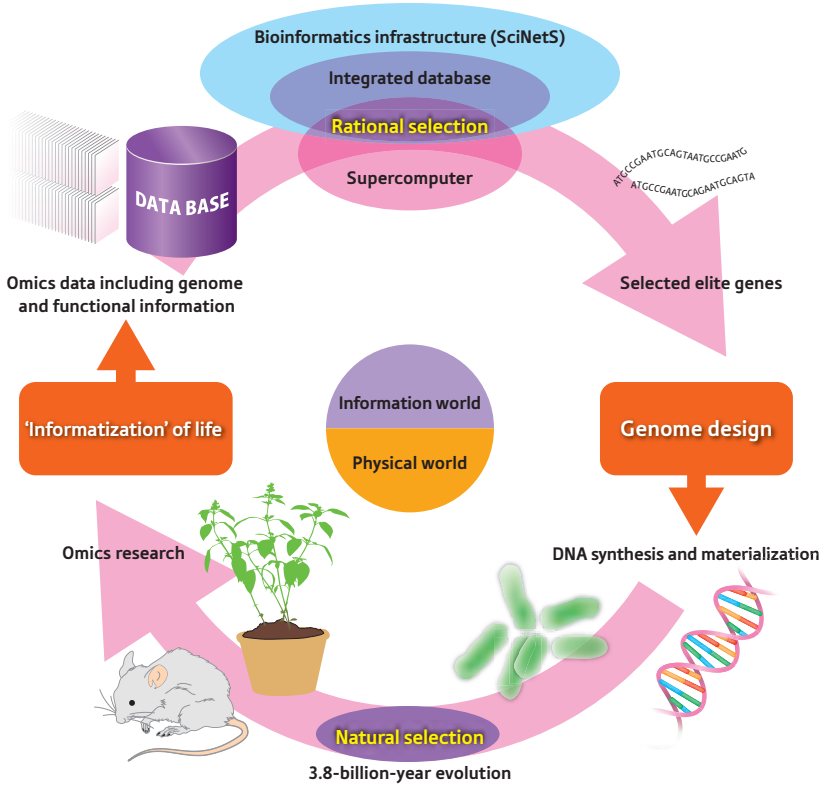


Figure 1: Omics-driven evolution expands the cycle of gene evolution to the information realm. Life is the propagation of genetic information, which evolves in the physical world by repeated replication and selection. The omics technology revolution has enabled the same replication and selection in databases. Useful biological resources can be created by selecting useful genes from the information world and returning them to the world of living organisms under proper safety control standards.

the purpose of establishing complete databases for certain organisms in order to construct a comprehensive of each organism. Toyoda saw these databases as a place in which the evolution of new life could occur.

Databases as a realm for the evolution of life

Organisms evolve through the repeated process of replication of genetic information and natural selection. Genetic information is naturally recorded in the structure of DNA and RNA, but the same information can now be recorded in databases (Fig. 1). "Recording media have expanded from the natural physical world to the information world. We are at the point where the medium of life evolution has changed significantly." Genetic information recorded in databases has been replicated on a global scale through the internet, allowing useful gene information to be plucked from a database. On this basis, could it be possible to create new and useful biological resources by using selected gene information to rationally design a genome that could then be transferred to an organism using DNA-synthesis technology? "A database can be regarded as a place for the replication and selection of genetic information—or a place where the evolution of life occurs," says Toyoda.

Toyoda's ideas are not just dreams—life is actually beginning to evolve with the help of databases (Fig. 2). "We selected the genetic information of groups of enzymes that produce the sticky paste of fermented soybeans called γPGA from a database, and rationally designed the genetic information, which was then transferred to the genome of a plant, thus successfully creating a new plant that is drought tolerant." Induced pluripotent stem (iPS) cells, which are now expected to be applied in regenerative medicine, are also created using the same concept. The original creation of iPS cells was based on the complete record for a particular cDNA, a DNA created from a template mRNA into which a gene region of DNA is transcribed, stored in a RIKEN database. Shinya Yamanaka and his laboratory staff at Kyoto University used the cDNA record to select special genes expressed in embryonic stem (ES) cells and transferred those special genes into grown human skin cells. This process resulted in iPS cells, which, like ES cells, are able to differentiate into any cell type coded for in the organism's genome.

"Designing a database is equivalent to designing a place suitable for the evolution of life. To create new biological resources that can support the Earth and society from information resources, we need databases, and we will find it very interesting if a database is designed from the perspective that it is a place for the evolution of life."

Establishing a data-sharing infrastructure—a global issue

The BASE was inaugurated in April 2008 following a reorganization of the GSC based on advice that came out of the 2006 RIKEN Advisory Council (RAC) meeting. The RAC is an external advisory body consisting of world-leading scientists and eminent individuals from outside of RIKEN. The RAC evaluates the overall activities of RIKEN and delivers its recommendations to the RIKEN president. "In 2006, the RAC pointed out that although high-quality data were provided in each of the 100 or so data-release web sites operated by RIKEN, the data were not presented in an effective way. Everybody was really surprised because they believed their data was disclosed properly."

Most data-rerelease web sites operated by RIKEN were designed for people who wanted to view data directly; no connections could be made with the database to allow automated data analysis. In that regard, the databases were not being used effectively because there was no systematic system provided to standardize and share the various data sets. They were also insufficient from the perspective of displaying study

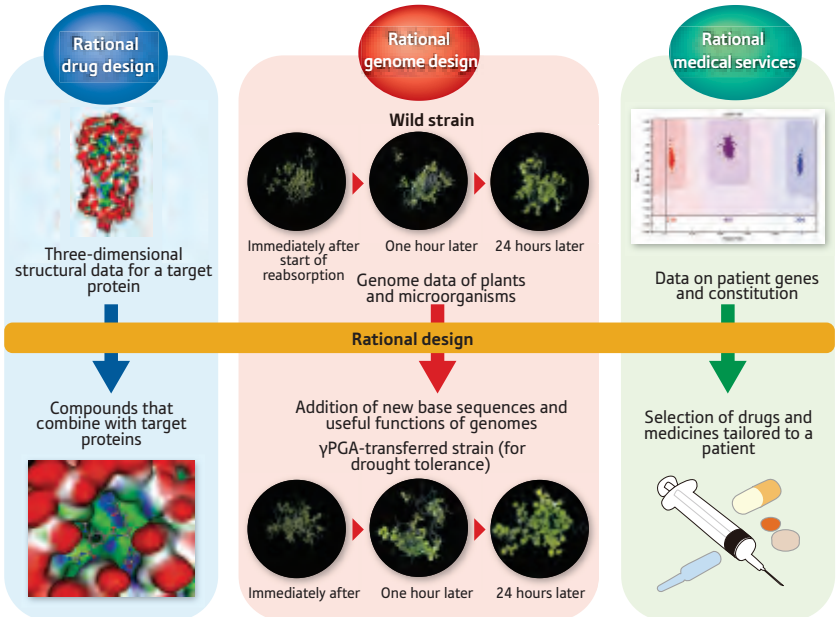


Figure 2: Rational design of drugs, medicines and genomes on the basis of logically created programs. Based on the rational design of genomes, Toyoda transferred the genomes of three enzyme groups that can produce γPGA (the sticky paste of fermented soybeans) to the genomes of Arabidopsis thaliana, a plant used often in experiments. γPGA-transferred strains absorb water more efficiently, resulting in a higher survival rate.

results. These were the problems that the RAC identified and requested database experts to address, but they were also problems that were common among databases around the world. "The RAC asked RIKEN to solve a problem that had not been solved before, and I have been officially selected as the director responsible for solving the database-related problems."

At that time, most data-release web sites failed to keep pace with fast-changing web standards. As the number of disorganized web sites increased, information management was quickly spiraling out of control. Database maintenance costs were also becoming a heavy burden. "We needed to integrate our databases, but that was the most difficult issue," says Toyoda. "I have seen many failures with respect to integration approaches. There are already hundreds of databases, and it is impossible to standardize all of them. So I adopted a new concept and started to develop an integration database consisting of a versatile database container that is compatible with international standards. This container automatically enables the standardization, collection and disclosure of data, and also facilitates data sharing when data is moved into it."

SciNetS captures the world's attention

Toyoda started by developing a 'total incubation infrastructure system' for life science-related databases called the Scientists' Networking System, or SciNetS (Fig. 3). "The greatest advantage of SciNetS is the adoption of the semantic web, a next-generation international web standard, and cloud computing."

The semantic web is an extension of the widely used world wide web (WWW). The WWW is suitable for use by people, who read, understand and search for information by following hyperlinks to documents. However, automated computer-based data analysis is ineffective using hyperlinks because there are no relationships defined among documents. In the semantic web, all data has meaning, and every link refers to the relationships between the data, enabling computers to search data effectively for automated data analysis.

Cloud computing is a complementary technology that provides a new way of using computer applications through web browsers. Researchers do not need to maintain their own servers; they instead prepare a virtual laboratory in the SciNetS and enter their data, which are then processed automatically and disclosed as a database that meets international standards. "Papers are published through the medium of academic journals, but no dedicated medium has been established in the world of databases. SciNetS thus became the world's first academic medium for databases," says Toyoda.

As soon as SciNetS was made operational in March 2009, it attracted attention from around the world. "The semantic web has been known for many years, but building large-scale databases for the semantic web was said to be difficult. We succeeded in building such large-scale databases for the first time by adding a new function that enabled security management on a per-item basis." Database sharing and the financing of maintenance costs are universal issues, but each research body conducts its own activities and deals with its own field-specific characteristic data, so there has been little organization until now. "The world's attention is now focusing on SciNetS because it is a total incubation infrastructure system for databases that can be used by all fields based on the semantic web and cloud computing."

International Rational Genome Design Contest

The virtual laboratories in SciNetS can be used for many purposes: as a substitute for personal databases, a repository for electronic laboratory notes of unreported data, or for joint research or 'medical clouds'—an electronic health chart network among medical specialists and clinicians. Such uses are supported by the per-item security function. Another use for SciNetS virtual laboratories is the International Rational Genome Design Contest, or GenoCon. "ROBOCON is a well-known robot contest in which individually developed robots compete on the basis of excellence in certain skills. GenoCon is the life-science version, where researchers are expected to compete on the excellence of their rational skills in designing genome base sequences."

GenoCon has been running since the end of May 2010 and will continue

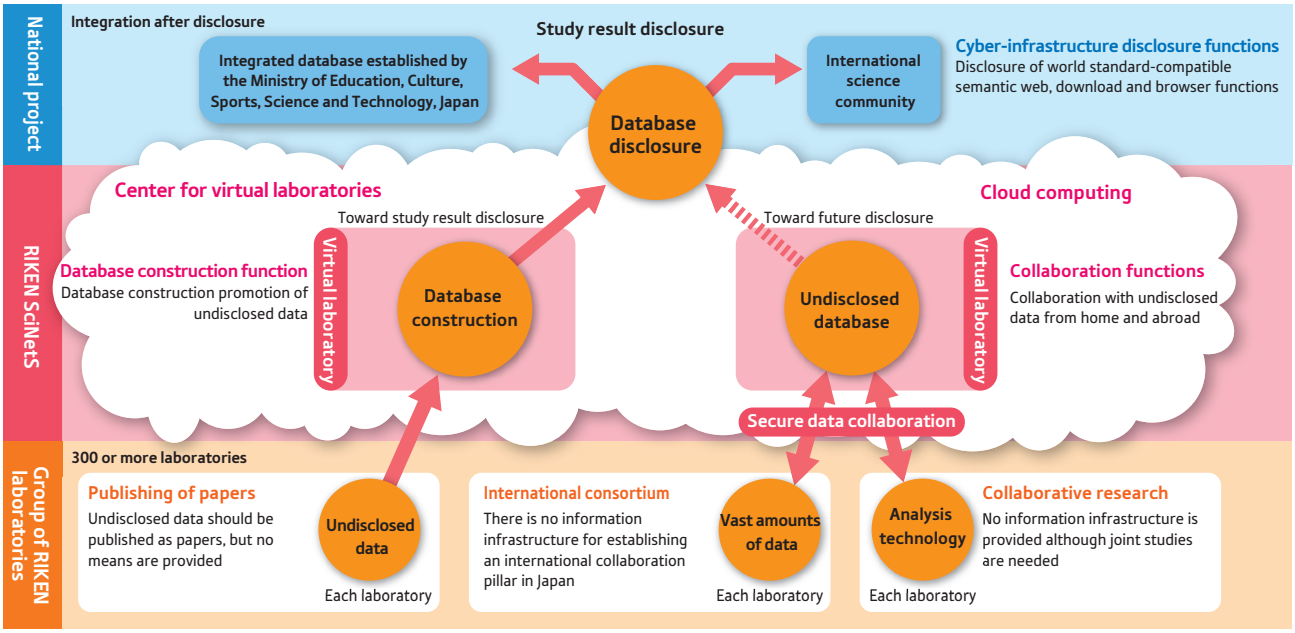


Figure 3: Center for virtual laboratories (SciNetS).
SciNetS provides incubation functions from database construction to the integration of databases in computing clouds or a group of large-scale servers, and discloses databases using interfaces compatible with international standards, thus contributing to the establishment of cyber-infrastructure for integrating worldwide databases.

through to the end of September 2010. The assignment: to design a DNA sequence conferring to the model organism Arabidopsis thaliana the functionality to effectively eliminate and detoxify airborne formaldehyde, which causes sick building syndrome. Participants need to take advantage of genome and protein databases in SciNetS to find out which genes should be optimized to enhance functionality for eliminating and detoxifying airborne formaldehyde. They also need to program via a web browser in order to rationally design part of the genome. The best design results will be used by RIKEN and other research institutes and actually transferred into a plant for functional verification under proper statutory safety control standards. The invitation to participate has been extended not only to researchers and university students in Japan and around the world, but also to high-school students. "I will be pleased if GenoCon could give high-school students with good programming skills the opportunity to become interested in life science and join the world of life sciences to become 'genome designers'. Many useful genes have been patented, but all current patents will expire by 2030, and this will bring about a genome design boom. Genome designer will become a glamorous job in the near future."

GenoCon will provide participants with the opportunity to enjoy the most advanced science and take on an open optimization challenge. Although genome designs for conferring the functionality to effectively eliminate and detoxify airborne formaldehyde to a plant have been published and some even patented, there may be better embodiments of the technology. The contest aims to search for better and more suitable embodiments with easier practical applications.

Toyoda is also a member of the RIKEN Biomass Engineering Program, which was initiated in April 2010. Through the program, Toyoda aims to improve the efficiency of producing bioplastic materials based on rational genome design methods for plants. Genome design methods and programs collected through GenoCon would also be used for that purpose.

"We intend to establish infrastructure for synthetic biology," says Toyoda. "Synthetic biology is a newly emerging field of science in which bioinformatics and biology are combined, and deals with the whole range of information and biological resources. We are now required to use the collaboration network of SciNetS to connect groups at RIKEN's technical bases, and to establish a structure that enables the creation of useful biological resources as a social asset from information resources. To begin with, I want to create an easily grown plant that can yield environmentally friendly bioplastic materials."

More information can be found at the SciNetS and GenoCon websites. ■

Tetsuro Toyoda

Tetsuro Toyoda was born in Tokyo, Japan, in 1968. He graduated from the Faculty of Pharmaceutical Sciences at The University of Tokyo in 1992, and obtained his PhD in 1997 from the same university. He started as a researcher at the Institute of Medical Molecular Design in 1997, and joined RIKEN as team leader in the Genomic Sciences Center in 2001. He became director of the RIKEN Bioinformatics And Systems Engineering Division when it was established in 2008. His expertise is in bioinformatics and computer-aided rational design of biomolecules, including rational database-supported drug design based on protein structural information and rational genome design in synthetic biology for biomass engineering. He promotes Japan's database integration projects as a member of several national database committees.

RIKEN–RAL Muon Facility celebrates 20 years of collaborative research

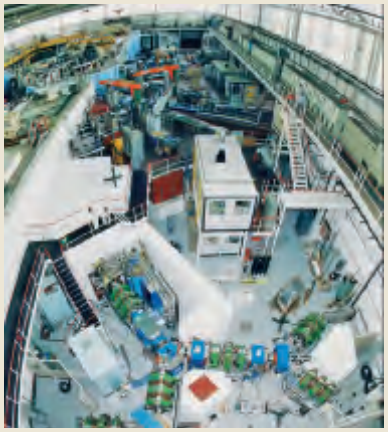
After two decades of successful collaboration on one of the largest joint research projects between Japan and the UK, a new agreement signed on 2 July 2010 extends the collaboration between RIKEN and the Rutherford Appleton Laboratory (RAL) out to 2018. The new agreement, formalized by RIKEN President Ryoji Noyori and Science and Technology Facilities Council (STFC) Chair Keith Mason, sets out a renewed path for advanced muon science.

RIKEN's involvement in muon science dates back to the 1930s and to the groundbreaking work of RIKEN physicists Hideki Yukawa and Yoshio Nishina. The existence of the muon was first hinted at in 1935 when Yukawa predicted the existence of a particle with similar properties later identified as the pion. The age of muon science was ushered in with Nishina's discovery in 1937 of the first muon, which was also discovered in independent work by Carl D. Anderson and Seth Neddermeyer in the USA.

The collaboration between RIKEN and RAL carries on in the footsteps of these early discoveries in muon science. The RIKEN–RAL Muon Facility was established to make use of the ISIS 800 MeV proton synchrotron, which while originally created for pulsed neutron scattering experiments, can also be harnessed to generate an intense source of muons.

Construction of the RIKEN–RAL Muon Facility at the ISIS pulsed neutron and muon source began in November 1991 following the signing of the first ten-year collaborative research agreement between RIKEN and the UK Science and Engineering Research Council (SERC). The first muon beam was extracted in November 1994, and experiments at three experimental ports began in April 1995. These experiments allow research on muon-catalyzed fusion, condensed matter and molecular science, and low-energy muon production. An additional experimental port added in the early 2000s now makes it possible to conduct fundamental muon-based nuclear and atomic physics studies.

STFC Chair Keith Mason (left) and RIKEN President Ryoji Noyori (right) sign an agreement extending the RIKEN–RAL collaboration until 2018.



Inside the RIKEN–RAL Muon Facility.

The first agreement was extended for another ten-year term, and third agreement signed in 2010 extends the partnership for another seven and a half years, assigning to RIKEN the roles of construction, operation, maintenance and administration of the facility. RAL continues with the role of providing the high-intensity proton beam from the ISIS accelerator. This new arrangement grants greater independence to each of the collaborating institutions, with RIKEN and RAL both having their own experiment review committees and freedom to pursue their own directions in muon research.

The signing ceremony for the new agreement was held as part of an event celebrating 20 years of collaborative research at the RIKEN–RAL Muon Facility. The event included speeches and presentations by Mason, Noyori, Andrew Taylor (ISIS director), Philip King (group head of the ISIS Muon Group) and Teiichiro Matsuzaki (director of the RIKEN Facility Office at RAL). Noyori gave a lecture on "Science and Technology for Future Generations" and expressed his gratitude to RAL for its contribution to 20 years of successful collaborative research.

The next seven years of research at the RIKEN–RAL Muon Facility promises to push the boundaries of muon science. The production of low-energy muons will be ramped up, while newly installed instrumentation will make it easier for scientists to study materials under extreme conditions of pressure and temperature. Research will also use muons to delve into the fundamental properties of new functional materials, and open up possibilities for energy generation based on muon-catalyzed fusion experiments. Just as it has over the past 20 years, the partnership between RIKEN and RAL will continue to set the standard for international collaboration in scientific research well into the future. ■

Masafumi Tsujimoto
Laboratory Head (former), Laboratory of Cellular Biochemistry
RIKEN Advanced Science Institute, Wako, Saitama, Japan
(Now at Faculty of Pharmaceutical Sciences, Teikyo Heisei University, Ichihara, Chiba, Japan)

Dear Prof. Tsujimoto,

It's my great pleasure to be able to write a postcard to you from my new office in the Department of Microbiology at Chulalongkorn University in Bangkok, Thailand. How are you? The time has passed so quickly, I still remember the day when I first joined your laboratory in 2008.

I first came to RIKEN in April that year, just in time for early spring. The sakura cherry blossom trees in full bloom around the RIKEN campus were so beautiful and wonderful. I was told some time later that RIKEN has a special kind of sakura with a deep pink bloom. It was so beautiful, I remember the cherry blossoms vividly.

In the Laboratory of Cellular Biochemistry, researchers were studying various types of proteins, including aminopeptidases, scavenger receptors and mRNA binding proteins. Each member performed their own research on proteins that they had discovered themselves. I focused on plant aminopeptidase. The lab was classic but extensive, and its research output was outstanding. The knowledge that I gained through my time in your lab is now being transferred faithfully to my students here in Thailand.

During my stay at RIKEN, I enjoyed not only the research but also many other activities. The other lab members were very welcoming and I got the chance to take part in many Japanese culture activities — two that I remember clearly are the traditional Japanese tea ceremony and fireworks festivals in summer. At the tea ceremony I got to wear a wonderful kimono. It was my first time to experience wearing a kimono, and it was really an amazing way to experience the tea ceremony.

Just before I left RIKEN, I remember we went to a Japanese restaurant where I had some of the best sashimi I've ever eaten. As I told you at the time, I love Japanese food, particularly sushi and sashimi, and I remember eating a lot of sashimi that night — my Japanese colleagues were surprised that I knew so many different kinds. In Thailand, sashimi is very expensive and I have not had the chance to eat it since returning home. I have to say that I miss sashimi very much.

I am really grateful for the scientific experience I acquired while working in your laboratory. I sincerely thank you and all the members in the lab. I always look forward to coming back to Japan one day and seeing my RIKEN friends. Best wishes to you all.

Yours sincerely,

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