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Defining diabetic diversity

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Different roads to diabetes

A specific genetic variant puts individuals of Asian ancestry at risk of developing diabetes—but not their European counterparts

Type 2 diabetes is relatively widespread in Japan, and that nation's Ministry of Health, Labour and Welfare estimates that nearly one-third of all individuals over the age of 40 are either diabetic or pre-diabetic. Intriguingly, Japanese patients as a whole are less prone to obesity, a condition commonly associated with onset of type 2 diabetes in the Western world, indicating that there may be some significant differences in disease pathology between these two groups.

Two primary mechanisms contribute to the onset of type 2 diabetes. Fat, muscle and liver cells lose the ability to respond to the hormone insulin, a state known as 'insulin resistance' that greatly reduces the efficiency with which excess glucose is taken up from the bloodstream; in parallel, the capacity of the pancreatic beta cells to produce and secrete additional insulin is impaired (Fig. 1). However, Shiro Maeda of the RIKEN Center for Genomic Medicine in Yokohama points out that the relative importance of these mechanisms appears to differ between Eastern and Western populations. "Accumulating clinical evidence suggests that disability of insulin secretion contributes more to the pathogenesis of Japanese type 2 diabetes," he says, "whereas insulin resistance seems more important for European type 2 diabetes."

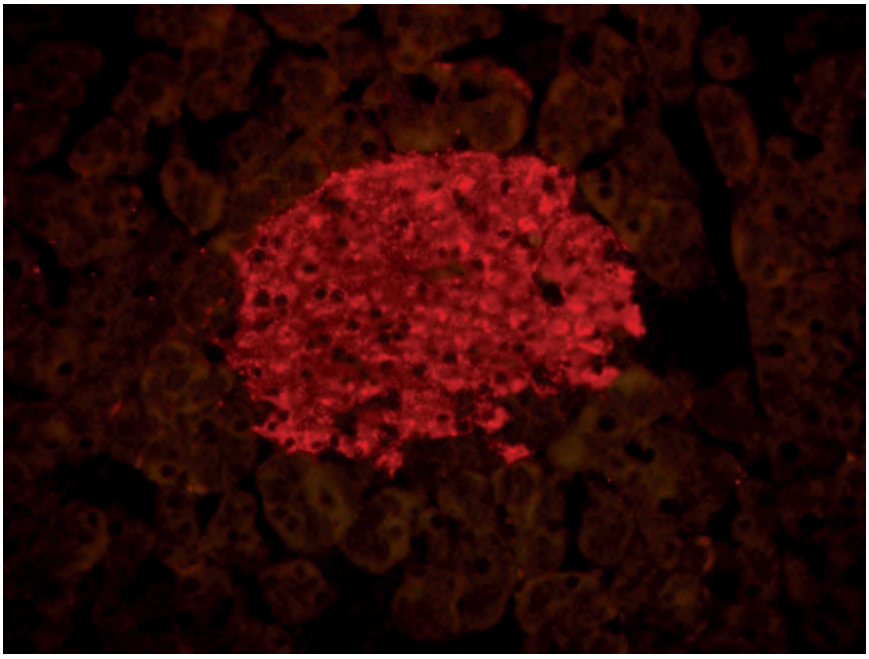

Figure 1: Beta cells (red) in pancreatic islets are responsible for the production and release of the blood sugar-regulating hormone insulin.

Getting the big picture

Very little is known about the genetic-level differences in pathology between the Japanese and Europeans. From several previous genome-wide association studies (GWAS), geneticists have identified several small sequence changes, also known as single-nucleotide polymorphisms (SNPs), which might be located near or within genes involved in type 2 diabetes. They have also identified several of these 'susceptibility loci'. However, with the exception of a few small-scale Japanese studies, nearly all of these data were obtained exclusively from individuals of European ancestry.

In an effort to collect more information about Japanese-specific risk factors, Maeda and Takashi Kadowaki of The University of Tokyo recently headed up a large GWAS that matches the scale of its European counterparts both in terms of the numbers of subjects involved and in the number of genomic markers examined[1]. According to Maeda, their study of some 5,000 type 2 diabetes subjects and 3,000 controls for 459,359 SNPs, is one of the largest sample sizes for a single GWAS worldwide.

Maeda, Kadowaki and colleagues subjected 98 candidate SNPs with the strongest association to type 2 diabetes to an additional round of analysis in a second, independent, set of disease and control cohorts. Based on these data, they identified statistically significant disease linkage for SNPs at a number of different genomic loci (Fig. 2). One of these SNPs, *KCNQ1*, was identified in both of the previous Japanese GWAS as a risk factor in both Asians and Europeans, and this gene appears to participate in the regulation of glucose-induced insulin secretion.

The researchers' study also flagged SNPs at two additional loci for which no association to type 2 diabetes had been previously reported. Both of these loci were subsequently validated in yet a third round of genomic screening, reinforcing the likelihood of their connection to diabetes.

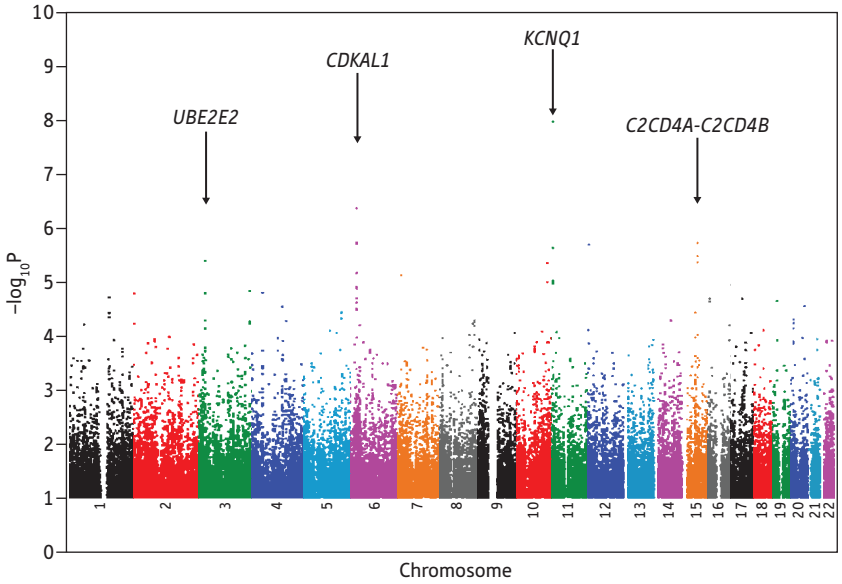

Figure 2: A 'Manhattan' plot of GWAS results for the Japanese population. The horizontal axis indicates each SNP's chromosomal location, while the vertical axis indicates the statistical significance of disease association; SNPs positioned higher on the plot have stronger association with type 2 diabetes.

Enigmatic risk factors

One of the newly identified loci, *UBE2E2*, encodes an enzyme that targets proteins for destruction by marking them with individual molecules of the small protein ubiquitin. It is expressed in a variety of tissues, including the liver, pancreas, muscle and fat. Based on several recent studies, researchers have suggested that the ubiquitination pathway may contribute to the efficient synthesis and secretion of insulin. Maeda, Kadowaki and colleagues noted that study subjects with the diabetes risk-associated *UBE2E2* allele appeared to exhibit impairments in insulin regulation.

Strikingly, although the SNPs at this locus were also determined to be associated with type 2 diabetes for three other East Asian populations, in addition to various Japanese study groups, this association was not statistically significant for two European groups, consisting of a total of 6,980 subjects and 8,615 controls. "Although this population-specific effect needs to be validated further, the present study is the first to show the existence of a disease-susceptibility locus [for diabetes] in a population-specific manner with genome-wide significant levels of association," says Maeda.

Despite escaping detection in previous large-scale GWAS, the second newly identified locus, *C2CD4A-C2CD4B*, showed significant association with type 2 diabetes in both Asian and European cohorts. *C2CD4A-C2CD4B* produces a pair of factors that appear to contribute to the maintenance of cell structure, and although relatively little is known about their function, both factors are expressed in many of the same tissues as *UBE2E2*. This represents the first indication that they might contribute to the pathology of type 2 diabetes.

Digging deeper

Kadowaki, Maeda and colleagues are now scanning these two loci more carefully in an effort to identify potential mechanisms by which sequence variants in these regions might contribute to onset of diabetes. Maeda indicates that they are particularly keen to understand the biological basis for the apparent ethnicity-specific disease association of SNPs in the *UBE2E2* locus.

At the same time, the identification of a novel locus that appears to contribute to risk of diabetes across population lines suggests that there may be a number of other susceptibility genes waiting to be discovered. "The identification of *C2CD4A-C2CD4B* as a common locus is really surprising, because this important locus has been missed in European GWAS," says Maeda, "[and] we are now participating in international collaborations, both East Asian and trans-ethnic, to identify additional type 2 diabetes susceptibility loci." ■

1. Yamauchi, T., Hara, K., Maeda, S., Yasuda, K., Takahashi, A., Horikoshi, M., Nakamura, M., Fujita, H., Grarup, N., Cauchi, S. *et al.* A genome-wide association study in the Japanese population identifies susceptibility loci for type 2 diabetes at *UBE2E2* and *C2CD4A-C2CD4B*. *Nature Genetics* **42**, 864–868 (2010).

About the researcher

Shiro Maeda was born in 1960 and is currently Laboratory Head at the Laboratory for Endocrinology and Metabolism, RIKEN Center for Genomic Medicine, where his research area is genetics for diabetic nephropathy and type 2 diabetes. He received his MD and PhD from Shiga University of Medical Science. He worked as a physician at Koka Public Hospital and Shiga University of Medical Science for next three years. From 1993 to 1996 he was a research fellow at the Department of Pathology, University of Michigan, where he researched osmotic response elements within the *ALR2* gene. For the next two years, he did a residency at Shiga University of Medical Science in internal medicine, and emergency and critical medicine. In 1999, he was an instructor for the Third Department of Medicine, Shiga University of Medical Science, and then joined RIKEN the following year as a research scientist in the Laboratory for Genotyping, SNP Research Center. He was a Laboratory head at Laboratory for Diabetic Nephropathy between 2001 and 2008, and has been in his current post since 2008.

Finding hope in a meltdown

Theoretical physicists find evidence of a new state of matter in a simple oxide

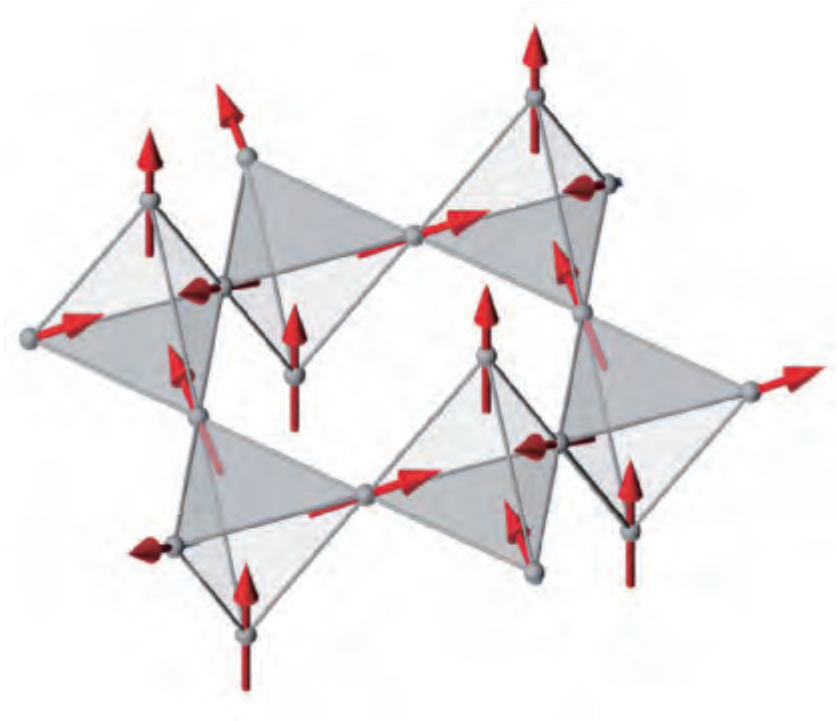

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Figure 1: Schematic of the spin-ice structure of the oxide $Pr_2Ir_2O_7$. Large quantum fluctuations of the spins (red arrows) are predicted to melt the spin-ice structure and form a new state of matter, called a chiral spin liquid.

Symmetry is a fundamental concept in physics. Our 'standard model' of particle physics, for example, predicts that matter and anti-matter should have been created in equal amounts at the big bang, yet our existing universe is mostly matter. Such a discrepancy between the symmetry of known physical laws, and what we actually observe, are often the inspiration for realizing that new interactions are important or that new phases of matter can exist.

Shigeki Onoda, a theorist at the RIKEN Advanced Science Institute in Wako, recognized that experimentalists at The University of Tokyo had possibly discovered a new state of matter, called a 'chiral spin liquid' when they reported evidence of time-reversal symmetry breaking[1]—a difference between the trajectory of a particle moving along one path or its inverse—in the oxide called $Pr_2Ir_2O_7$. If a material is magnetic, or in a magnetic field, its electrons will not obey time reversal symmetry; but in $Pr_2Ir_2O_7$, neither contribution was present to explain what the experimentalists had observed.

Now, Onoda and colleague Yoichi Tanaka have explained how a chiral spin liquid could emerge from so-called 'quantum spin fluctuations'—the motion of spins that occurs even at absolute zero[2]. "The possibility of a chiral spin liquid was first proposed twenty years ago and many physicists had lost hope to find it," explains Onoda. "This is a revival of a phase that was found in a totally different system than where it was first expected."

The interesting properties of $Pr_2Ir_2O_7$ are rooted in its crystal structure, called a pyrochlore lattice: four praseodymium (Pr) ions, each of which carries a magnetic 'spin', form a tetrahedral cage around an oxygen (O) ion. At low temperatures, the spins of materials with this structure often 'freeze' into what is called a 'spin ice' (Fig. 1) because of its similarity to the way hydrogen ions form around oxygen in water ice.

Onoda and Tanaka predict, however, that the quantum fluctuations in the spins melt the spin ice structure of $Pr_2Ir_2O_7$. They proposed a realistic model of Pr spins on a pyrochlore lattice and suggested that both the geometry of the crystal and the small size of the spin on the Pr ion allowed the quantum fluctuations to grow so large that they melted the spin ice into a chiral spin liquid.

If their prediction is correct, $Pr_2Ir_2O_7$ will be the first material in which one can study this new state of matter. ■

1. Machida, Y., Nakatsuji, S., Onoda, S., Tayama, T. & Sakakibara, T. Time-reversal symmetry breaking and spontaneous Hall effect without magnetic dipole order. *Nature* **463**, 210–213 (2010).
2. Onoda, S. & Tanaka, Y. Quantum melting of spin ice: Emergent cooperative quadrupole and chirality. *Physical Review Letters* **105**, 047201 (2010).

When old is new again

A fundamental effect associated with electrons also occurs in non-charged particles—a potential boon for spintronics

Just as electronics revolutionized computing and communications technology, spintronics is touted to follow suit. This relatively new field involves manipulating the flow of a magnetism-related property called 'spin'. In magnons, a spintronic counterpart of electrons, Naoto Nagaosa from the RIKEN Advanced Science Institute (ASI) in Wako and his colleagues have observed an effect first seen with electrons over 130 years ago: the Hall effect[1]. The Hall effect is used in sensitive detectors, so the researchers believe their finding could lead to new applications for magnetic insulators.

The Hall effect arises because a charge-carrying particle such as an electron experiences a force perpendicular to its direction of motion as it moves through a magnetic field of a conducting material. The result is a build-up of charges of opposite signs on either side of the material, which creates a measureable electric field. Magnons, however, have no charge, so an analogous effect had never been observed previously.

"The Hall effect is one of the most fundamental phenomena in condensed matter physics," explains Nagaosa. "It is important to study to what extent we can apply ideas from conventional electronics to spintronics." Nagaosa, along with Yoshinori Tokura also from ASI, Yoshinori Onose and co-workers from The University of Tokyo, and Hosho Katsura from the University of California, Santa Barbara, USA, studied the magnetic and thermal properties of the insulating ferromagnet $Lu_2V_2O_7$ at low temperatures. Rather than the electric field associated with the conventional effect, the Hall effect manifested in this material as a thermal conductivity gradient across the sample (Fig. 1). This difference occurs because the magnons carry heat, rather than charge.

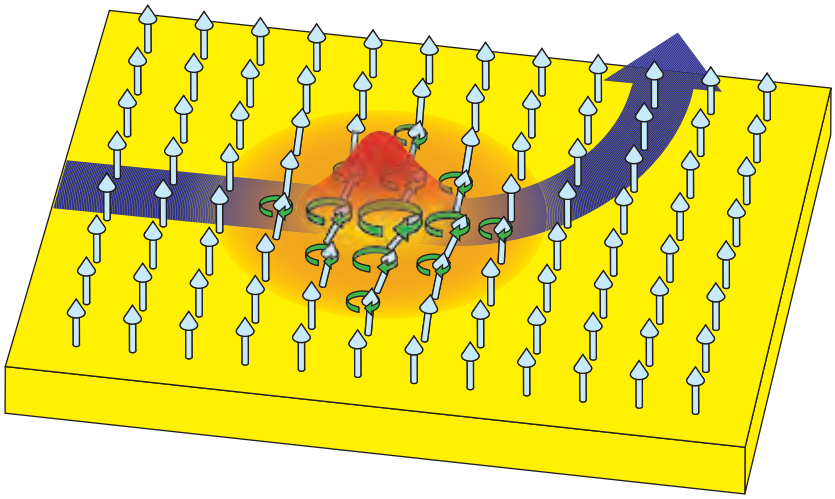

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Figure 1: A schematic depiction of the Hall effect in a ferromagnet (yellow). A magnon (red)—a localized area in which the spins (arrows) point in a different direction to those in the rest of the material—is diverted by the relativistic effect (blue arrow).

The researchers showed that the size of the effect is not proportional to the applied magnetic field, but has a maximum at relatively low fields. This supports the hypothesis that magnons, influenced by the relativistic interaction, are responsible because the number of magnons is known to be reduced at these low-level magnetic fields. They also observed that the conductivity gradient started to decrease at higher fields. This observation allowed Nagaosa and colleagues to rule out lattice vibrations, or phonons, as another possible underlying cause of the experimental results: a phonon-induced thermal conductivity gradient would be expected to continue to increase with magnetic field.

"According to our theoretical prediction, only certain types of the crystal structure show this magnon Hall effect," says Nagaosa. "To confirm this theory, we next aim to check that the phenomenon is absent in more conventional structures such as a cubic lattice." ■

1. Onose, Y., Ideue, T., Katsura, H., Shiomi, Y., Nagaosa, N. & Tokura, Y. Observation of the magnon Hall effect. *Science* **329**, 297–299 (2010).

Heads up, tails down

Advanced laser spectroscopy exposes the unique organization of water molecules under model membrane surfaces

The behavior of water molecules as they contact biological substances has long puzzled scientists. The first few layers of interfacial water can display complex arrangements that distinctly influence biochemical reactivity and function. Mapping these interfaces, however, is extremely difficult because chemical signatures of surface-bound water are often swamped by bulk liquid signals. Now, researchers led by Tahei Tahara from the RIKEN Advanced Science Institute in Wako have developed a laser spectroscopy technique that conclusively determines the orientation of water molecules beneath charged lipid layers—the primary components of cell membranes[1].

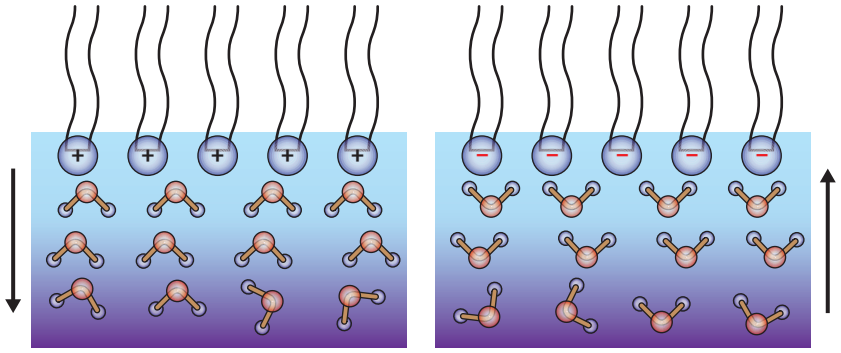

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Figure 1: Schematic diagram showing that, when the air–water interface is positively charged (top left), the water molecules (bottom) will orientate 'hydrogen-down' (left), and when it is negatively charged they are 'hydrogen-up' (right).

Phospholipids are fatty acid molecules that contain two parts: hydrophobic 'tails' made of long hydrocarbon chains and hydrophilic 'heads' comprised of charged phosphate groups and other organic units. At the air–water interface, phospholipids spontaneously form into monolayer films, with their tails extending into the air and their heads immersed in water. The structure and orientation of water molecules below such monolayers has been a matter of controversy. Some investigators suggest that the partially positive-charged hydrogen atoms of water orientate 'up' or 'down' to align with the lipid head charge, while others suggest the opposite outcome.

Tahara and colleagues resolved this debate by using an optical technique called heterodyne-detected vibrational sum frequency generation (HD-VSFG) spectroscopy, which has extremely high surface sensitivity. HD-VSFG combines two laser beams with different frequencies at an interface to generate a sum-frequency signal; when vibrations of surface molecules resonate with the applied laser, the sum-frequency signal rapidly shoots up—instantly identifying which chemicals are present. Because this signal originates from non-linear surface polarization effects, it contains only contributions from interfacial species. "HD-VSFG automatically probes the depths of water layers that are different from the bulk," says Tahara.

Determining the orientation of surface water required heterodyne detection, a method that determines the phase of weak signals via interference with a reference beam. According to Tahara, performing such measurements required precisely sensing changes to the signal light's optical phase—meaning the researchers had to control the laser beams with nanometer-scale accuracy.

The teams' experiments on three different lipid monolayers revealed that the interfacial structures are governed by the net charge of the heads: water hydrogen atoms pointed up with anionic lipid heads, and faced downwards in the presence of cationic lipids (Fig. 1). "This is totally different from the situation for reactions in aqueous solutions," says Tahara, who believes that the results will shed light on important reactions that take place at cell membranes, such as enzyme activation. ■

1. Mondal, J.A., Nihonyanagi, S., Yamaguchi, S. & Tahara, T. Structure and orientation of water at charge lipid monolayer/water interfaces probed by heterodyne-detected vibrational sum frequency generation spectroscopy. *Journal of the American Chemical Society* **132**, 10656–10657 (2010).

Solving single molecule mobility

A versatile formula describes the energetic conditions needed to transport molecules laterally on surfaces

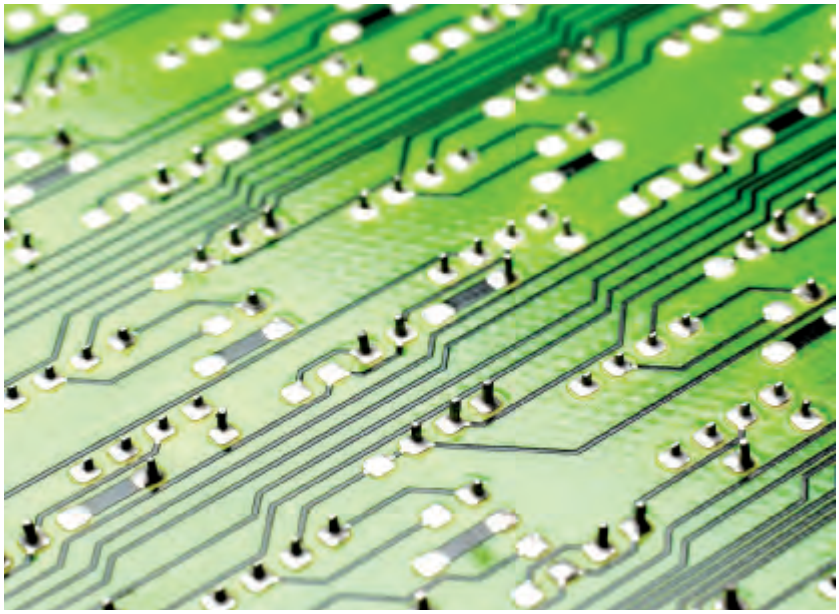

Figure 1: Molecular-scale versions of devices such as computer chips are now a step closer thanks to a new microscopy study by researchers in Japan.

Nanotechnologists assemble intricate nanodevices, such as computer chips (Fig. 1), molecule by molecule using 'bottom-up' techniques that mirror nature. One approach shuttles molecules along surfaces into new and functional arrangements using electrons from a scanning tunneling microscope (STM) tip. However, because energy transfer between the atomic-scale tip and the surface chemical involves many complex interactions, laborious efforts are currently needed to understand even the simplest reactions.

Results from a new theoretical and experimental study, however, may soon allow non-specialists to easily construct molecular devices. Kenta Motobayashi and Yousoo Kim from the RIKEN Advanced Science Institute in Wako and their colleagues from RIKEN and Japanese universities have developed a mathematical formula that describes how STM-induced molecular vibrations couple with dynamic movements on surfaces—enabling precise calculation of the energy and number of electrons needed to initiate single molecule motions[1].

When scientists use an STM to perform a straightforward molecular movement—for example, making carbon monoxide (CO) compounds 'hop' on palladium surfaces—they see that the fraction of successful movements depends heavily on the applied voltage. For CO, this is because hopping from one surface site to another requires a tunneling electron to initiate a specific stretching vibration. In the voltage range corresponding to this vibrational energy, CO hopping can increase exponentially, giving rise to so-called 'action spectra': curves of movement yields versus voltage with shapes characteristic to particular surface reactions.

Motobayashi, Kim and colleagues sought to uncover the microscopic mechanisms behind STM-stimulated diffusion by proposing a formula that relates movement yields to the energy transfer efficiency needed to excite reaction-triggering vibrations, while also accounting for thermal interactions. Fitting the CO action spectra to this formula revealed the exact magnitudes of critical reaction properties, like vibrational energies and rate constants, because the spectral curves were highly sensitive to small modification of the fit parameters.

Furthermore, the team's new equation proved versatile enough to analyze the more complex motions of butene (C_4H_8) molecules on palladium, a process that involves multiple excitations. Analyzing the butene action spectra with the formula showed the presence of three distinct vibrations and enabled calculation of the reaction order—a fundamental chemical property that identifies the number of tunneling electrons needed to initiate surface movement.

According to Motobayashi, the surprising abilities of this simple method should expand bottom-up nanotechnology practices. "STM-based action spectroscopy, which can precisely identify chemical species thanks to our spectral fittings, promises to contribute greatly to the technique of composing molecular devices," he states. ■

1. Motobayashi, K., Kim, Y., Ueba, H. & Kawai, M. Insight into action spectroscopy for single molecule motion and reactions through inelastic electron tunneling. *Physical Review Letters* **105**, 076101 (2010).

Entangled frameworks limber up

The degree of interconnectivity of molecular frameworks in microporous materials influences their structural flexibility and gas sorption

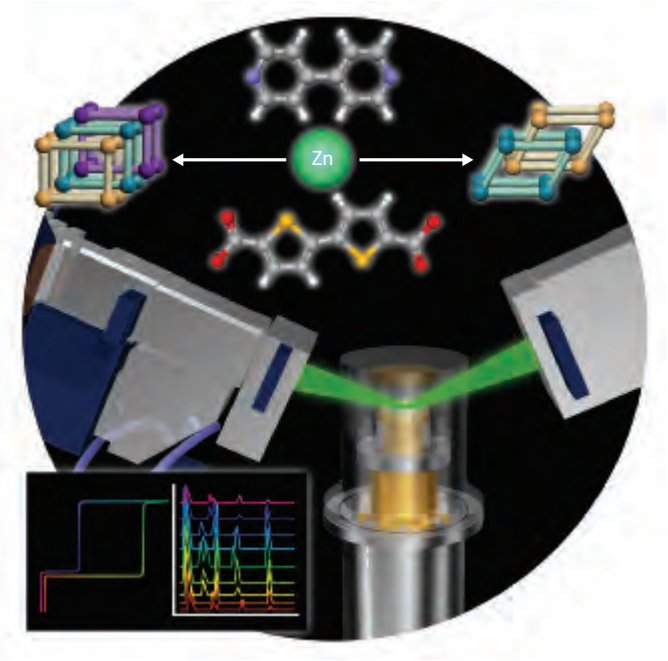

Figure 1: Schematic representations of the synthesis of the two-fold and three-fold interpenetrated porous coordination polymers, structural analysis by x-ray powder diffraction and adsorption and desorption profiles.

The quest to tune the three-dimensional (3D) molecular frameworks of materials called porous coordination polymers (PCPs) has taken a step forward thanks to a research team led by Ryotaro Matsuda and Susumu Kitagawa at the RIKEN SPring-8 Center in Harima and Kyoto University, Japan. The team, with members from Osaka Prefecture University, has described the influence of interpenetration of PCPs on the structural flexibility and gas sorption behavior of these materials[1], which show great potential for use in gas storage, heterogeneous catalysis and as separation materials.

The interpenetrated molecular frameworks of PCPs are composed of metal ions and bridging organic ligands. Materials scientists initially thought that interpenetration would reduce the available capacity of the voids within the structure. However, other researchers showed recently that such entangled structures exhibit high gas-uptake, as a result of increased internal surface area. Interpenetration also increases the thermal stability of flexible frameworks.

These findings prompted Matsuda, Kitagawa and colleagues to make PCPs with the same chemical components but with either two-fold or three-fold interpenetration. Both forms of the 3D frameworks were made using a solvent templating method and were composed of zinc atoms and carboxylate- and pyridyl-based organic ligands (Fig. 1). The two forms allowed the researchers to test the correlation between various physical properties and the degree of entanglement of the polymers.

Crystal structure analyses of the two forms indicated that non-covalent interactions, namely π–π interactions, in the three-fold structure are more significant than in the two-fold structure. Consequently, the two-fold structure has a more flexible structure and is of lower thermal stability than the more rigid three-fold PCP.

Using coincident x-ray powder diffraction and adsorption measurements, the team also showed that the two forms of structures have completely different carbon dioxide (CO_2) adsorption behavior. The two-fold structure can adsorb four times the amount of saturated CO_2 than the three-fold structure, owing to its greater flexibility and dynamic capability. Sorption occurs as a stepwise progression as a result of crystallographic transformations triggered by the addition and removal of guest molecules.

"The next challenge is the control of adsorption properties by external stimuli such as light or magnetic field to realize on-demand gas separation and storage," says Matsuda. "This kind of material could be used to separate CO_2 which is discharged from steelworks or to remove CO_2 and hence keep air fresh in a spaceship." ■

1. Bureekaew, S., Sato, H., Matsuda, R., Kubota, Y., Hirose, R., Kim, J., Kato, K., Takata, M. & Kitagawa, S. Control of interpenetration for tuning structural flexibility influences sorption properties. *Angewandte Chemie International Edition* **49**, 7660–7664 (2010).

Jekyll and Hyde material

A porous polymer network that researchers can make reactive at will can store gases and hasten chemical reactions

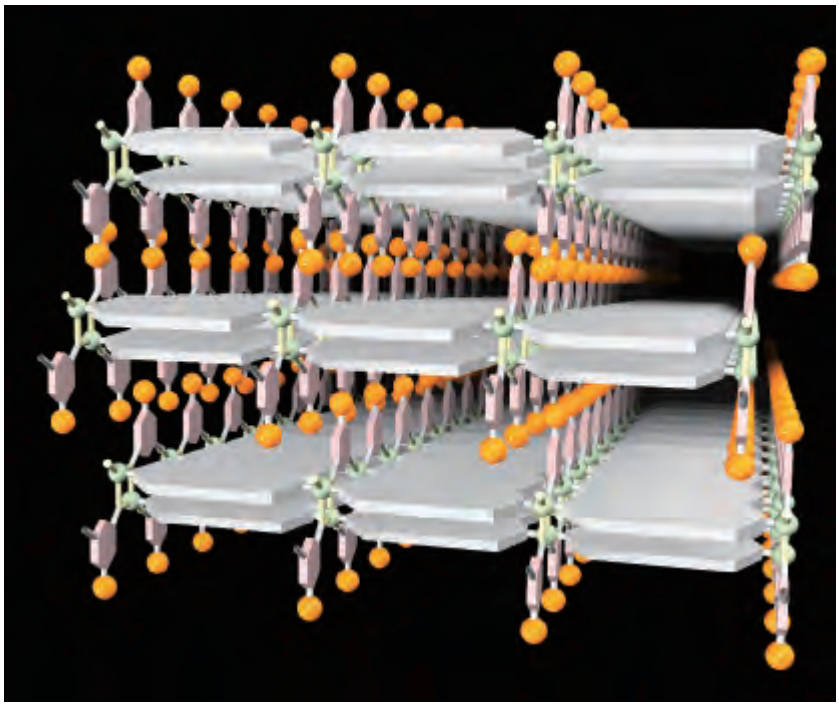

Figure 1: A schematic representation of a polymer network after irradiation by ultraviolet light. Orange balls, light green balls and pink panels represent the nitrogen atoms of nitrene, zinc ions, and azide molecules, respectively. The three-dimensional porous structure is constructed by mutual interdigitation of two-dimensional layers.

A team of researchers in Japan has developed a porous material, decorated with a highly reactive 'species' of molecules that can be activated remotely using a technique called photoactiviation[1]. Since porous materials have large surface areas for a given volume, they can be used for gas storage, and for the acceleration of chemical reactions. The ability to turn these molecular species 'on' or 'off' increases their utility. The novel porous material is also unique for its high degree of reactivity, which traditionally has been difficult to achieve while maintaining material stability.

Ryotaro Matsuda, Susumu Kitagawa of Kyoto University, the Japan Science and Technology Agency and the RIKEN SPring-8 Center and their colleagues, from these institutes and Japan Synchrotron Radiation Research Institute, made their porous material from a polymer network with an interlinked structure (Fig. 1) constructed from aryl azide molecules, which are relatively inactive but produce the highly reactive molecule aryl nitrene when irradiated with ultraviolet light.

The researchers exposed a single crystal or crystalline powder of their novel polymer to ultraviolet light and then measured the result with infrared spectroscopy, spin resonance and x-ray diffraction. Their results indicated that the irradiation converted a significant fraction of the dormant azides into reactive nitrenes, without disrupting the underlying porous network. The product was therefore a set of dense, highly reactive nanoscale pores.

The reactivity of these pores imparted new functionality to the polymer network, explains Matsuda. For example, the polymer's oxygen storage capacity increased by a factor of 29 after irradiation. The researchers also observed nitrenes reacting with carbon monoxide, suggesting that the polymer could be used to detect or filter this dangerous gas. Furthermore, because reactive species besides nitrenes can also be activated in this way, the technique has the potential to allow the capture and conversion of a variety of gases. The ability to increase the storage capacity or the speed of a chemical reaction remotely, and at a particular time, also significantly increases the range of available applications, he notes.

The approach represents the confluence of well-understood photochemistry and the materials science behind porous networks, with potential implications for devices such as sensors and purifiers. However, while the initial results are promising, several critical features need to developed, according to Matsuda. "In addition to demonstrating the trapping of gases besides oxygen and carbon monoxide, we need to make our material reusable," he says. "Currently, it cannot desorb gas molecules after the photoreaction, and therefore cannot be reused." ■

1. Sato, H., Matsuda, R., Sugimoto, K., Takata, M. & Kitagawa, S. Photoactivation of a nanoporous crystal for on-demand guest trapping and conversion. *Nature Materials* **9**, 661–666 (2010).

Improving pore 'vision'

The formation of nuclear pores in dividing human cells is being illuminated by new visualization techniques

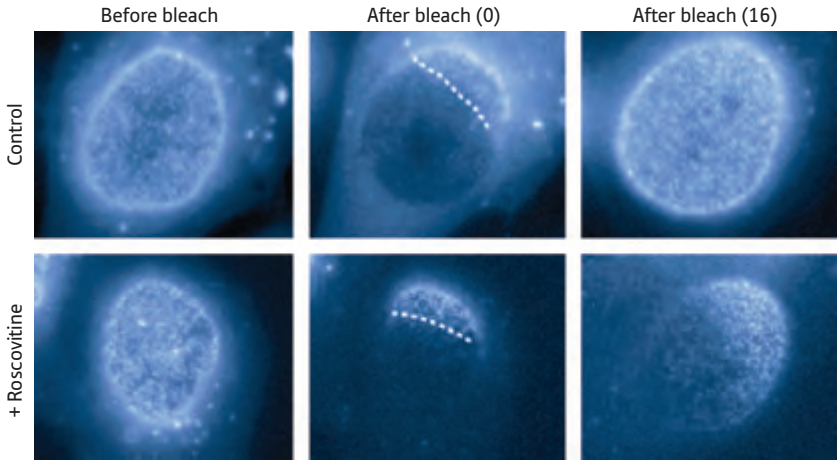

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Figure 1: Interphase NPC formation can be visualized using a laser photobleaching method that involves fluorescently tagged Nups (central panels). The fluorescence of the bleached area recovered after 16 hours incubation in control cells (top right), whereas addition of the CDK inhibitor roscovitine prevented the recovery (bottom right).

A team led by Naoko Imamoto of the RIKEN Advanced Science Institute in Wako has uncovered processes governing the formation of functionally important structures called nuclear pore complexes (NPCs) in dividing human cells[1].

Mitosis, the process of mammalian cell division, is followed by a period called 'interphase' during which the volume of the cell nucleus almost doubles; as does the number of NPCs on the nuclear envelope separating the 'nucleoplasm' from the cytoplasm—the rest of the cell's contents.

Innumerable molecules shuttle between the nucleus and cytoplasm through pores formed by NPCs, which are large octagonal structures composed of multiple copies of around 30 different proteins called 'nucleoporins' (Nups). NPCs form at the end of mitosis, when previously disassembled NPCs are reassembled, and during interphase when their number increases in preparation for another round of cell division.

"We wished to understand how NPCs form on the nuclear envelope of interphase cells, which is much less understood than post-mitotic NPC formation initiating on mitotic chromosomes," says Imamoto.

The researchers began by developing fluorescence-based NPC visualization methods. Their first approach involved using a laser to photobleach certain nuclear surface areas of early human interphase cells expressing fluorescently tagged 'scaffold' Nups. This allowed them to monitor the formation of new NPCs, which appeared as bright dots in the bleached areas (Fig. 1).

A second method involved monitoring NPC formation in fused cells called 'heterokaryons'. "Our heterokaryon method allows quantitative analysis of many nuclei without risking laser damage," explains Imamoto.

By combining these visualization techniques with cell engineering experiments they found that the formation of NPCs in human interphase cells is promoted by known cell-cycle regulators called cyclin-dependent protein kinases (Cdks).

Cdk inhibition experiments further revealed that CdK1 and CdK2, in particular, govern NPC formation during interphase, but not in post-mitotic NPC assembly, suggesting that different regulatory mechanisms are at play. Interestingly, Cdks also govern the behavior of nuclear envelope proteins, suggesting an as yet unknown mechanistic connection between NPC formation and membrane dynamics.

Cdks appear to act early in NPC formation because their inhibition suppressed the generation of small 'nascent' pores observed on early interphase nuclear envelopes using scanning electron microscopy.

"We believe that these 'nascent pores' are probably immature nuclear pores, although this needs to be confirmed," says Imamoto.

Cdk inhibition did not greatly affect nuclear growth, suggesting that its mechanism of regulation is distinct from that of NPC formation, which is something that future research must also address. ■

1. Maeshima, K., Iino, H., Hihara, S., Funakoshi, T., Watanabe, A., Nishimura, M., Nakatomi, R., Yahata, K., Imamoto, F., Hashikawa, T., Yokota, H. & Imamoto, N. Nuclear pore formation but not nuclear growth is governed by cyclin-dependent kinases (Cdks) during interphase. *Nature Structural & Molecular Biology* **17**, 1065–1071 (2010).

The brain lights up

The dynamic activity of electrical signals in neuronal populations can now be visualized with a powerful tool

Information processing in the brain relies on the coordinated activity between populations of different types of neurons, each with distinct electrical properties and connections. Understanding how complex neuronal circuitry processes information is challenging, as it requires measuring the activity of groups of specified cells.

Thomas Knöpfel of the RIKEN Brain Science Institute, Wako, and his colleagues have developed a genetically encoded voltage sensor that can be used to probe the electrical of activity from selected populations of defined neurons within the brains of living animals[1]. The sensor is based on voltage-sensitive proteins that insert themselves into the membrane of genetically targeted nerve cells and emit a fluorescent signal in response to the changes in membrane voltage that occur during neuronal activity.

Knöpfel's group validated the sensor by introducing it into cultured mouse hippocampal neurons. By inserting electrodes into the cells and observing them under the microscope, they found that single spontaneous nervous impulses were accompanied by an increase in yellow fluorescence.

The same results were obtained in brain slices prepared from mice transfected with DNA encoding the sensor while still in the womb. These experiments also showed that the sensor is capable of detecting circuit activity in the slices. When nervous impulses were induced in specified cells using electrodes, fluorescent signals were observed in the cells connected to them.

Finally, the researchers demonstrated that the sensor can detect the activity of specific groups of cells in the brains of live mice in response to natural sensory stimuli. Again, they transfected embryonic mice with the sensor, targeting a brain region called the barrel cortex, which receives information from the whiskers.

When the mice became adults, the researchers stimulated their whiskers and monitored activity in the barrel cortex through thinned regions of the animals' skulls. Deflection of individual whiskers was found to produce fluorescent signals in the corresponding area of the cortex (Fig. 1).

Other optical methods available for monitoring neuronal activity have disadvantages. Voltage-sensitive dyes can be toxic to cells, while genetically encoded calcium indicators, which fluoresce in response to the localized calcium signals characteristic of neuronal activity, can interfere with signaling pathways by buffering calcium and provide information only on a slower time scale. The voltage sensor developed by Knöpfel and his colleagues therefore improves on them.

"This will facilitate the investigation of fundamental questions of information processing in the brain," says Knöpfel, "and will also be applicable to directly visualize cognitive function." ■

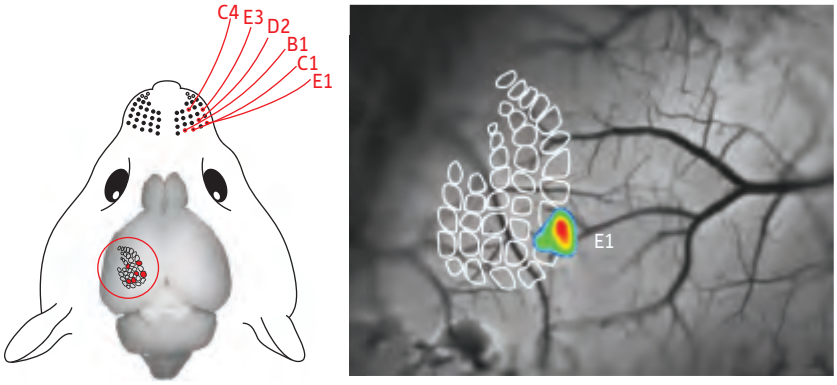

Figure 1: In mice transfected with the voltage sensor (left), deflection of a whisker leads to fluorescence in the corresponding region of barrel cortex (right).

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1. Akemann, W., Mutoh, H., Perron, A., Rossier, J. & Knöpfel, T. Imaging brain electric signals with genetically targeted voltage-sensitive fluorescent proteins. *Nature Methods* **7**, 643–649 (2010).

Rocking the death dance

Identification of the mechanism that triggers suicide in dissociated human stem cells should assist their development as therapeutics

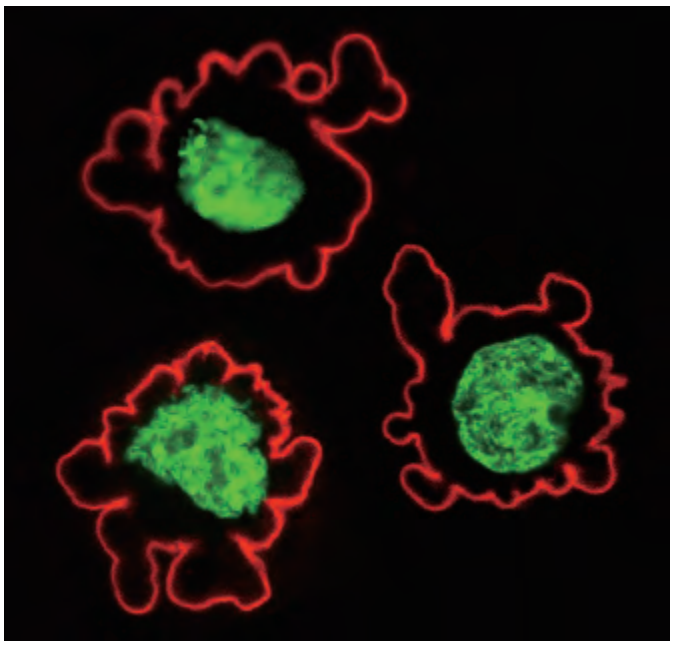


Figure 1: Fluorescent imaging of blebbing in live, dissociated human stem cells. The plasma membrane is red, and the nucleus is green.

A RIKEN-led team of molecular biologists has determined why human embryonic stem cells (ESCs) and induced pluripotent stem (iPS) cells undergo apoptosis, or programmed cell death, when separated from each other. The finding should allow more efficient culturing of human stem cells, making them easier to maintain, more flexible to handle, and generally improving their survival.

At present, human ESCs, unlike those derived from mice, must be cultured in clumps, which makes them difficult to manipulate. When they lose contact with neighboring cells, human ESCs immediately go into apoptosis.

The research team, led by Yoshiki Sasai from the RIKEN Center for Developmental Biology in Kobe, and including members from Kyoto University, showed that this apoptotic response could be countered by application of an inhibitor of the enzyme ROCK (Rho-dependent protein kinase). Now, using a combination of live-cell imaging and laboratory analysis, the team has elucidated the onset and progress of dissociation-induced apoptosis[1].

They found that within a few hours of separation, human ESCs began blebbing—a process whereby the membrane spontaneously bulges in finger-like projections causing the cells to jiggle around (Fig. 1). Blebbing occurs when the membrane breaks away from the internal cytoskeleton, and can vary in its duration and severity. In this case, blebbing lasted for hours and inevitably ended with the cell bursting. The researchers dubbed it the death dance, and traced its onset to hyperactivation of myosin, a contractile protein associated with cell movement.

By studying the levels of ROCK after dissociation, as well as the regulation of its activity by the compounds with which it interacts, Sasai and colleagues determined that myosin hyperactivation—hence the blebbing and apoptosis—is caused directly by ROCK. It can be suppressed by the myosin inhibitor, blebbistatin. Further, the whole process is triggered by loss of intercellular contact, and regulated by a compound known as Abr.

The molecular mechanism that the researchers have unraveled should be susceptible to manipulation, potentially allowing human ESCs to be separated and handled without risking their certain death. Interestingly, they found that the difference in susceptibility to apoptosis of dissociated human and mouse ESCs had nothing to do with species, but could be attributed to the stage of development from which the parent stem cells were derived.

"We are now planning further work to understand the detailed mechanism of Abr activation," says Sasai. "Another question we wish to study is why cells die upon myosin hyperactivation." ■

1. Ohgushi, M., Matsumura, M., Eiraku, M., Murakami, K., Aramaki, T., Nishiyama, A., Muguruma, K., Nakano, T., Suga, H., Ueno, M., et al. Molecular pathway and cell state responsible for dissociation-induced apoptosis in human pluripotent stem cells. *Cell Stem Cell* **7**, 225–239 (2010).

Great leap forward

Cells sourced from adult rabbits and recast as stem cells show good potential for laboratory use

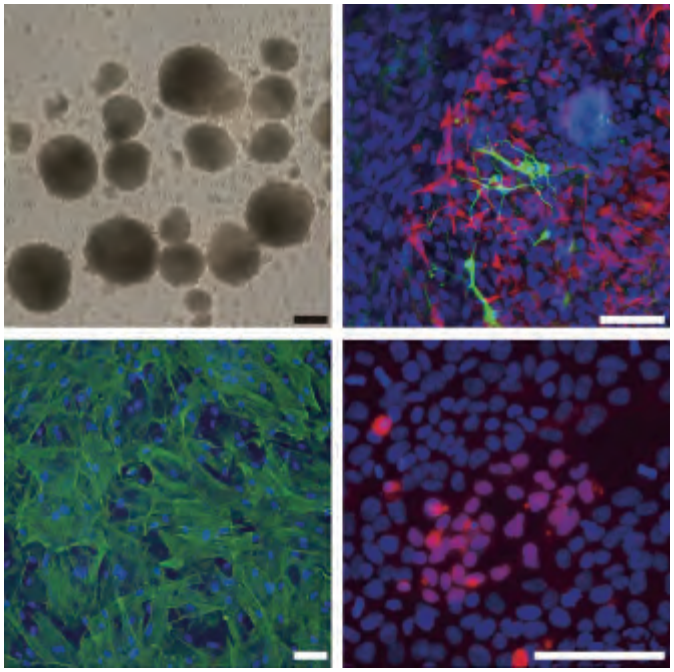


Figure 1: Rabbit iPS cells, differentiated in vitro, which specialize into a variety of cell types from the three basic germ layers (top left). These include ectoderm—neural cells (top right); mesoderm—smooth muscle cells (bottom left); and endoderm (bottom left) (scale bar, 100 μm).

RIKEN molecular biologists have successfully reprogrammed adult rabbit body cells to form colonies of fully pluripotent cells that are highly similar to rabbit embryonic stem cells (ESCs). These induced pluripotent stem (iPS) cells of rabbits are likely to be used as a laboratory model of human iPS cells, the researchers say—in particular, for comparisons with ESCs to evaluate the feasibility of iPS cells for regenerative medicine.

In fact, the researchers consider the development of these rabbit iPS cells as an important tool for human medical research, as rabbits are much closer to humans physiologically than mice and can be handled much more easily in the laboratory than other animals used as models of humans, such as pigs and monkeys.

The research team from the RIKEN BioResource Center in Tsukuba, which was led by Atsuo Ogura, used lentiviruses modified as vectors to introduce four human genes into adult rabbit liver and stomach cells[1]. The genes—for transcription factors that guide reading of the DNA—effectively reprogrammed the adult cells as iPS cells, and these proved easy to handle and maintain in culture. But the result was dependent on the initial adult cell-type, according to Arata Honda, a researcher in Ogura's team.

Honda says that the researchers first tried without success to reprogram adult rabbit fibroblasts, the most common cells in connective tissue.

They tested the properties of the rabbit iPS cells by using them to generate the tumors known as teratomas that contain differentiated or specialized cells of all three types of germ layers—ectoderm, endoderm and mesoderm (Fig. 1). Marker compounds that are characteristic of stem cells were present in the iPS cells.

Ogura, Honda and colleagues also determined which genes were active in their rabbit iPS cells. When they compared the profile of this activity with that found for rabbit ESCs, they found that, although not the same, the two types of cells were very similar.

At least three types of pluripotent cells, generated from rabbits by different methods, are now available to researchers—ES cells, iPS cells, and nuclear transfer ES cells. "Thus, using rabbits, we can fully characterize these different pluripotent cells in parallel under the same experimental conditions," Ogura says.

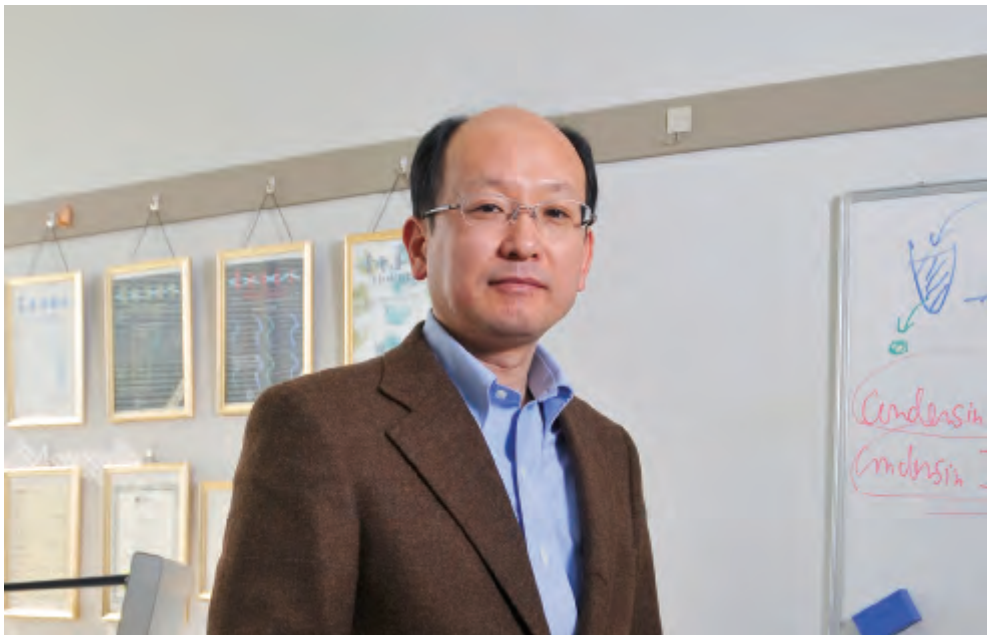
Honda adds that: "We can now assess the efficacy and safety of new cell-based treatments for degenerative diseases in human. We hope that we will finally identify which type of cells is best suited for each purpose of regenerative therapy in humans." ■

1. Honda, A., Hirose, M., Hatori, M., Matoba, S., Miyoshi, H., Inoue, K. & Ogura, A. Generation of induced pluripotent stem cells in rabbits: potential experimental models for human regenerative medicine. *Journal of Biological Chemistry* published online 29 July 2010 (doi: 10.1074/jbc.M110.150540).

Unlocking the chromosome

Tatsuya Hirano

Chief Scientist
Chromosome Dynamics Laboratory
RIKEN Advanced Science Institute



The DNA is a remarkable molecule in many ways. It encodes our entire genome, and if stretched out in a thin thread would measure 1.8 m in length. Yet in each and every cell, less than a tenth of a millimeter in size, the DNA must be replicated and segregated precisely in order for the cell to divide and create two daughter cells. Key to this process is the chromosome. It had widely been believed that the action of unknown protein(s) would be required in order to condense the replicated DNAs to form a chromosome. However, it wasn't until a discovery in 1997 by Tatsuya Hirano, currently chief scientist of the Chromosome Dynamics Laboratory at the RIKEN Advanced Science Institute, that the long-sought protein machinery condensing the chromosome was finally unveiled. Hirano's discovery of the 'condensin' protein complex is marked as a major breakthrough that has led to rapid advances in the elucidation of not only basic biological processes involving the chromosome but also human diseases involving chromosome aberrations.

The chromosome black box

"In the early 1980s, when I entered university, I found the molecular structure of the DNA double helix on the first page of my biology textbook," says Hirano. "The textbook also described the then recent finding that DNA wind around histone proteins to form a structure known as chromatin." It was also known at that time that chromatin should condense further to form a chromosome (Fig. 1), a structure that had been observed by optical microscopy as early as the nineteenth century. Nonetheless, there was a huge gap in our understanding between the chromatin and the chromosomes, and the proteins involved in chromatin condensation and their mechanism of action remained a black box. "I wanted to open that black box, and this is how I became interested in the chromosome."

Hirano entered Kyoto University's Graduate School and joined the laboratory of Mitsuhiro Yanagida, where he engaged in chromosome research by means of yeast genetics. "My initial project was to generate lots of mutants of fission yeast. In the search for mutants displaying abnormal shapes of chromosomes, I was able to discover many genes involved in chromosome morphogenesis and segregation. During my thesis work, however, I was not completely satisfied with the genetic approach, and became interested in analyzing the chromosome using a more direct technique, namely, biochemistry. I quickly learned, though, that conventional biochemical methods do not allow us to isolate the chromosome from a cell in a very pure form."

The discovery of condensin

In 1989, Hirano joined Tim Mitchison's lab as a postdoctoral fellow at the University of California in San Francisco, USA, where an *in vitro* experimental system for chromosome assembly was under development. "Frog eggs were squashed to obtain an extract of high protein concentration. Amazingly enough, by placing the DNA in the extract, it was possible to produce the chromosome in a test tube. Because the chromosome produced in this *in vitro* system could be taken out in a pure form quite easily, analysis of their

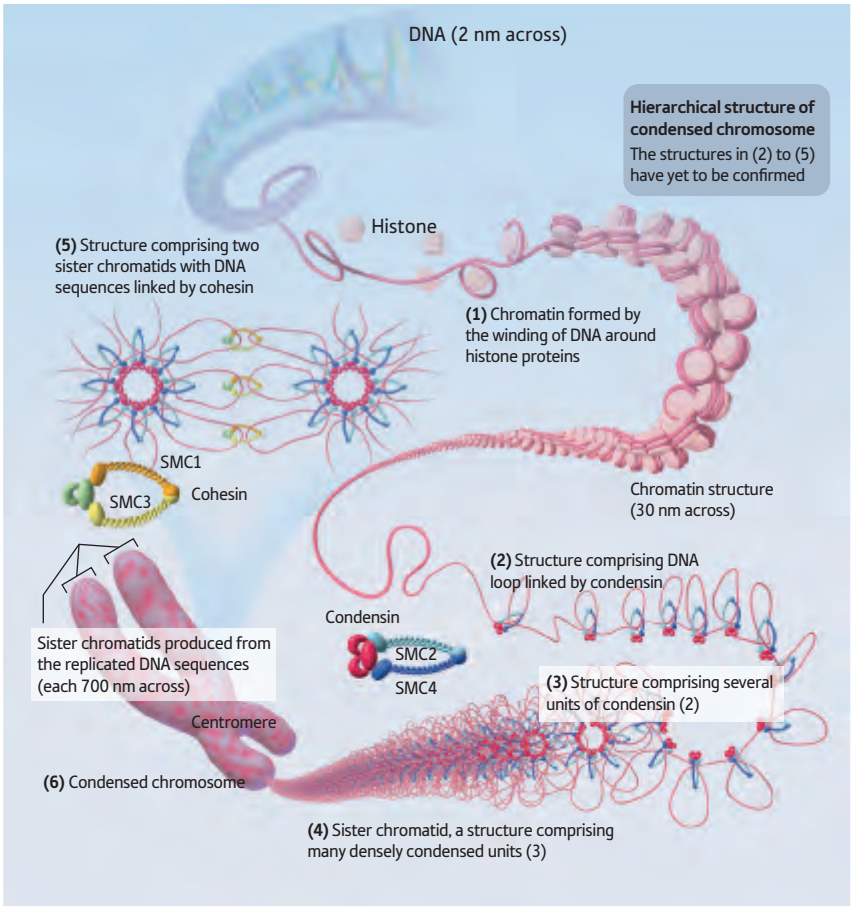


Figure 1: Condensation of DNA into chromatin and chromosome.

protein components became far easier than before," says Hirano.

By taking advantage of this experimental system, in 1994, Hirano discovered two novel proteins essential for chromosome condensation: SMC2 and SMC4. "Just around that time, studies using budding yeast and nematodes were starting to provide evidence that some SMC-related genes are somehow involved in chromosome functions. One day my thesis advisor gave me an international call, telling me that he had also found two SMC-related genes while analyzing fission yeast mutants with morphologically abnormal chromosomes. Even more surprisingly, one of the mutants was identical to the one I discovered myself when I was a graduate student."

Thus, the biochemical analyses of the frog chromosomal proteins by Hirano was supported by the genetic analyses of yeast and nematodes, leading him to the very general conclusion that SMC proteins play a central role in chromosome dynamics. However, this work was a mere introduction to an even larger story.

In 1995, Hirano moved to Cold Spring Harbor Laboratory on the East Coast of the USA and set up his own laboratory. In 1997, he discovered that SMC2 and SMC4 form a complex with three other proteins, and that the complex, which he named condensin, plays an essential key role in chromosome condensation. "When we examined the condensin complex under an electron microscope, we found that it had a shape like a small clip. Until that time, no one had ever seen a protein complex with such a strange shape. However, on reflection, this shape is perfectly suitable for bundling a long chromatin fiber to make a compact chromosome."

The cohesin glue

The mid-1990s marked the dawn of genome research. In 1996, the budding yeast genome had been completely decoded and it had been revealed that there are four types of SMC in the yeast genome. "To investigate the roles of the other two types, SMC1 and SMC3, we conducted a series of experiments using a frog egg extract. We found that these two types of SMC also form a large complex with other proteins, but that the complex works in a completely different way from condensin in the process of cell division."

From 1997 to 1998, the combined achievements of several research groups demonstrated that the complex works as a glue to link two sister chromatids. This complex is now known as cohesin (Fig. 1). "Classical research had led us to predict that there must be proteins responsible for chromosome condensation and cohesion. However, it turned out that the proteins responsible for the two processes, condensin and cohesin, contain SMC as their core components, hence sharing a structural similarity to each other. This was completely unexpected."

Emergence and evolution of life from the viewpoint of condensin

"Even more surprisingly, condensin-like proteins were also found in prokaryotic organisms, including *Escherichia coli* and other bacteria, which evolved earlier than eukaryotic organisms," continues Hirano. The replication and segregation of genetic information are fundamental to life, and while the mechanism for cell division apparently differs between prokaryotes (organisms lacking a cell nucleus) and eukaryotes (organisms bearing a cell nucleus), "the proteins responsible for condensing the chromosome were found to be common."

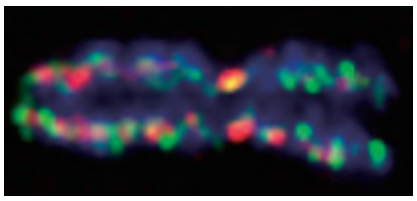


Figure 2: Condensin I and II in the human chromosome.
Condensin I (green) and II (red) are arranged alternatively along the axis of the chromosome (blue). A fraction of condensin II concentrates in the vicinity of the centromere (two red oblong regions). Although condensin I and II share SMC2 and SMC4, they differ in the three other proteins.

Self-proliferation is regarded as one of the fundamental properties that define 'life'. "When the primordial organism capable of replicating DNA emerged on Earth, it would have encountered a major problem of how to organize replicated DNAs and separate them into two daughter cells. The ancestor protein from which condensin originated might have appeared to resolve the problem soon after the emergence of life."

In prokaryotes, however, no protein corresponding to cohesin has been found. "A prokaryotic condensin could serve for both condensation and cohesion, and cohesin seems to have emerged from the ancient condensin to take a differential part during evolution, perhaps around the birth of eukaryotes."

Another noticeable finding was that higher animals and plants possess a second type of condensin. "In 2003, we discovered this new type of condensin in human cells. The original type is now called condensin I, and the second type is called condensin II. Intriguingly, condensin I and II are arranged alternatively along the axis of the chromosome. When condensin I or II is experimentally removed from the cell, the chromosome becomes morphologically abnormal in distinctive manners, indicating that the two condensin complexes have non-overlapping functions."

Organisms with relatively small genomes, such as yeast, have condensin I only, whereas many higher animals and plants with larger genomes have both condensin I and II (Fig. 2). "Condensin II may have emerged more recently in the history of evolution. In higher animals and plants, the chromosome lengthened, so it became necessary for condensin I and II to cooperate in condensing the chromosome."

Unlocking the black box

Hirano made the discovery of the first key to unlocking the black box of chromosome condensation. "I was lucky to have been able to discover condensin and demonstrated that it mediates chromosome condensation in 1997. But it was just a start of a long journey. Even now, the exact mechanism of chromosome condensation remains to be fully elucidated. Although a model has been proposed in which several condensin proteins linking DNA loops come together in densely arranged assemblies, we still do not know whether that model is indeed correct."

Returning to Japan in 2006, Hirano established the Chromosome Dynamics Laboratory at the RIKEN Advanced Science Institute. "In my laboratory, we have specialists covering a broad range of research areas, including protein crystallography, biochemistry and cell biology. I want to elucidate the mechanism behind chromosome condensation by making the best use of all the techniques available. That will be my lifework."

Of the many approaches taken by researchers at the laboratory, frog egg extract remains the most powerful experimental system. "Using this system, we can analyze the processes of chromosome condensation and segregation in great detail at an unprecedented level."

Research over the last ten years or more has firmly established that condensin and cohesin work together to support the processes of chromosome

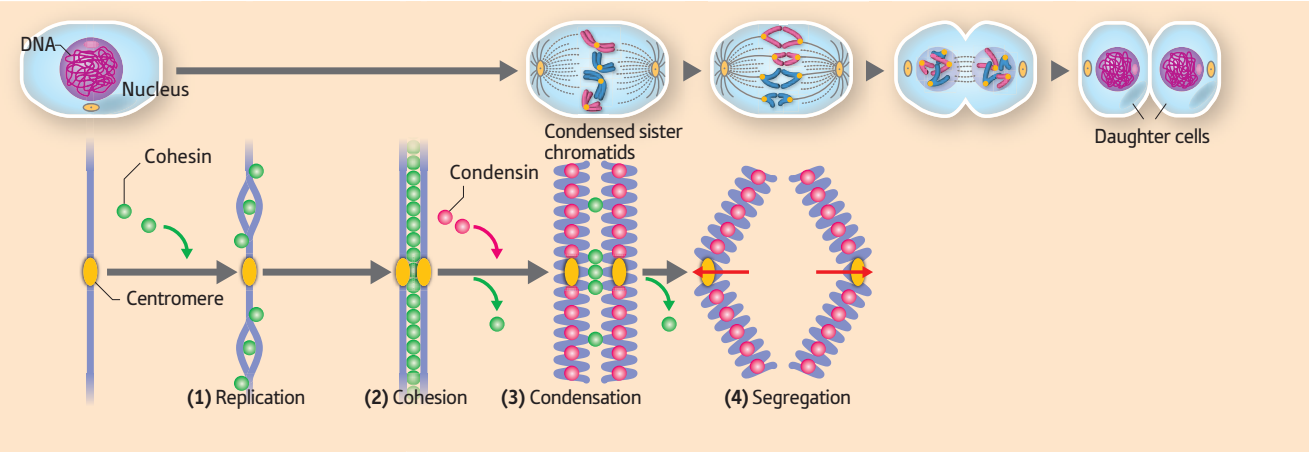


Figure 3: Chromosome condensation and segregation processes (lower) in cell division (upper).
Upon DNA replication, cohesin works as a glue to bond together the pair of DNA sequences ((1) and (2)). The cohesin then detaches, except near the centromere, and condensin acts to condense the sister chromatids ((3)). The remaining cohesin then detaches and the sister chromatids are segregated, with one set distributed to each of two daughter cells ((4)). Stage (3) corresponds to the sequence shown in Fig. 1.

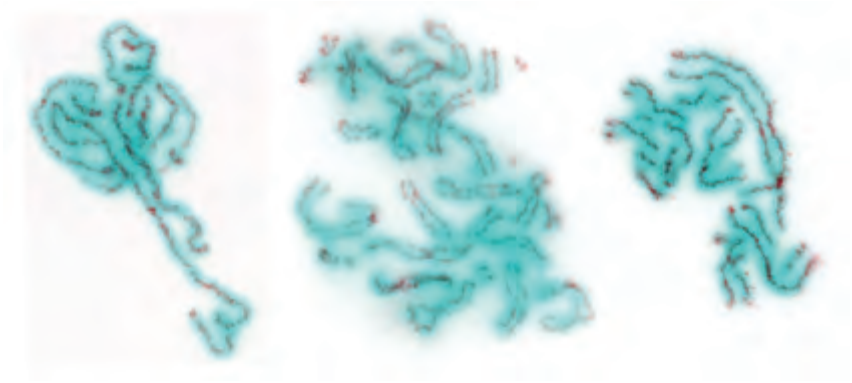


Figure 4: Manipulation of chromosomes to alter cohesin function.
Left: A chromosome with enhanced cohesin function. Division of sister chromatids is inadequate. Middle: A normal chromosome. Sister chromatids are arranged in parallel over the entire length of the DNA. Right: A chromosome with weakened cohesin function. Sister chromatids are divided too far apart. All chromosomes were prepared in frog egg extract.

condensation and segregation (Fig. 3). Upon DNA replication, cohesin holds the replicated pair of the DNA (sister chromatids) together. When the cell prepares for division, almost all of the cohesin detaches, except for a small amount near the center of the chromosome (centromere), while condensin condenses the sister chromatids. Finally, the residual pool of cohesin detaches, and the sister chromatids are segregated and distributed to two daughter cells.

"By manipulating the level of proteins regulating the cohesin function in the frog egg extract, or adding a mutant form of the regulatory proteins with a different functionality, the action of condensin or cohesin can be altered as desired," says Hirano (Fig. 4). "Through this type of experiments, we are endeavoring to elucidate the mechanism by which condensin and cohesin cooperate to condense and segregate the chromosomes."

Chromosome-mediated diseases and biological phenomena

In recent years, several examples of human diseases associated with misregulation of condensin or cohesin have been reported. "Microcephaly is a disease characterized by a marked reduction in the size of the frontal lobe of the brain. A German research group examined the cells of patients with this disease and found that there is a remarkable change in the mode of chromosome condensation. We started a collaboration with that group and found that condensin II is abnormally activated in the patient cells," says Hirano.

If condensin becomes dysfunctional and causes a condensation defect during the development of the brain, a critical gene in the formation of the brain may fail to work normally, which may in turn cause microcephaly. "Hence, it is possible that condensin is also involved in gene expression."

The proper formation and function of an organism can only be achieved when the right genes work in the respective cells whenever necessary. Recently, the discovery that methylation of the DNA or histones affects the state of gene expression has stimulated a cascade of research in many fields of life science. "Moieties such as methyl groups control the expression of individual genes. Condensin, on the other hand, may control gene expression more globally by governing the condensation of chromosomes."

Research into condensin and cohesin is expected to be useful in understanding the etiology of diseases involving chromosome aberrations. Normally, an individual inherits two sets of chromosomes, one from the father and the other from the mother. However, in patients with Down's syndrome, for example, three copies of chromosome 21 are inherited, causing a range of pathological conditions. "The exact cause for abnormal inheritance of chromosomes in the patients remains unknown, but it may be that cohesin fails to work correctly on chromosome 21."

In fact, mice deficient in cohesin have been reported to encounter an increased frequency of chromosome aberration with maternal aging. A mouse model for Down's syndrome could therefore contribute to clarifying the etiology of this disease and the development of therapies. "Our team is also working on meiosis using the mouse as a model organism. It is known that the probability that the chromosome is inaccurately distributed in meiosis is ten times higher in humans than in mice, and that this is one of the major causes of infertility. Although the reason is still unknown, investigations into condensin and cohesin may lead to a solution to the riddle."

Stimulated by the discovery of condensin and cohesin by Hirano, chromosome research has advanced dramatically in recent years. "Considering the fundamental role of the chromosome in life, further research could lead to a series of unexpected discoveries in the years to come." ■

Tatsuya Hirano

Tatsuya Hirano was born in Chiba, Japan, in 1960. He graduated from Kyoto University in 1984, and received his PhD in 1989 from the same university. He then moved to the University of California, San Francisco, USA, and worked as a postdoctoral fellow in the laboratory of Tim Mitchison. In 1995, he obtained an independent position at Cold Spring Harbor Laboratory, New York, USA, to set up his own research group. He was promoted to full professor in 2003 and stayed there until 2007. He then returned to Japan as visiting chief scientist at RIKEN in 2006 and became chief scientist in 2007. His research focuses on understanding the molecular mechanisms of chromosome architecture and dynamics through multidisciplinary approaches, such as biochemistry, cell biology, structural biology and genetics. He is known for his discovery of condensin.

RIKEN–McGill University joint scientific workshop

In celebration of a new general agreement on collaboration in research between RIKEN and McGill University in Canada, the two partners held a joint scientific workshop in Mont Tremblant, Quebec, Canada, on 22–23 September 2010 with the aim of strengthening their collaborative relationship through greater communication between their respective research communities.

Founded in 1821 in Montreal, Quebec, McGill University—an English-speaking university in a French-speaking Canadian province—is well-known for its high-quality education and cutting-edge scientific research. With Nobel laureates such as physicist Ernest Rutherford (chemistry, 1908) and biologist Jack Szostak (medicine, 2009), the university's long list of respected alumni has earned it a worldwide reputation for excellence in research. Today, McGill University comprises 11 faculties, 10 schools, more than 35,000 students, and over 1,600 tenure and tenure-stream staff.

Building on already strong ties in the areas of developmental biology, RIKEN and McGill University now seek to collaborate in the areas of green chemistry and nanotechology. The starting point for this new collaboration is an agreement for collaborative research in these fields signed on 15 July 2010 following a visit to McGill University by RIKEN President Ryoji Noyori in 2009.

This year's workshop was attended by 14 members of RIKEN from the Wako, Yokohama and Harima institutes, including Executive Director Yoshiharu Doi, Zhaomin Hou from the Organometallic Chemistry Laboratory, Koji Ishibashi from the Advanced Device Laboratory and Takuo Tanaka from the Metamaterials Laboratory. The Canadian group numbered 17 including McGill University Vice-Principal Rima Rosen, associate deans Andrew Kirk and Peter Grütter, and Chair of the Department of Chemistry Bruce Lennox. The workshop was also attended by Chao-Jun Li, a famous chemist and winner of the 2001 United States Presidential Green Chemistry Challenge Award. Many of the McGill researchers arrived from places outside of Canada, including China, France and Switzerland.

RIKEN executive director Yoshiharu Doi addresses the RIKEN–McGill University joint scientific workshop.

The workshop included presentations by Rosen and Doi introducing their respective organization's research activities. The workshop itself included parallel sessions on green chemistry and nanotechnology, and featured topics presented by both Canadian and Japanese researchers. Spirited discussions continued throughout the day, covering not only research itself but also the research environment in Japan and Canada.

On the last day of the workshop, after a morning of discussions on how to foster stronger ties between the two organizations, attendees returned to Montreal to attend a poster session at the university by students of McGill. The workshop was brought to a close with dinner at the McGill University Faculty Club hosted by Principal and Vice-Chancellor Heather Munroe-Blum.

RIKEN attendees returned to Japan with a strong sense of the university's history and tradition, as well as with fond memories of the lakeside view at Mont Tremblant, and of the red and yellow of Quebec's autumn leaves. Today's generation faces many serious challenges, ranging from water and food shortages to climate change, the solutions to which demand a significant contribution from the world's scientific community. Responding to these demands, Doi cited the new agreement with McGill University as a step in the right direction. "We hope our partnership in green chemistry and nanotechology," he said, "will lead to results that will make significant contributions to resolving pressing global issues and pave the way for a sustainable society."

Takashi Nakatsukasa
Associate Chief Scientist, Theoretical Nuclear Physics Laboratory,
RIKEN Nishina Center for Accelerator-Based Science

Dear Prof. Nakatsukasa,

It has been only a few months since I left RIKEN and my many memories of life in Japan are still very vivid. As you know, I now hold a permanent position in Surrey. This was one great outcome of working at your laboratory.

I still remember when we first met, back in September 2007, during a Japanese–German workshop at the beautiful Lake Chiemsee, near Munich. At that time, I had been thinking about doing research in Japan but found few opportunities. Then I learned about your laboratory and the foreign researcher program at RIKEN, one of the most modern and prestigious institutions for the study of radioactive beams. We had a warm chat and very interesting discussion about time dependent Hartree-Fock theory, and you encouraged me to apply.

When I arrived to Wako, almost a year later, I was astonished by the high-quality research environment. There, I learned how RIKEN bets on revolutionary projects and young researchers, giving them ample opportunities to develop themselves as scientists. While benefitting from your advice and experience, it was also possible to meet most members of the nuclear physics community in Japan and work side by side, everyday, with our experimental colleagues. Having been there, and seen all the passion you put in research, it is a pleasure to see new results coming out from your laboratory, like your recent paper on canonical-basis formulation of the time-dependent Hartree-Fock-Bogoliubov (TDHFB) theory. Working at RIKEN was like a dream come true for me.

I also have to thank all of my Japanese colleagues for teaching me so much about Japanese life and culture in general. And, without the secretaries at the Main and RIBF buildings—and with my poor Japanese—I would never have been able to solve even the smallest administrative issue.

Life in England is nice and we enjoy the beautiful countryside very much. Sadly, I am missing a lot the onsens and all the tasty Japanese food. This is quite hard, considering that I have become pretty much sushi dependent over the last two years! Fortunately, here one can find lots of ethnic restaurants and even some Japanese food stores in London that (can you believe it?) sells natto!!!

I thought you might like to receive some pictures from our last weekend in London. I am including them here, together with those we took at the farewell party in Wako.

Thank you for everything. I hope you are doing well and that you can continue inspiring young researchers to pursue research at the highest levels. And, of course, I look forward to meeting you again at the next workshop or conference!

All the best,

Carlo Barbieri
University of Surrey, Guilford, UK

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

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