Life at zero gravity

HIGHLIGHT OF THE MONTH

Stability reigns

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Stability reigns

First-ever observation of electronic states in aqueous acetic acid reveals water's unexpected influence

Water is life's most essential nutrient, and one of its key roles is to regulate acidity concentrations, or pH levels, within our bodies. But directly observing how water solvents participate in critical pH-dependent processes, such as protein assembly, is no easy matter. Scientists need a technique that can resolve the mutual interactions occurring between acids and millions of chemically similar surrounding water molecules.

Now, researchers led by Shik Shin from the RIKEN SPring-8 Center in Hyogo Prefecture, Japan, have combined state-of-the-art technical engineering with x-rays to detect quantum electronic state changes during an aqueous acid–base reaction[1]. The resulting data paint a surprising picture of water-induced stability that differs sharply from typical views of chemical dynamics.

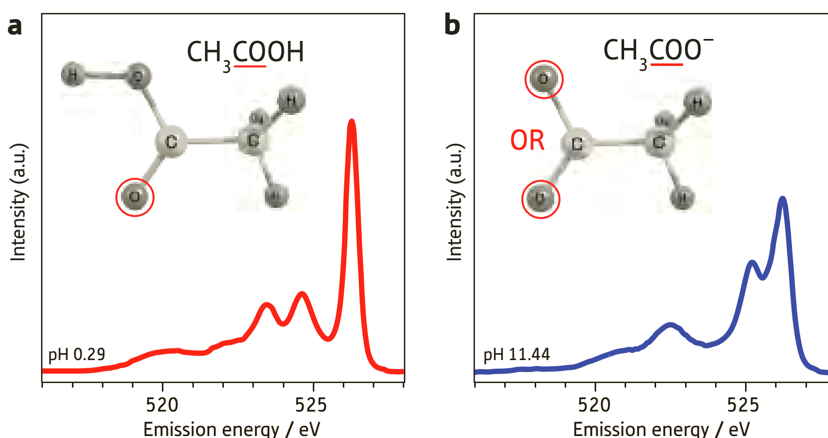

Figure 1: Schematic of x-ray emission spectroscopy results of (a) neutral acetic acid and (b) anionic acetic acid. Owing to precise energy tuning, soft x-rays selectively excite the oxygen molecule circled in red in panel a, and by one or other of the oxygen molecules circled in red in panel b.

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A delicate balance

The researchers used a molecule called acetic acid—best known as the main component of vinegar—as a model system to explore pH-dependent reactions. When acetic acid is dissolved in water, it can either remain intact or it can break its oxygen–hydrogen (O–H) bond and become a charged anion. At acidic pH, the neutral species dominates whereas at basic pH, the anion form prevails (Fig. 1). Equilibrium always exists between the two states, however, regardless of the pH level.

During this chemical reaction, acetic acid moves through and interacts with a multitude of water molecules. Shin and colleagues found that the best way to understand how water influences the acid–base equilibrium was to probe the quantum electronic states that hold acetic acid together.

X-ray vision

The imaging abilities of x-rays are familiar to anyone who has visited a dentist's clinic. However, x-rays generated by electrons accelerated to near the speed of light in RIKEN SPring-8 synchrotron also can be used to peer deep inside molecules to reveal their underlying chemical framework.

In x-ray emission spectroscopy (XES), a photon first excites an electron, which is tightly bound to the nucleus, to a higher, excited-energy state. This creates a 'hole'—an absent electron—in the core level. This hole is quickly filled by a valence electron in the molecule, and, to conserve energy, a new x-ray photon is emitted.

The emitted x-rays are uniquely dependent on the chemical and physical environment of the excited atom—a characteristic that let the researchers distinguish the electronic signal of acetic acid from the surrounding water molecules. Following this signal at varying pH levels provided nearly instantaneous information on chemical interactions during the acid–base reaction.

Because the intensity of x-rays emitted by light elements such as carbon, nitrogen and oxygen is quite low, it was essential to use high-energy synchrotron light to produce detectable electronic signals. "Observing such kinds of weak x-ray emissions requires an intense excitation light source and precise energy tuning of the incident light," says co-author Takashi Tokushima, a research scientist at RIKEN.

Engineering at the interface

To perform the experiment, the researchers developed a new liquid cell[2] that could be bolted onto the vacuum chamber and connected to the beamline of the synchrotron light source (Fig. 2). According to Tokushima, two challenges had to be overcome during the engineering process.

"First, we had to make a window as thin as possible to separate the vacuum chamber from the liquid cell,"

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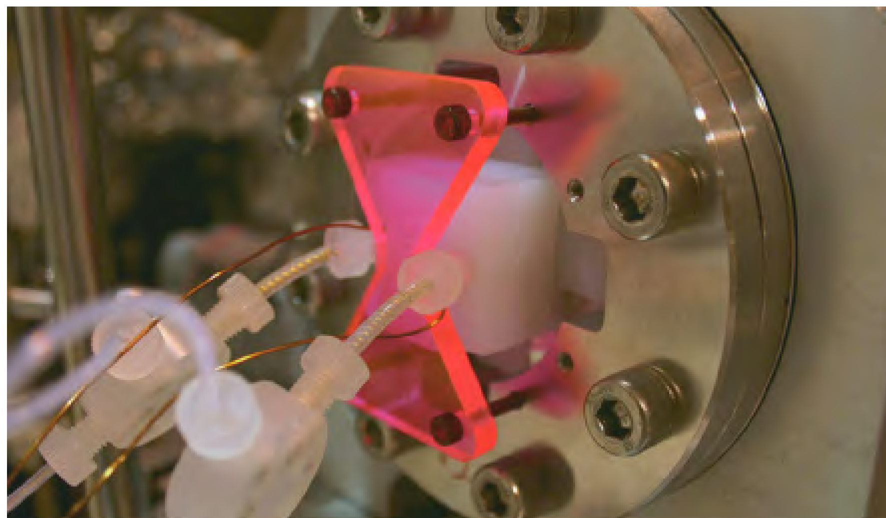

Figure 2: Photograph of the liquid reaction cell used to measure the electronic states of acetic acid solutions.

says Tokushima. Once the team had determined the optimum window—a 150 nm-thick silicon-nitride film—they faced their second challenge: how to flow a liquid sample past it.

"The 150 nm window is very fragile," states Tokushima. "Our liquid cell is designed to make liquid flow parallel to the window, with no pushing on its surface." Otherwise, the window cracks—spilling water into the vacuum chamber (Fig. 3).

Unexpected influence

Using their liquid cell, the scientists recorded x-ray emissions from a double-bonded oxygen atom located in acetic acid. The XES spectra showed significant differences between low and high pH levels—reflecting the change in chemical structures between the neutral and ionized species.

Figure 3: Photograph of a flood in a vacuum chamber after the liquid cell window breaks.

"Each peak structure mostly corresponds to a unique valence electronic state," says Tokushima. "Thus, we can use XES to fingerprint the forms of acetic acid."

At intermediate pH levels, chemists typically picture a dynamic acid–base equilibrium, in which the neutral and ionized forms of acetic acid rapidly change states. According to this model, the XES spectra of aqueous acetic acid at intermediate pH should be quite different than the acidic or basic spectra.

However, the researchers saw only gradual, systematic changes to the original XES spectra. In fact, the spectra from intermediate pH levels could be reproduced as ratios of either the pure neutral or ionic form. These results indicated that the two states of acetic acid did not interact with each other; instead, each state was static and stable, with populations determined by the solution pH.

Tokushima says that the unexpected perfect match between the ratio analysis of the XES spectra and a static acid–base equilibrium equation provides strong evidence that water molecules stabilize the different acetic acid states, possibly by forming a 'shell' of water around the acid molecules.

Fast company

The next plan for the researchers is to install a microfluidic mixer into their liquid cell to study chemical reactions involving two different reagents. Because the XES technique is extremely fast, the researchers hope to observe electronic structure changes with real-time precision. "In XES, the emission process occurs within the core hole lifetime—a few femtoseconds (10^{15} seconds)," says Tokushima. "That means it is possible to observe dynamics down to this timescale." ■

1. Horikawa, Y., Tokushima, T., Harada, Y., Takahashi, O., Chainani, A., Senba, Y., Ohashi, H., Hiraya, A. & Shin, S. Identification of valence electronic states of aqueous acetic acid in acid–base equilibrium using site-selective X-ray emission spectroscopy. *Physical Chemistry Chemical Physics* **11**, 8676–8679 (2009).
2. Tokushima, T., Horikawa, Y., Harada, Y., Takahashi, O., Hiraya, A. & Shin, S. Selective observation of the two oxygen atoms at different sites in the carboxyl group (–COOH) of liquid acetic acid. *Physical Chemistry Chemical Physics* **11**, 1679–1682 (2009).

About the researcher

Shik Shin obtained his doctorate of science in 1983 from the University of Tokyo in Japan. He then joined the Research Institute of Measurement at Tohoku University as a research associate, and was later promoted to associate professor. In 1991, he returned to the University of Tokyo as an associate professor in the Institute for Solid State Physics, where he later obtained the position of professor. Shin joined RIKEN in 2008 as team leader of the Excitation Order Research Team in the Quantum Order Research Group.

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Sifting out the sources of spin

High-energy proton collisions help solve the puzzle of what determines the proton's spin

Along with the neutron, the proton is the basic building block of the atomic nucleus. But, the source of the proton's spin—a fundamental property, along with charge—has been a longstanding puzzle to scientists.

By analyzing data from the BNL Relativistic Heavy Ion Collider (RHIC), the PHENIX collaboration at Brookhaven National Laboratory in Upton, USA, including scientists from the RIKEN BNL Research Center and the RIKEN Nishina Center for Accelerator-Based Science, has now ruled out gluons—the particles that exchange the so-called strong force between the quarks that make up the proton—as the dominant contributor to proton spin[1].

There are three quarks—two 'up' quarks and one 'down' quark— in the proton (Fig. 1). Each quark has a spin of ½ and they configure with respect to each other—two up and one down—so the sum of their spins is ½. For this reason, scientists thought the spin of the proton, which is also ½, came entirely from quarks.

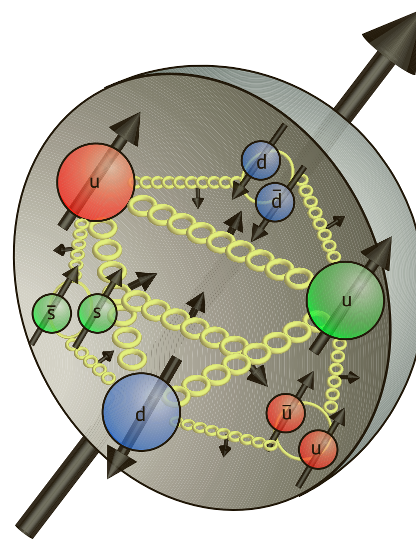

Figure 1: A schematic of the internal structure of a proton, which consists of two up (u) and one down (d) quark that interact via gluons (beige chains). Experiments in the 1980s showed that quarks contribute only about one-fourth of the entire spin of the proton (indicated by the large black arrow).

Experiments in the 1980s, however, showed that quarks only contribute about 25% of the total spin of the proton. "This result was so surprising that it was called the 'spin-crisis'," says Yasuyuki Akiba, a member of the PHENIX team. Particle physicists were therefore confronted with a fundamental question: What else contributes to the spin of the proton?

Some models predict that the missing spin comes mainly from gluons, while others suggest that the contribution from the orbital angular momentum of quarks within the proton may also be significant. To determine the contribution from gluons, the PHENIX team analyzed data from a year-long experiment performed at RHIC in 2006.

In this experiment, protons were collided at very high energies approximating 200 GeV, where 1 GeV is equal to one billion electron volts, to produce particles called pions. The researchers derived the gluon contribution to the proton spin from the difference between the number of pions produced by colliding protons with their spins lined up in the same direction to one another versus those lined up in opposite directions.

The important result from the PHENIX team's work is that the gluon contribution to the proton spin is actually small. "The best estimate is that it is about 40%, but the data don't rule out that it is zero," explains Akiba. "Although there is still a significant uncertainty in this result, our data show that models predicting large gluon spin can now be firmly excluded." ■

1. Adare, A., Afanasiev, S., Aidala, C., Ajitanand, N.N., Akiba, Y., Al-Bataineh, H., Alexander, J., Aoki, K., Aphecetche, L., Asai, J. *et al.* (PHENIX Collaboration). Gluon-spin contribution to the proton spin from the double-helicity asymmetry in inclusive π^0 production in polarized $p + p$ collisions at $\sqrt{s}$ = 200 GeV. *Physical Review Letters* **103**, 012003 (2009).

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Unearthing chemistry's rare gems

Combining rare-earth clusters with traditional metal catalysts reveals secrets of chemical transformation

Metal catalysts, with their ability to both speed up chemical reactions and influence product structures, have revolutionized manufacturing of essential goods such as petroleum and pharmaceuticals. The constant search for new catalysts that can improve existing methods has spurred chemists to investigate a relatively unknown part of the periodic table—the rare-earth elements.

Rare earths, named for the uncommon minerals in which they were first discovered, possess remarkable chemical properties owing to their internal electronic configuration. Now, Zhaomin Hou from the RIKEN Advanced Science Institute in Wako and colleagues have used an yttrium-based rare-earth cluster to generate a new series of complexes that hold vital structural clues towards improving catalytic reactions[1].

Hou and co-workers studied one of industry's most critical reactions: the reduction of carbon monoxide (CO) molecules attached to transition metal catalysts. In this process, a reagent known as a hydride causes CO to gain electrons or hydrogen, producing useful liquid hydrocarbons. Scientists know little about the mechanism of this reaction, however, and industry greatly desires more efficient catalysts.

First, Hou's team developed a new molecular rare-earth hydride—a large cluster containing several yttrium, hydrogen, and organic groups—to investigate CO reduction. According to Hou, the rare-earth hydride is extremely reactive towards molecules with triple bonds such as CO.

When mixed together, the rare-earth hydride incorporated the metal–CO complex into its own framework, creating structurally well-defined organic–multimetallic molecules with various degrees of CO reduction. The researchers believe that these new hybrid compounds are important intermediates in the transformation of CO into hydrocarbon molecules.

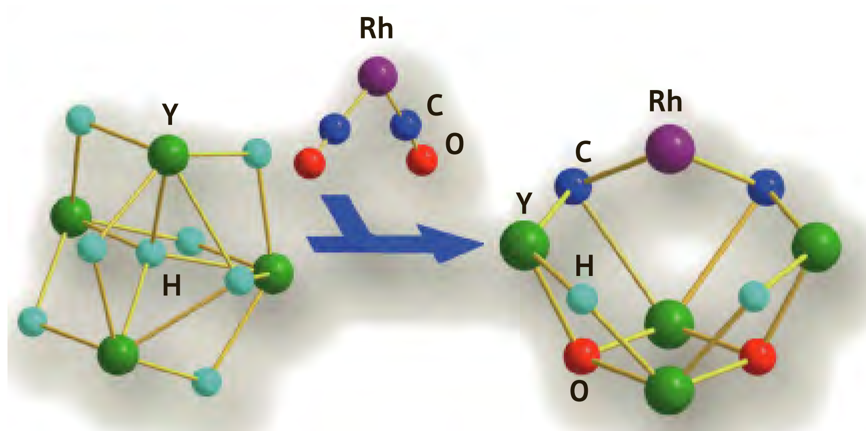

Figure 1: Schematic showing that a rare-earth yttrium hydride (Y–H) cluster reacts with a rhodium–carbon monoxide (Rh–CO) complex to give a new organic–multimetallic species, accompanied by cleavage of the C≡O triple bonds.

They also found that different metal–CO complexes generated unique structures with the rare-earth hydride. For example, tungsten–CO complexes added to the yttrium cluster as intact units by bonding oxygen atoms to yttrium sites. With a rhodium–CO complex, however, the C–O bond is cleaved after addition; carbon groups joined directly to yttrium while oxygen atoms moved deeper into the cluster framework (Fig. 1).

The ability of rare-earth hydrides to capture 'snapshots' of catalytic reactions through an extraordinary variety of metal, carbon, and oxygen bonding interactions promises to spark development of better organic synthetic techniques, a prospect that Hou and colleagues are actively investigating.

"These new organic–multimetallic structures provide well-defined examples of the individual first steps in the reduction of coordinated CO," says Hou. "And, our findings may give clues for the design of new catalysts for selective synthesis of hydrocarbons from CO reduction." ■

1. Takenaka, Y., Shima, T., Baldamus, J. & Hou, Z. Reduction of transition-metal-coordinated carbon monoxide by a rare-earth hydride cluster: Isolation of well-defined heteromultimetallic oxycarbene, oxymethyl, carbene, and methyl complexes. *Angewandte Chemie International Edition* **48**, 7888–7891 (2009).

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A beacon of change

A fluorescent sensor helps scientists track specific chromosomal modifications that can alter gene activity

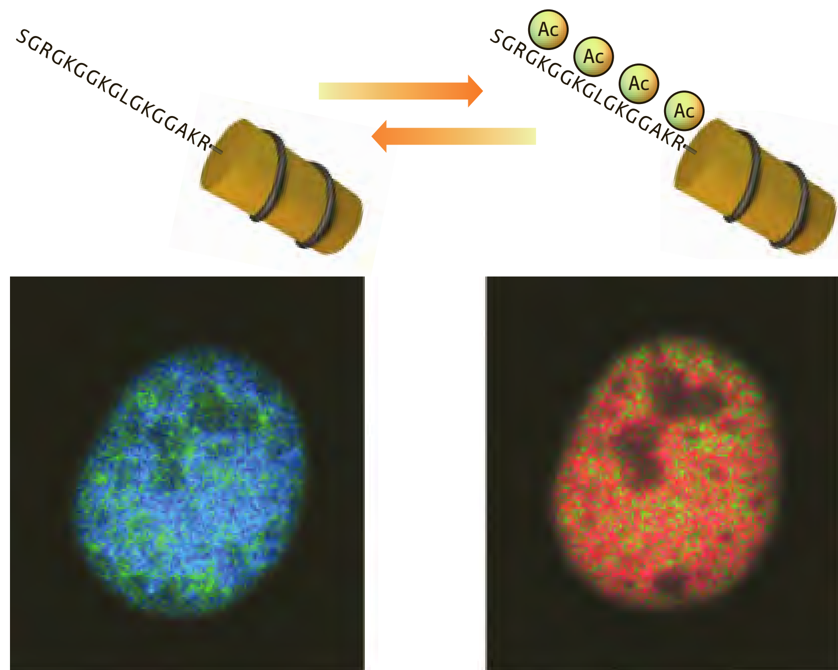

Figure 1: Tracking changes in histone acetylation. Histac produces a strong FRET signal (blue) when chromatin is unacetylated (left), but this signal diminishes when the chromatin is acetylated (right).

Chromosomal DNA is normally coiled and compacted via interactions with histone proteins to form material called chromatin. Chemical modification of the histones can profoundly alter how the chromatin is arranged, and this represents an important mechanism by which cells regulate gene expression.

One example is the addition of acetyl groups by histone acetyltransferase (HAT) enzymes. HATs mark histone H4 at multiple sites, and these acetylations are subsequently recognized by regulatory proteins of chromatin such as BRDT. However, this is a highly dynamic and reversible process, and these acetylations are routinely removed by histone deacetylase enzymes (HDACs).

To develop tools for tracking histone acetylation in live cells, Minoru Yoshida of the RIKEN Advanced Science Institute in Wako paired his knowledge of histone acetylation with colleague Kazuki Sasaki's expertise in fluorescent biosensor design. The result was Histac—a highly sensitive genetically encoded indicator that enables real-time imaging of changes in H4 acetylation[1].

Their sensor is based on Förster resonance energy transfer (FRET), which makes use of two distinct 'donor' and 'acceptor' fluorescent proteins. When the donor is excited by light, it releases energy that excites the acceptor and causes it to produce fluorescent signal, but only when the two are close together. In Histac, they are linked by H4 and a chunk of BRDT; they can interact in the unacetylated state, but when the H4 segment is acetylated, subsequent interaction with BRDT separates the fluorescent proteins and greatly decreases the FRET signal (Fig. 1).

Yoshida and colleagues demonstrated that Histac effectively incorporates into cellular chromatin, and subsequent treatment with HDAC inhibitor trichostatin A yielded a strong signal change, indicating successful detection of elevated histone acetylation. Subsequent experiments enabled Yoshida and Sasaki to directly observe histone deacetylation as cells undergo mitosis, followed by an increase in acetylation once cell division was complete—a somewhat surprising finding. "This dramatic decrease in histone H4 acetylation in mitosis was a matter of dispute," says Yoshida. Interestingly, they were even able to observe subtle variations in the rate of histone deacetylation at different regions within a single cell nucleus.

Having demonstrated its effectiveness and sensitivity, Yoshida and colleagues are expanding their work with Histac and developing additional tools that will enable them to analyze complex patterns of chromatin modification relating to embryonic development. "It has been proposed that epigenetic changes—including histone acetylation—are important for embryonic reprogramming and tissue differentiation," says Yoshida. "We therefore would like to visualize histone acetylation in embryonic cells under various conditions." ■

1. Sasaki, K., Ito, T., Nishino, N., Khochbin, S. & Yoshida, M. Real-time imaging of histone H4 hyperacetylation in living cells. *Proceedings of the National Academy of Sciences USA* **106**, 16257–16262 (2009).

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Secrets of separation

Surprising complexity underlies the seemingly simple process of chromosome pair separation during cell division

When chromosomal DNA duplicates, the resulting identical 'sister chromatids' are tethered together by the cohesin protein complex until the onset of mitosis. Cohesin is then released in multiple stages, enabling the sister chromatids to condense into distinct rod-shaped structures as a precursor to separation from each other during cell division.

Several years ago, a team led by Tatsuya Hirano—now at the RIKEN Advanced Science Institute in Wako—identified human protein Wapl as a regulator of cohesin and likely participant in this process of 'sister chromatid resolution'[1]. In order to better understand the function of this and other cohesin-modulating proteins, Hirano and postdoctoral fellow Keishi Shintomi have now performed a systematic series of experiments using extracts derived from frog eggs[2]. "In this experimental system, a protein of interest can be depleted and [the effects of] loss-of-function can easily be analyzed," says Hirano. "Moreover, wild-type or mutant forms of the protein can be added back into the depleted extracts to test whether they might be able to rescue the loss of function."

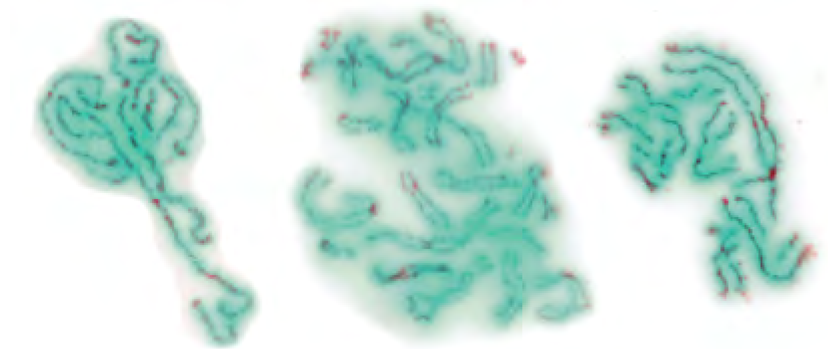

Figure 1: Chromosomes assembled in frog egg extracts. Pairs of duplicate chromosomes are normally aligned in parallel along their entire length prior to cell division (center). However, when the regulatory processes underlying this organization are disrupted, the duplicates fail to be resolved (left) or come apart (right).

Hirano and Shintomi noted that Wapl and another protein, Pds5, normally load onto chromosomes in a cohesin-dependent manner, and that targeted depletion of either protein impairs release of cohesin complexes. Subsequent experiments helped them to identify specific segments of Wapl that mediate this activity; Wapl variants with mutations in these key domains lost the ability to bind cohesin or Pds5, and therefore failed to restore normal chromatid resolution in Wapl-depleted extracts.

The researchers also gained new insights into Sgo1, a protein that contributes to the staged release of cohesin by protecting a subset of complexes from dissociation until late mitosis. Although Sgo1 was thought to act primarily at the central portion of chromosomes, this protein was observed along the entire length of the chromosome in Wapl- or Pds5-depleted extracts, suggesting that it may also stabilize interactions between the arms of sister chromatids.

Collectively, their findings reveal a bounty of hidden complexity in this important process (Fig. 1). "At first glance, removing cohesin from chromosomes during mitosis looks like a simple task," says Hirano. "But our current work shows that this process is regulated by an intricate network composed of many positive and negative regulators." Having delved below the surface of this network, his long-term goal is ambitious: reconstructing the full assembly line of proteins involved in cohesin assembly and disassembly. "This is a big challenge," says Hirano, "but nonetheless, it has to be done." ■

1. Gandhi, R., Gillespie, P.J. & Hirano, T. Human Wapl is a cohesin-binding protein that promotes sister-chromatid resolution in mitotic prophase. *Current Biology* **16**, 2406–2417 (2006).
2. Shintomi, K. & Hirano, T. Releasing cohesin from chromosome arms in early mitosis: opposing actions of Wapl–Pds5 and Sgo1. *Genes & Development* **23**, 2224–2236 (2009).

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Stacking at the flick of a switch

A switch that controls formation of stacks from nucleic acid strands has potential applications in gene expression and molecular machines

Small, structured nucleic acid strands designed to interact with specific target molecules, known as aptamers, are particularly interesting to scientists as therapeutics against viruses and blood clotting disorders. Aptamers that bind strongly to target enzymes or proteins can prevent their activity, thereby regulating the biological process they control.

Aptamers frequently contain stacks of hydrogen-bonded guanine-rich sequences, called quadruplexes, which enable effective binding to targets. When these structures are disrupted or destroyed—perhaps through poor replication—the aptamer can no longer bind. Interestingly, the reversible formation and disruption of these quadruplexes have the potential to be controlled using external stimuli, leading to a switching on and off of the aptamer's binding. Using this idea, Shinzi Ogasawara and Mizuo Maeda at the RIKEN Advanced Science Institute in Wako, have developed a light-controlled switch to control quadruplex formation in an aptamer that binds specifically to thrombin[1], the protein in blood that control blood coagulation.

The researchers developed a range of modified aptamers that contained various numbers of potential quadruplex structural units. Using light they were able to switch and select between a stable quadruplex and a non-structured state. Using light to control a switch allows accurate and easy control of the location, dosage, and timing. They then screened their variant aptamers to determine the best number and positions of the structural units to give effective regulation.

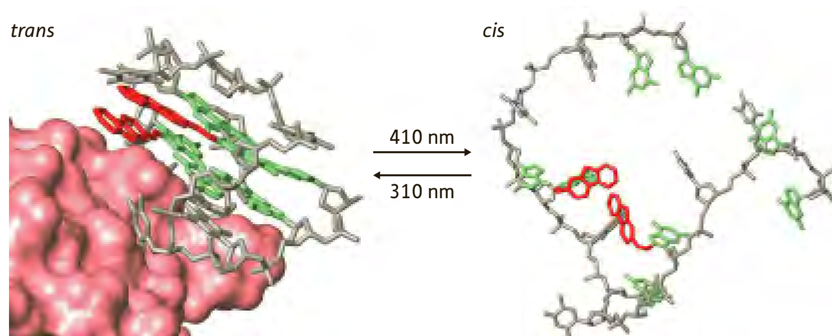

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Figure 1: Schematic of the reversible switch. The aptamer on the left, which has a so-called G-quadruplex structure, is bound to thrombin (pink). Light-induced photoswitching leads to destabilization of structure and loss of binding; the process can be reversed via further irradiation.

More specifically, Ogasawara and Maeda were able to control the switching behavior using light of specific wavelengths. In solution, they found that when the quadruplex is present, the aptamer binds strongly to thrombin. When the sample was irradiated at 410 nanometers, the double bonds within the complex changed from a so-called *trans* configuration to a *cis* configuration that disrupted the quadruplex and suppressed binding of the aptamer (Fig. 1). Irradiation at 310 nanometers, however, changed the bonds back into the *trans* form, allowing the quadruplex to re-form and bind once again.

Ogasawara and Maeda realized that the release-and-bind steps could be repeated over two cycles. First, they irradiated a reaction sample at 410 nanometers for 5 minutes and then at 310 nanometers for 2 minutes. After repeating the irradiation cycle, they found that the switch was completely reversible, and they detected no side reactions, thus demonstrating its potential in living cells.

Ogasawara and Maeda now plan to apply this technique to investigate other important biological events involving quadruplexes, such as gene expression and the construction of molecular machines.■

1. Ogasawara, S. & Maeda, M. Reversible photoswitching of a G-quadruplex. *Angewandte Chemie International Edition* **48**, 6671–6674 (2009).

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The simple truth

The plant hormone abscisic acid makes use of a surprisingly elegant and straightforward system to regulate its many essential functions

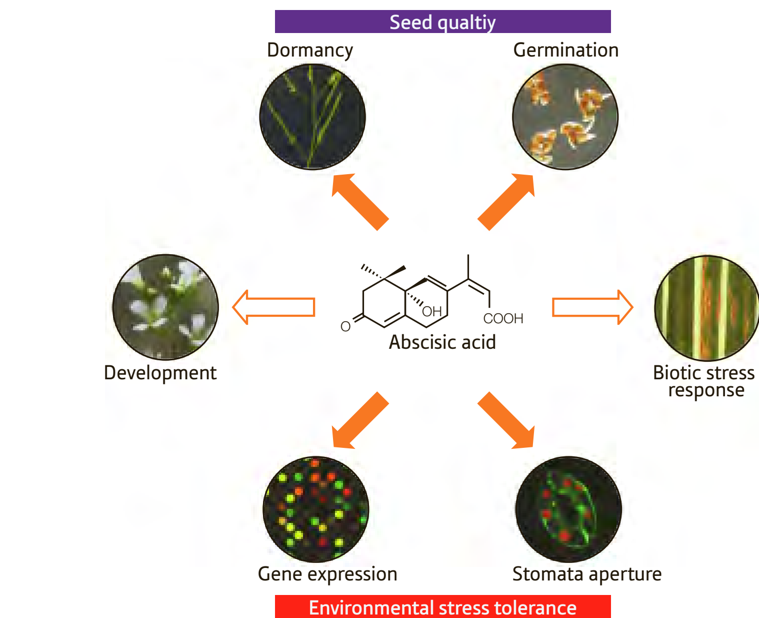

Figure 1: Schematic outlining the importance of the plant hormone abscisic acid (ABA), which controls a broad variety of crucial activities relating to plant development and survival.

Abcisic acid (ABA) has an awful lot on its plate. This plant hormone handles a staggeringly diverse array of jobs, from controlling seed germination to combating pathogen infection to dealing with drought conditions (Fig. 1), and scientists have assumed that the mechanisms regulating its activity must be equally elaborate. "ABA signal transduction pathways were thought to be a web-like, complex network in which many components have 'some role'," says Kazuo Shinozaki of the RIKEN Plant Science Center in Yokohama.

Taishi Umezawa of Shinozaki's team recently revealed the involvement of a subset of SNF1-related protein kinase 2 (SnRK2) proteins, which get activated via addition of phosphate groups in the aftermath of ABA signaling, enabling them to turn on downstream transcriptional activators. However, these signals also need to be turned off, and Takashi Hirayama's team at the RIKEN Advanced Science Institute in Wako identified several protein phosphatase 2C (PP2C) enzymes that may constrain ABA signaling.

By combining their expertise, these two teams have now successfully sketched out the surprisingly simple processes underlying ABA regulation[1]. Thale cress plants lacking the three members (kinases) of SnRK2 subclass III are minimally responsive even to high ABA levels, highlighting their central role in this pathway. Subsequent experiments revealed that these kinases physically interact with a subset of PP2C enzymes, which directly remove the phosphate group; mutations that inactivate these PP2Cs lead in turn to SnRK2 hyperactivation.

Under normal conditions, PP2C appears to keep SnRK2 in a constant state of inactivation—but when ABA binds its receptor, the resulting complex interacts with PP2C in a manner that lifts this inhibition. Accordingly, the researchers found that mutant forms of PP2C that lack an ABA receptor-binding domain establish resistance to ABA signaling.

The investigators were taken aback by the simplicity of the model they identified. "We think this is the major ABA signaling pathway—and may even be the only ABA signaling pathway—[and it] consists of only four components: soluble ABA receptors, PP2C, SnRK2, and [downstream] transcription factors or other enzymes," says Shinozaki. "That is very simple compared with our previous understanding."

Of course, some additional levels of complexity still remain to be uncovered—most notably, understanding ABA's part in the greater ecosystem of plant hormonal regulation. "We are interested in the molecular basis of cross-talk among plant hormone responses," says Shinozaki. "Now that one major ABA signaling pathway is established, we can investigate this cross-talk at the molecular level." ■

1. Umezawa, T., Sugiyama, N., Mizoguchi, M., Hayashi, S., Myouga, F., Yamaguchi-Shinozaki, K., Ishihama, Y., Hirayama, T. & Shinozaki, K. Type 2C protein phosphatases directly regulate abscisic acid-activated protein kinases in *Arabidopsis*. *Proceedings of the National Academy of Sciences USA* **106**, 17588–17593 (2009).

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Putting the brakes on cell death

Internal degradation within plant cells and their response to aging and disease are linked

The internal degradation process within cells of higher plants known as autophagy plays a role in limiting programmed cell death in response to infection and aging, according to recent findings by a RIKEN-led research group[1]. The group has also uncovered important molecular components of the mechanism by which this occurs. Autophagy is known to form the core of plant responses to starvation, so understanding how it works has significant consequences for plant survival under changing environmental conditions.

During autophagy a part of the cell—often including organelles, such as endoplasmic reticulum, Golgi bodies and mitochondria—is engulfed into a vesicle surrounded by a double membrane. This structure then fuses with and delivers its contents to another specialized vesicle full of enzymes that break down the plant molecules into simpler components. The plant can then use these components to build new molecules. Thus, it was long thought that autophagy was simply a mechanism for plants to recycle their molecular resources during times of scarcity.

The *ATG* genes involved in autophagy, however, are still active under nutrient-rich conditions. To investigate why, Kohki Yoshimoto and colleagues at the RIKEN Plant Science Center in Yokohama, together with researchers from the Japanese National Institute for Basic Biology and the Max Planck Institute for Plant Breeding Research in Germany, undertook biochemical, pharmacological and genetic studies in the plant *Arabidopsis* of two *atg* mutants in which autophagy was defective.

Figure 1: Comparison of a normal *Arabiadopsis* plant (left) with two mutants under nutrient-rich conditions. All plants were grown under the same environmental conditions for five weeks.

Under nutrient-rich conditions, the researchers found that the mutant plants grew quite normally, but showed early signs of senescence or aging and increased sensitivity to infection (Fig. 1). In short, the cells became more likely to undergo programmed cell death from these two causes. They also displayed a build-up of salicylic acid (SA), a signaling compound that stimulates others into action, suggesting that autophagy plays a role in regulating SA signaling.

The researchers then studied plants that were both SA deficient as well as *atg* mutants, and found this combination suppressed the *atg* mutant features. The suppression of the mutant characteristics was restored when they added a compound that triggers the same receptors as SA. In addition, autophagy could be induced in wild-type plants by this compound, but not when a critical downstream protein, NPR1, was missing or defective.

Yoshimoto says the team proposes that: "During aging or infection, SA signaling is accelerated, but that also induces autophagy via NPR1, which in turn modulates the SA signaling, limiting cell death." ■

1. Yoshimoto, K., Jikumaru, Y., Kamiya, Y., Kusano, M., Consonni, C., Panstruga, R., Ohsumi, Y. & Shirasu, K. Autophagy negatively regulates cell death by controlling NPR1-dependent salicylic acid signaling during senescence and the innate immune response in *Arabidopsis*. *The Plant Cell* **21**, 2914-2927 (2009).

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Getting past inspection

By being picky about its binding partners, an RNA-modifying enzyme brings an important measure of quality control to the protein production process

Messenger RNA (mRNA) molecules are the key intermediaries that enable translation of gene-encoded information into functional protein, but this process also relies on another essential family of RNAs—the various transfer RNAs (tRNAs) that physically 'read' the mRNA sequence and facilitate delivery and addition of appropriate amino acids to the nascent protein chain.

Although there is a specific tRNA for each individual amino acid, they all share a common L-shaped structure, which has a so-called anticodon mRNA-reading domain and an amino-acid-laden acceptor domain located at opposite ends. The assembly of a mature, functional tRNA depends on the addition of numerous targeted chemical modifications by a variety of specialized enzymes, such as Trm5, which tacks a methyl chemical group onto a specific guanine nucleotide in the anticodon region. For many tRNAs, this Trm5 modification is a crucial step, and new research from Shigeyuki Yokoyama and colleagues at the RIKEN Systems and Structural Biology Center in Yokohama has revealed unexpected insights into why this is the case[1].

The researchers began by collecting high-resolution structural data about the complex formed by Trm5 from the archaea *Methanocaldococcus jannaschii* (aTrm5) with a target tRNA and the methyl-group donor molecule AdoMet. As expected, their analysis revealed that the target guanine of the tRNA, G37, is positioned at a highly exposed orientation near the AdoMet molecule's methyl group at the catalytic center of aTrm5.

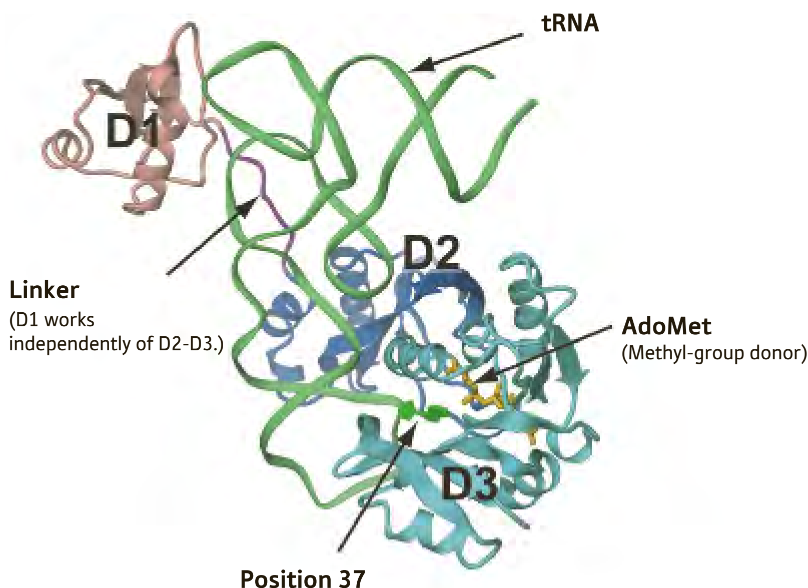

Figure 1: Schematic of the structure of the aTrm5 complex showing that the tRNA residue G37 (bright green) is positioned near the methyl-donor AdoMet within the catalytic domain (D2 and D3 of the enzyme), while sensor domain D1 recognizes the tRNA 'corner' of the L-shaped structure.

However, Yokoyama's team also characterized additional interactions between an enzymatic 'sensor' domain and structural elements located at the corner of the tRNA 'L', suggesting that aTrm5 selectively interacts only with fully folded target molecules (Fig. 1). Follow-up biochemical analyses confirmed the importance of this interaction in facilitating catalysis; at temperatures between 70 and 80 °C, where *M. jannaschii* typically thrives, aTrm5 variants entirely lacking the sensor domain exhibited dramatically reduced tRNA methylation activity relative to full-length enzyme, as did aTrm5 mutants with mutations at specific corner-binding positions.

Given the fundamental importance of G37 methylation in preventing 'typos' during protein production and the strong structure-dependence of Trm5 tRNA recognition and catalysis, Yokoyama and colleagues hypothesize that this enzyme may play a vital role in quality control. "The tRNA maturation process has a 'checkpoint' composed of the aTrm5 sensor-effector system to verify whether the tRNA assumes the proper L shape, in order to send only qualified tRNAs out to the translational stage," they conclude, adding that this may ultimately represent just one of many such 'inspection' steps over the course of maturation. ■

1. Goto-Ito, S., Ito, T., Kuratani, M., Bessho, Y. & Yokoyama, S. Tertiary structure checkpoint at anticodon loop modification in tRNA functional maturation. *Nature Structural & Molecular Biology* **16**, 1109–1115 (2009).

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Embryonic development—lost in space?

Experiments simulating zero-gravity conditions reveal developmental difficulties arising from mammalian reproduction in space

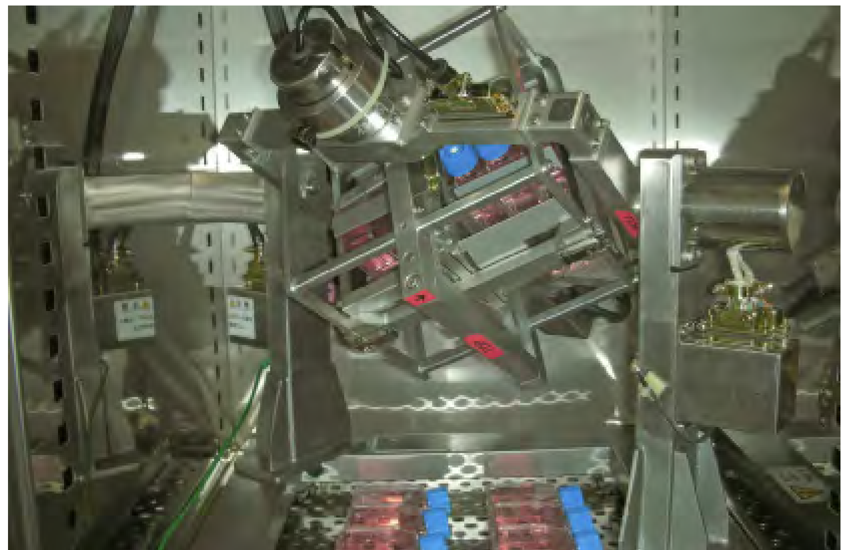

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Figure 1: The 3D clinostat apparatus uses rotation in all three dimensions to enable cell culture experiments under conditions that recreate zero gravity.

Despite other challenges, biological difficulties may be the primary obstacle to successful mammalian reproduction and development in orbit, according to new findings by Teruhiko Wakayama and his colleagues at the RIKEN Center for Developmental Biology in Kobe.

Wakayama's research is primarily focused on cloning, but he has long sustained an interest in outer space. "I took the exam to be an astronaut more than ten years ago," he says. Although his career ultimately followed a more earthbound path, an encounter with scientists working in space research provided the opportunity to tackle a long-standing question pertaining to life in outer space: can mammals reproduce successfully in zero gravity?

Previous studies have demonstrated successful reproduction by fish, amphibians and birds in zero-gravity conditions (also called microgravity), and that already pregnant rats can deliver healthy offspring aboard a space shuttle. On the other hand, experiments from a 1979 mission indicated that rats can get impregnated in space, but are seemingly incapable of bringing these pregnancies to term—although it was unclear at which stage complications arose[1].

Now, thanks to an apparatus known as a 3D clinostat (Fig. 1), which simulates microgravity via continuous three-dimensional rotation, Wakayama and colleagues were able to study fertilization and the earliest stages of embryonic development under conditions that replicate space travel[2].

They performed *in vitro* fertilization (IVF) experiments with mouse sperm and ova, both within the clinostat and at regular gravity (1G), and determine that microgravity had minimal effects on fertilization. It may prove detrimental to subsequent development, however. Microgravity-cultured embryos successfully reached the two-cell stage and yielded viable offspring upon implantation into female mice, but at a significantly lower rate than their 1G counterparts. The researchers observed more severe negative effects when embryos were transplanted following longer culture periods in the clinostat.

Microgravity led to an overall reduction in the rate of blastocyst formation after 96 hours of culture, and closer examination of these blastocysts revealed that the differentiation of embryonic cells into trophectoderm—the tissue that nourishes the embryo and ultimately contributes to placenta formation—was markedly impaired.

Given the successful development of non-mammalian embryos in microgravity, these findings were surprising, and Wakayama and colleagues intend to pursue further gravity-manipulation studies to zoom in on the source of the developmental problem. "We are planning to perform similar experiments at different gravities, such as Moon gravity (1/6G) or Mars gravity (1/3G)," he says. "I want to know how much gravity is necessary to perform normal reproduction." ■

1. Serova, L.V. & Denisova, L.A. The effect of weightlessness on the reproductive function of mammals. *Physiologist* **25**, S9–12 (1982).
2. Wakayama, S., Kawahara, Y., Li, C., Yamagata, K., Yuge, L. & Wakayama, T. Detrimental effects of microgravity on mouse preimplantation development *in vitro*. *PLoS ONE* **4**, e6753 (2009).

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Keeping the circadian clock ticking

The daily rhythm of mammals is maintained by a key molecular process that is unaffected by changes in ambient temperature

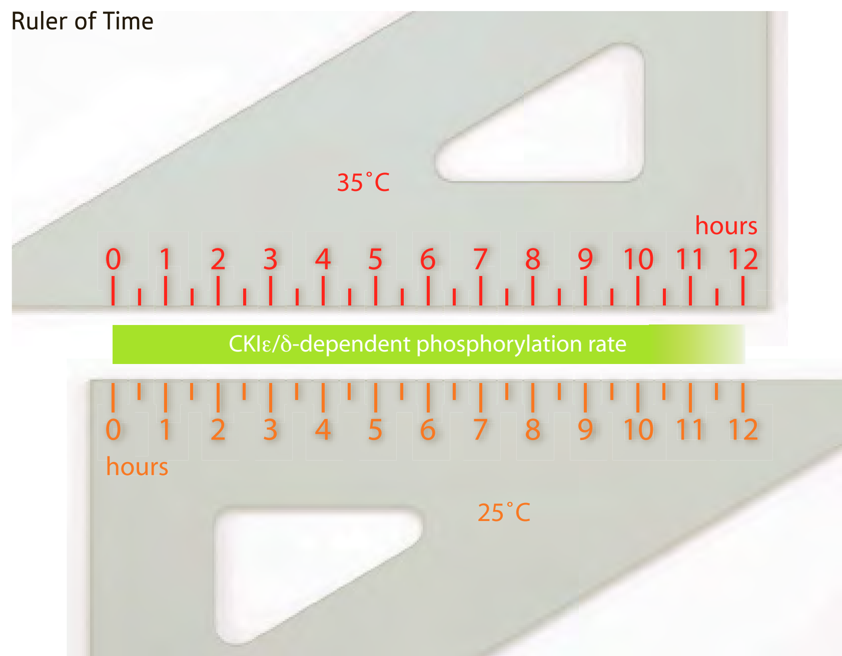

Figure 1: Schematic illustrating that phosphorylation by CKIε and CKIδ occurs at the same rate regardless of temperature, meaning that the daily cycle is not disrupted by changes in temperature. The process is therefore essential for maintaining the circadian rhythm in mammals.

The daily cycle of waking and sleeping in mammals, including humans, is governed by a complex array of cellular processes, which have only recently been uncovered.

One important molecular process that acts to keep the circadian rhythms of mammals 'up to speed' with the sun—within a period close to 24 hours—has now been discovered by Masato Nakajima and Hideki Ukai at the RIKEN Center for Developmental Biology in Kobe and their co-workers. Importantly, this process is independent of temperature, meaning that the daily cycle is not disrupted by the changing seasons.

According to Ukai, "Circadian clocks regulate many physiological phenomena such as sleep–awake cycles, blood pressure, hormonal secretions and drug metabolism." To investigate this important system, he and his co-workers tested 1,260 different compounds, chosen from the Sigma-Aldrich Library of Pharmacologically Active Compounds (LOPAC), by adding them to cells from both humans and mice[1].

The team identified ten compounds that lengthened the circadian period of cells. At least nine of the compounds were found to suppress the activity of casein kinase enzymes called CKIε and CKIδ. By increasing the concentration of these compounds even further, the researchers almost doubled the circadian period to 48 hours.

The results imply that, under normal circumstances, both CKIε and CKIδ act to set the speed of circadian rhythms. They do this by adding a phosphate group, or phosphorylating, and thus deactivating, a protein called PERIOD in a process that is completely independent of temperature (Fig. 1).

Such temperature insensitivity is a rare trait for biochemical reactions but was recently observed in enzyme reactions in the cyanobacterial clock. Similar findings for mammals by this team suggest that temperature insensitivity could be a conserved evolutionary principle across species, maintaining a consistent circadian clock even under the normal physiological range of environmental changes.

The researchers suggest that the accelerating effect provided by CKIε and CKIδ probably dominates other reactions that decelerate the circadian clock. Nakajima hopes that by quantifying the effects of all these processes, the research could have widespread medical applications.

"These compounds may become the seeds to develop new drugs for circadian rhythm abnormalities or insomnia," he says. With this goal in mind, the team is already assessing the importance of CKI-dependent phosphorylation in defining circadian rhythms in cells and tissues. "At the same time, we are focusing on how the temperature-insensitive phosphorylation works at an atomic level." ■

1. Isojima, Y., Nakajima, M., Ukai, H., Fujishima, H., Yamada, R.G., Masumoto, K., Kiuchi, R., Ishida, M., Ukai-Tadenuma, M. Minami, Y. *et al.* CKI ε / δ -dependent phosphorylation is a temperature-insensitive, period-determining process in the mammalian circadian clock. *Proceedings of the National Academy of Sciences USA* **106**, 15744–15749 (2009).

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Exploring symbiotic mechanisms through the genes of unculturable microorganisms

Moriya Ohkuma

Head of the Japan Collection of Microorganisms
RIKEN BioResource Center

Investigations are under way all over the world to produce biofuels by degrading the cellulose contained in plant waste, such as wood chips, thinned wood and rice straw, rather than producing biofuels from cereals such as corn and sugarcane. However, as the technology currently available for this purpose consumes a great deal of energy, the efficiency of the production process must be increased. In nature, one organism in particular obtains nutrients by degrading cellulose with extremely high efficiency: termites (Fig. 1). These insects exhibit astonishingly high reproduction rates while feeding on wood alone. The key to this efficiency in the production of nutrients is the assemblage of several hundred kinds of symbiotic microorganisms living in the termite gut. However, because most of these microorganisms are unculturable, their identities and natures cannot be examined in detail. For this reason, the symbiotic mechanisms between termites and microorganisms remain unclear. In 2008, Moriya Ohkuma, head of the Japan Collection of Microorganisms at the RIKEN BioResource Center, and colleagues succeeded for the first time in completely decoding the genome (the total genetic information of a set of chromosomes) of a symbiotic microorganism in the termite gut. Described below is his genetic analysis of the mechanisms behind the symbiosis between termites and microorganisms.

99% of microorganisms remain uncharacterized

Koki Horikoshi, researcher emeritus and former chief scientist at RIKEN, was the first to discover a microorganism capable of surviving in an alkaline environment. The cellulose-degrading enzyme produced by it is utilized in a household detergent.

"Japan and RIKEN have traditionally been active in research into microorganisms that degrade cellulose. However, there have been no studies at all on gut microorganisms in termites, which are known to degrade cellulose with extremely high efficiency. This is because most gut microorganisms cannot be cultured by any existing technique," says Ohkuma.

The properties of a microorganism can be examined in detail by isolating and culturing it to increase its population. If cultivation is not possible, even the very existence of the microorganism may not be known. "Microorganisms that can be cultured by currently available techniques account for less than 1% of all microorganisms identified so far. More than 99% are unculturable, so their identities and properties remain unknown. There are still large frontiers in microbial research."

Gut microorganisms found to have different habitats in the gut

In 1994, Ohkuma joined RIKEN and began studying microorganisms in the termite gut, an entirely new area of research. "My work at RIKEN started with the capture of termites. I attempted to culture the microorganisms I found in their guts, but I was able to culture less than 1% of them."

Ohkuma then decided to examine the properties of unculturable microorganisms by genetic analysis. The gut microorganisms in termites can be classified into two types by size: major-member protozoans, which have nuclei, and minor-member bacteria, which lack nuclei (Fig. 2). Ohkuma first analyzed genes serving as indexes for classification and identification of the microorganisms. "Surprisingly, new bacterial species were discovered that had not been known, even at the level of phylum, the highest taxonomic unit of bacteria."

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Figure 1: **Formosan subterranean termites.**
Although termites dispose of their gut microorganisms at the time of each ecdysis, they reacquire a full set of microorganisms by nutrient exchange—an interesting process of eating the excreta from the anus of another termite in the nest. It is hypothesized that the social behaviors of termites, including nutrient exchange and community life, have evolved in order to perpetuate the set of gut microorganisms essential for their survival.

Examination of the distributions of the new bacteria in the termite gut revealed their symbiotic association with particular protozoans. "It was found that particular bacterial species occur as symbionts with respective protozoans. Different bacteria have different habitats in the termite gut."

A world first in decoding a microbial genome from a trace amount of sample without culturing the microorganism

In recent years, many researchers have investigated unculturable microbial populations by a technique known as metagenomic analysis, resulting in major achievements including some related to the study of human enteric bacteria. "Metagenomic analysis, a method of analyzing a mixture of genes of various microorganisms, is a valuable tool for clarifying the functions and other features of a microbial population as a whole and thereby discovering useful genes. However, the symbiotic mechanisms involved cannot be explored without knowing the capabilities and interactions of individual microorganisms. Metagenomic analysis does not provide information on which genes are present and which ones are lacking in a particular microorganism," says Ohkuma.

In response, Ohkuma began studying ways of decoding the genome of a microorganism using very few microbial cells and without culturing the microorganism, a goal that no-one in the world had yet achieved. The genome cannot be decoded unless amplified to increase its quantity. If the number of cells of a microorganism can be increased by culturing it, a sufficient amount of the genome can be obtained to allow it to be decoded. In decoding the genome of an unculturable microorganism, the key issue is how to obtain the required amount of the genome.

Ohkuma and his colleagues chose Rs-D17 as the first target of their project to decode microbial genomes. This is a newly discovered bacterial species occurring as a symbiont in the gut of the Japanese subterranean termite (Reticuliformes aperatus), for which no strain in culture had been available even at the phylum level (Fig. 2). The microorganism lives in the bodies of certain protozoans that degrade cellulose, and accounts for 4% of all symbiotic bacterial cells in the gut of the Japanese subterranean termite. However, the genomic information of Rs-D17 varies slightly depending on its protozoan host, and the presence of this dissimilar genomic information

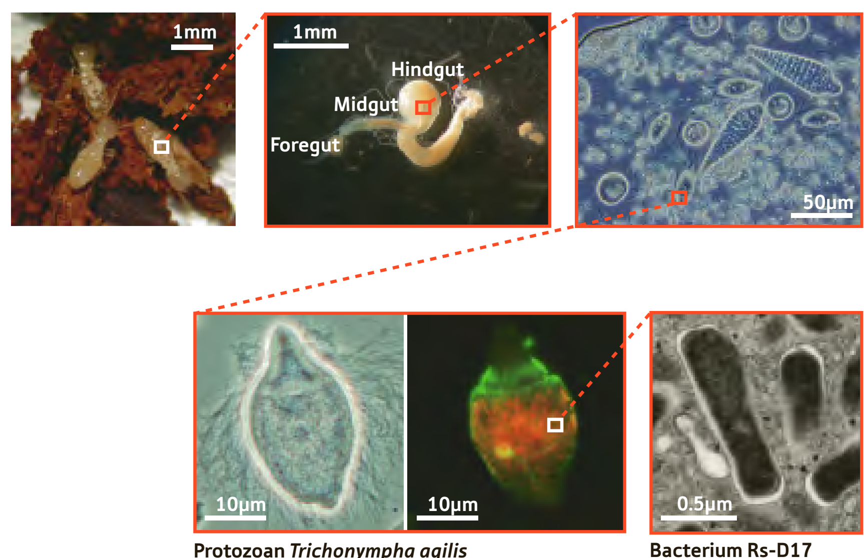

Figure 2: **Symbiotic protozoans and bacteria in the gut of the Japanese subterranean termite.**

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interferes with the decoding. Studies on how to decode the genome from a single bacterial cell have yet to be successful because the amount of genome extractable from a single cell is at present insufficient for analysis.

"Fortunately, Rs-D17 bacterial cells having exactly the same genomic information congregate densely at a particular site in one protozoan. We recovered several hundred Rs-D17 cells from that site. Even so, the amount of genome obtained was extremely low, just one picogram. Decoding the genome requires more than one microgram of genome sample, one million times the amount we had available. Using a new enzyme that allows genome amplification, we amplified the genome ten million times, giving us ten micrograms, and attempted to decode the genome in cooperation with the RIKEN Yokohama Institute. We were thus able to decode the genome of a microorganism completely from very few microbial cells without culturing the microorganism for the first time in the world."

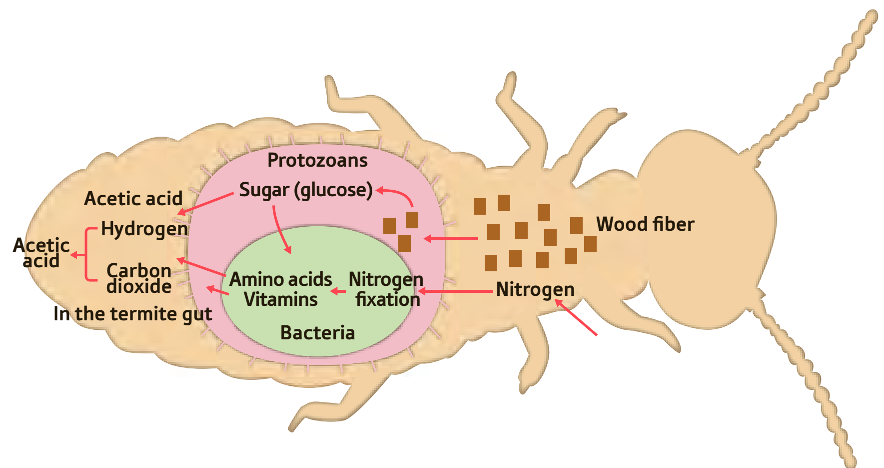

Figure 3: Symbiotic mechanisms in gut microorganisms in the termite intestine. Fractions of wood eaten by termites are taken by protozoans, which decompose the cellulose component of the wood into sugar (glucose) and supply it to bacteria. The bacteria fix atmospheric nitrogen and synthesize amino acids and vitamins using the sugar as a source of energy, and supply the products to the termites and protozoans. The bacteria also produce acetic acid and hydrogen from the sugar; the protozoans produce acetic acid, hydrogen and carbon dioxide from the sugar. Other bacteria in the termite gut produce acetic acid from the hydrogen and carbon dioxide. The acetic acid thus produced serves as a source of energy for the termites.

Symbiotic mechanisms clarified in part

What has decoding the Rs-D17 genome revealed? The genome was estimated to comprise 761 genes for protein synthesis. Also discovered were 121 pseudogenes that had lost their original function for protein synthesis. Another important finding was that the genome is very small, only about a quarter of the size of the Escherichia coli genome. "It is hypothesized that evolution for genome shrinkage occurs, by which genes no longer necessary for Rs-D17 to survive in protozoans lose their function and are discarded. For example, a gene involved in the synthesis of the cell wall, which protects the cell body against environmental changes, has lost its function in Rs-D17. Living in the protozoan body, Rs-D17 does not require genes for environmental adaptation."

Conversely, still-functioning genes are thought to be indispensable for symbiosis with the termites and protozoans. Discovered among the 761 genes were genes for the synthesis of vitamins and amino acids that cannot be produced by termites or protozoans.

Ohkuma and his colleagues also succeeded in decoding the genome of CfPt1-2, a bacterium that occurs as a symbiont with a cellulose-degrading protozoan in the gut of the Formosan subterranean termite (Coptotermes formosanus). Accounting for 70% of the total cell count of symbiotic bacteria in the termite gut, CfPt1-2 was found to have a gene for the synthesis of nitrogen components, which are scarce in wood fiber. Nitrogen is an essential element for the survival of organisms. CfPt1-2 fixes atmospheric nitrogen and synthesizes ammonia, which serves as a starting material for vitamins and amino acids. It also incorporates ammonia and urea as nitrogen waste from the host protozoan and reuses them as sources of nitrogen. It is believed that by doing so, the termite and protozoan are able to feed exclusively on wood fiber without suffering a shortage of nitrogen.

"Termites eat wood fiber, and protozoans degrade the cellulose component into sugars such as glucose, which gets supplied to the bacteria. The bacteria fix atmospheric nitrogen and synthesize amino acids and vitamins using the sugars as a source of energy, and supply the products to the termites and protozoans. There is a firmly established association in a waste-free give-and-take relationship; the termites, protozoans and bacteria are mutually indispensable partners. Part of these remarkable symbiotic mechanisms of extremely high efficiency was clarified for the first time by our bacterial genomic analysis," says Ohkuma (Fig. 3).

"Our next target is the genomes of protozoans that degrade cellulose to sugars," he says. However, the protozoan genomes are more than 100 times larger than the bacterial genomes. Such large genomes cannot be amplified and decoded using existing techniques. Hence, Ohkuma and others are conducting research on how to extract individual genes expressed in a protozoan population and examine their functions, rather than pursuing genomic analysis. "We don't know which enzymes are degrading cellulose to sugars in a single protozoan. I want to find that out. Additionally, protozoans have been found to possess a strong ability to produce hydrogen. As for bacteria, Rs-D17 has also been shown

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to be a hydrogen producer, and hydrogen is likely to be used as a next-generation source of energy."

Utilizing the symbiotic mechanisms in microorganisms

Biofuels manufactured from cereal crops such as corn and sugarcane are attracting attention as a next-generation energy source. However, the production of biofuels from cereals is causing food prices to surge, and there is increasing deforestation as producers expand agricultural land. Accordingly, there are many investigations focusing on the generation of biofuels from cellulose, which is available in large quantities in plant waste, such as wood chips and rice straw. Provided that cellulose is degraded into sugars, it should be possible to produce fuel alcohol from the sugars using the conventional technology for making Japanese sake. However, the currently available techniques consume vast amounts of energy to achieve the rigorous processing conditions for cellulose degradation, including high pressure and high temperature. "That approach seems to be of no use in resolving the energy crisis. Termites degrade and use cellulose much more efficiently. If we can use the symbiotic mechanisms in microorganisms that allow their host termites to achieve this performance, it will be possible to manufacture biofuels and hydrogen by using the mechanisms artificially to degrade cellulose to sugars with even higher efficiency than termites.

"Humans are using only a part of the capabilities of microorganisms," continues Ohkuma. "Currently, only single microorganisms and some enzymes produced by microorganisms are being used in practice. Although combinations of two or more microorganisms are used for some purposes, including wastewater purification, the mechanisms involved in their interactions remain unknown. Like the gut microorganisms in termites, microorganisms with a wide range of different capabilities gather and interact with each other everywhere in nature, and build symbiotic systems of extremely high efficiency. If we can understand the mechanisms involved, we might be able to make better use of the excellent capabilities of microorganisms."

Making best use of information on unculturable microorganisms to compile bioresources

After working at the RIKEN Advanced Science Institute, Ohkuma became head of the Japan Collection of Microorganisms (JCM) at the RIKEN BioResource Center in April this year. The term 'bioresources' refers to the types of experimental materials (biological resources) that are indispensable in life sciences research, including animals, plants, microorganisms, cells and genes. Japan is promoting the National Bioresources Project, which aims to establish world-class fundamental infrastructure for bioresources by 2010. The JCM is serving as the hub for activities related to general microorganisms through the project.

"In recent years, there has been a dramatic increase in interest in microorganisms. This is because close mutual associations have been demonstrated between microorganisms and humans, other animals and plants. For example, microorganisms living in the human intestines and skin are closely associated with human health and immune functions. Plant productivity also relies on symbiotic microorganisms that occur around the roots of plants. Utilization of microorganisms is indispensable for resolving energy and environmental issues. In the US and Europe, researchers across a broad range of fields are partnering with microbiologists in large research projects. Unfortunately, Japan is no longer as active in microbial research as it used to be. The same is true at RIKEN, which now has fewer laboratories studying microorganisms. I am very concerned about the situation."

Ohkuma points out that in view of the situation, the JCM should play a major role. "I want to provide microbial bioresources for researchers working in diverse fields, including medicine, agriculture, energy and the environment, so as to create a new circle of cooperation in microbial research.

Ohkuma also wishes to develop a new type of bioresource. "Currently, only culturable microorganisms are preserved and made available by the JCM. Of course, such microorganisms will remain important and we will continue to expand their stock. But the scope of cooperation should be expanded if we are to develop, preserve and supply genetic information on bioresources from microorganisms that are currently unculturable. I want to make best use of our technical resources that have been nurtured through termite research in the development of new bioresources." Cooperative research based on new bioresources will open up the vast potential of microorganisms. ■

About the researcher

Moriya Ohkuma was born in Saitama, Japan, in 1964. He graduated from the Department of Agricultural Chemistry of the University of Tokyo in 1988, and obtained his PhD in 1993 from the same university. He was a special fellow of the Japan Society for the Promotion of Science at the University of Tokyo until 1994. He then moved to RIKEN as a research scientist and started his career in molecular microbial ecology. He was promoted to senior scientist in 2000, served as a team leader in two research groups at RIKEN, and is now head of the Japan Collection of Microorganisms at the RIKEN Bioresource Center. From 2002 to 2005, he served concurrently in the Japan Science and Technology Agency PRESTO program. His research background is in molecular biology, microbiology, microbial ecology and molecular phylogeny, and his current research interests include symbioses, microbial diversity and microbial functions.

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RIKEN and the Tokyo University of the Arts hold joint symposium on science and art

On Sunday, November 15, 2009, RIKEN and the Tokyo University of the Arts (TUA) held a joint symposium to commemorate an agreement, signed in April 2009, toward cooperation in research and education, with the aim of achieving broad-based advances benefitting all of society.

In the first of three dialogues making up the first half of the symposium, Kiyoshi Furukawa of the TUA and Kazuo Okanoya of the RIKEN Brain Science Institute (BSI) talked about music and language. Okanoya explained that there are rules, referred to as song syntax, underlying chirp repetitions in songs of the Bengalese Finch. Furukawa responded by noting that cadence takes on a cohesive form in Western music, a form which, as he showed using actual data, the brain recognizes.

The second dialogue brought together Masaaki Miyasako, a professor of the TUA, and Junji Sugiyama of the RIKEN SPring-8 Center. Under the theme of 'cultural assets', Sugiyama introduced research results on the identification of wood in a wooden figure or sculpture based on analysis of a single piece of wood using the SPring-8 facility. He also explained the mechanism giving rise to spirals in plant vines, and noted that Miyasako often used the spiral form in his paintings. Miyasako went on to point out that various spiral shapes are also hidden in the work of the famous Japanese painter Ogata Korin, and the discussion turned to the deep relationship between spirals, the basic structural form of DNA, and beauty.

A third dialogue delved into this theme of 'beauty' in more depth. Hideto Fuse of the TUA and Shigeru Kuratani of the RIKEN Center for Developmental Biology both quoted Leonardo da Vinci in the introductions to their work. Fuse pointed out that da Vinci studied anatomy to augment his painting. Kuratani highlighted anatomical and developmental problems in the way that da Vinci drew the wings on the angel Gabriel in his work "The Annunciation", and spoke about his own research on the turtle shell as an example of morphological evolution based upon a shared body plan.

The last half of the symposium featured a three-way dialogue between TUA President Ryohei Miyata, RIKEN President Ryoji Noyori and BSI Director Susumu Tonegawa. Again centering on similarities and differences between science and art, the speakers discussed issues such as reproducibility and objectivity in science versus subjectivity and individuality in the arts. ■

(From left to right) Ryohei Miyata, Ryoji Noyori, Susumu Tonegawa.

Strengthening ties with China

On October 24, RIKEN Executive Director Kenji Takeda visited Zhejiang University in China to sign an important collaborative agreement between the two institutions. The agreement sets down plans to establish a platform for identifying candidate compounds for drug discovery research using structural biology and imaging techniques. It also calls on the institutions to exchange research personnel, and will involve a future transfer of RIKEN nuclear magnetic resonance imaging facilities to Zhejiang University and the establishment of a laboratory for joint research.

The day before the signing of this agreement, on October 23, a two-day joint symposium for young researchers was held at Shanghai Jiao Tong University on the topic of nano materials and technology. The symposium provided a venue for young researchers from RIKEN and the university to present and actively discuss their research. Shigenori Fujikawa, who acted as symposium coordinator on behalf of RIKEN, reported that the event "changed everyone's perception of China. We were amazed by the transformation China has undergone."

Indeed, RIKEN has been working for some time toward strengthening its collaborative ties with Chinese research institutions. In 2006, RIKEN sent a representative to begin preparations for opening a RIKEN office in Beijing, and every year since then RIKEN President Noyori has given special lectures at Peking University and other major Chinese universities. In addition to the collaborative agreements already in place, a number of Chinese graduate students and researchers have come to work at RIKEN and to attend workshops.

These endeavors coincide with the Japanese government's initiatives toward the creation of an East Asian Community, and there is a general consensus on both sides that Japan and China must cooperate and strengthen their relationship. In China, the promotion of major research projects with Japan is clearly seen by the government as symbolic of the cooperative ties between the two countries. The Chinese government likewise hopes for more personnel exchanges including top-level scientists, and is eager to establish collaborative research laboratories. RIKEN is actively working now to draft concrete measures with the aim of realizing these objectives. ■

RIKEN holds joint symposium with the University of Liverpool

Over the years, researchers at RIKEN and the University of Liverpool (UoL) have fostered strong research ties through a number of productive collaborations centering on SPring-8, RIKEN's world-class synchrotron radiation facility in Harima, Japan. The joint symposium held on November 12, 2009 at the RIKEN Wako campus, featuring presentations by five visiting members of the UoL and five RIKEN researchers, grew out of this interdisciplinary research. Along with their latest research results, presenters shared their ideas on the importance of international collaboration in a world of increasingly globalized higher education.

One example of such collaboration was described by Samar Hasnain, professor at the UoL's School of Biological Sciences. Hasnain traced the path by which his discussions with Hiromichi Kamitsubo, a former executive director at RIKEN who played a leading role in the establishment of SPring-8, developed into key institutional partnerships. Stephen Holloway, pro vice chancellor of the UoL School of Natural Sciences, reiterated the importance of such partnerships in outlining his university's approach to internationalization, where he stressed the value not only of research links, but also of student and staff exchanges.

Reflecting the diversity of research conducted at RIKEN and the UoL, research-oriented presentations at the symposium covered a wide range of topics. The topic of bio-imaging techniques in particular sparked lively discussions. Mark White of the UoL introduced his latest achievement on spatial and temporal information coding by the Nf-κB system. Atsushi Miyawaki from the RIKEN Brain Science Institute described new fluorescent probes he developed recently, and also offered new perspectives on bioscience. Yasushi Sako from the RIKEN Advanced Science Institute (ASI) reported on his discovery of intracellular signal transduction using single-molecule imaging. The symposium ended with closing remarks by Holloway, who mentioned an exchange program between UoL students and young researchers at the RIKEN SPring-8 Center, scheduled to be launched soon.

At the banquet, it was announced that Yousoo Kim, one of the speakers from RIKEN ASI, had been awarded the Sir Martin Wood Prize the day before the symposium in recognition of his remarkable achievements in condensed matter physics. Laboratory Head Maki Kawai, who has supported Kim since his arrival from Korea, described Kim's achievement as among the most memorable events of her life. ■

Yousoo Kim and the British Ambassador, David Warren at the award ceremoney of Sir Martin Wood Prize.

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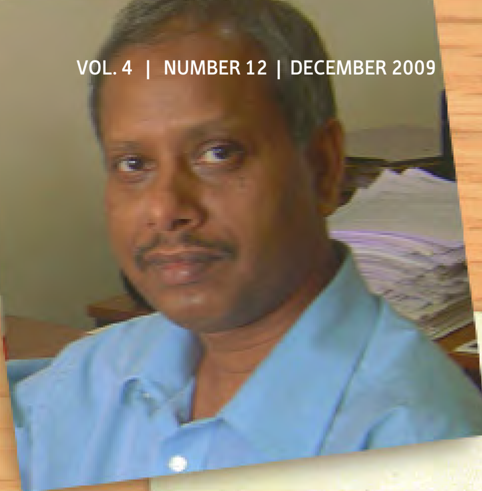

Dr Nobuyuki Nukina
Laboratory Head
Laboratory for Structural Neuropathology
RIKEN Brain Science Institute
2-1 Hirosawa, Wako-shi, Saitama 351-0198, Japan

Dear Dr Nukina,

It is with great pleasure that I send you this correspondence from my present position at the National Brain Research Centre in India. I hope this postcard finds you well. As a postdoctoral staff scientist in your laboratory from 1999 to 2001, I found the experience to be tremendously enjoyable, with an excellent research environment.

I can still remember my first day at the RIKEN Brain Science Institute (BSI) when you introduced me to your laboratory members, showed me different research centers, and later took me to lunch with all of the laboratory members, chatting over Indian curry. Taniguchi-san then took me to the Wako City Office for my Alien Registration Card, and because of her kind help I was settled in very quickly. Your laboratory was truly international, including researchers from Japan, Russia, Korea, China, Australia and India. Everybody was very cooperative and helpful.

Initially, we were working in the old research building of RIKEN, but we later moved to the fifth floor of the main BSI building. I have fond memories of the lab meetings and discussions we had every Monday at 9 am, and discussions on our research work with other team leaders like Dr Takahashi, Dr Yamakawa and Dr Takashima. The excellent animal house and experimental facility as well as the activities of the Advanced Technology Development Group also fascinated me. The expertise and experiences that I gathered at the RIKEN BSI helped me tremendously in creating my new laboratory at the National Brain Research Centre.

Apart from the research activities, I also enjoyed many cultural activities in Japan, including the tea making ceremony and the *tanabata* and coming-of-age festivals. Viewing *sakura* (cherry blossoms) and bathing at the *onsen* (natural hot spring) are some of my most unforgettable experiences.

Although I left the RIKEN BSI at the end of 2001, I continue collaborative work with the BSI and our recent joint research proposal under the India–Japan Cooperative Science Program will further boost our research collaboration and also promote cultural exchange.

I wish you all the best in the future. Please convey my best regards to all of your present laboratory members, and especially Ms Taniguchi.

Yours sincerely,

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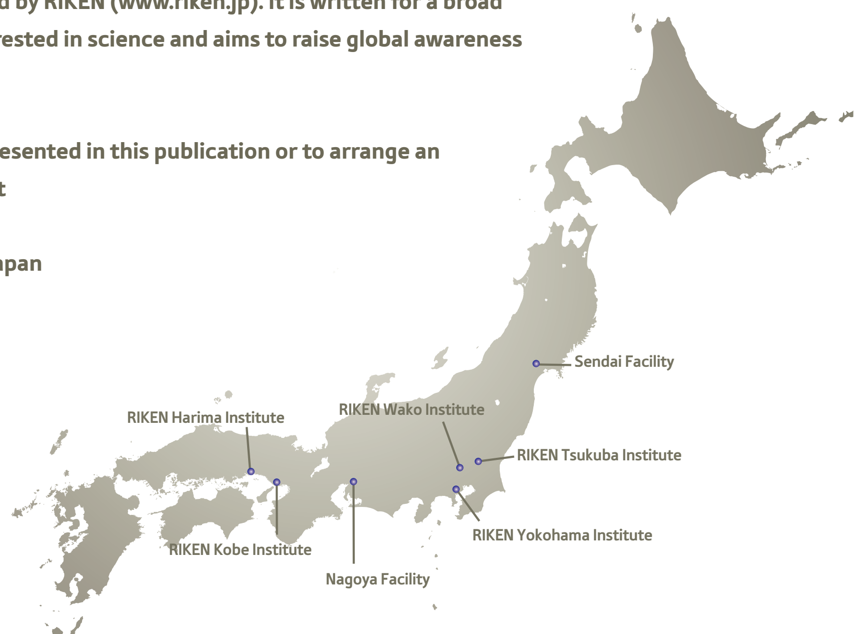

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