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Probing enzymes in the brain

HIGHLIGHT OF THE MONTH

Chemistry: Crystallizing the switch to hydrogen
Biology: 'Mind-readers' get a clearer view

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Chemistry

Crystallizing the switch to hydrogen

Real-time x-ray measurements of remarkable rare-earth/transition metal clusters expose the secrets of storing and releasing hydrogen gas

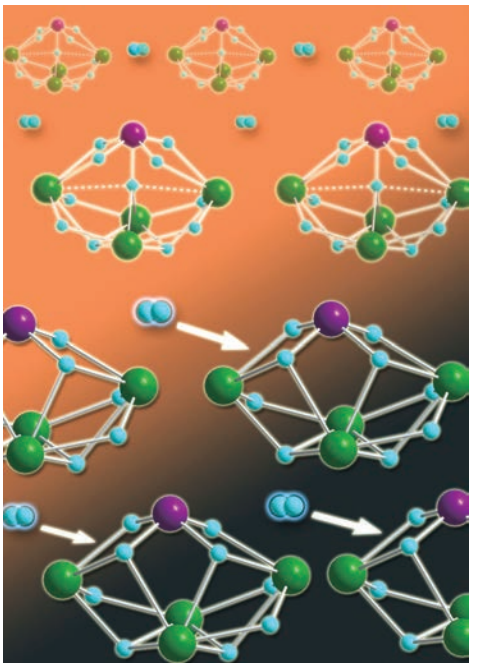

Figure 1: Heterometallic hydride clusters containing molybdenum atoms (purple spheres) and rare-earth yttrium metals (green spheres) are promising materials for on-board storage and release of hydrogen gas (light blue spheres).

Hydrogen gas is an almost infinitely inexhaustible fuel source that emits only clean water during combustion. Switching from hydrocarbon-based transportation to systems powered by state-of-the-art fuel cells therefore seems a natural choice, but numerous obstacles have kept this technology confined to laboratories. A prime example is the problem of on-board hydrogen storage for vehicles: because ambient hydrogen gas is roughly 10,000 times less dense than gasoline, it would require impractically large tanks to obtain comparable mileage.

Compressing hydrogen gas or liquefying it at -250 °C conditions are two ways to increase its energy content by volume. However, chemists are developing a more attractive strategy using specially designed compounds, called metal hydride clusters, to produce high hydrogen storage densities without extreme temperatures or pressures. The metal atoms within these molecules bind to large numbers of hydrogen atoms, producing a solid that can reversibly add or remove hydrogen using mild heating or cooling.

Now, Zhaomin Hou from the RIKEN Advanced Science Institute in Wako and an international team of colleagues have isolated a new class of 'heterometallic' hydride clusters (Fig. 1) that may spur development of lighter and longer-lived fuel cell devices[1]. By incorporating multinuclear rare-earth metals into their compounds, the team has produced the first high-density storage molecules that have hydrogen addition properties that can be monitored directly using x-ray diffraction—a technique that provides clear insights into cluster structure and functionality.

Rare combinations

For the past 25 years, chemists have paired so-called '*d*-block transition metals', such as tungsten (W) and molybdenum (Mo), with lightweight rare-earth metals, such as yttrium (Y), to increase the storage capacity of hydride clusters. Because the nuclei of rare earths are shielded by many electrons, these metals can pack high numbers of hydrogen atoms into small crystal volumes without suffering electronic repulsions. Unfortunately, once hydrogen gas binds to a rare-earth metal, it tends to stay there. Mixing in *d*-block metals alters the rare-earth reactivity so that on-demand hydrogen storage and release can occur.

Until now, most of these combined metal hydrides were constructed using mononuclear rare-earth building blocks, such as YH, with a mononuclear *d*-block metal. Using a different strategy, Hou and his colleagues recently devised innovative protocols to isolate polynuclear rare-earth hydrides using large molecular ligands to trap these typically unstable compounds in place[2]. Polynuclear hydrides feature dense, interconnected networks of 'bridging' hydrogen atoms connected to two or more metals—characteristics that led the

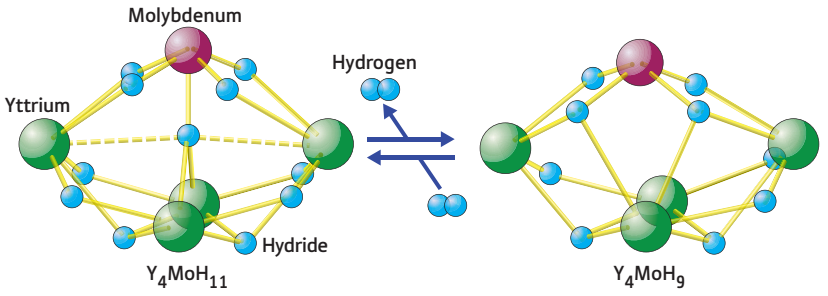

Figure 2: Monitoring the reversible addition and release of a hydrogen gas molecule to a molybdenum–yttrium cluster in real time with x-ray crystallography has revealed the first atom-resolved insights into hydrogen storage by organometallic crystals.

researchers to explore their potential for hydrogen storage applications.

"It is not difficult to imagine that hydrogen atoms could bond to multiple metal atoms in a polynuclear polyhydride complex, and the [mode of] bonding could be different with different metal combinations," says Hou. "However, it is not easy to prepare quality polyhydride samples for high-precision structure determinations. Hydride complexes containing both rare-earth and *d*-block transition metals are even more difficult to prepare because of their air- and moisture-sensitivity."

A five-way first

Performing their experiments inside nitrogen-filled and humidity-free enclosures, the team mixed one of their carefully prepared polynuclear complexes—four yttrium metals and eight hydrogen atoms held together by bulky organic ligands—with either a Mo or W pentahydride. After precipitating crystals out of the reaction, they used x-ray and neutron diffraction experiments to view their product's atomic structure. These measurements showed that the two metallic components fused together, yielding a Y_4MH_{11} (M = Mo, W) hydride with double-, triple-, and quadruple-bridged hydrogen atoms.

Zapping the penta-metallic polyhydride with ultraviolet light enabled the team to remove a protective phosphorus ligand and increase the hydrogen bridging density within the cluster. This produced the first hydride cluster where hydrogen is bonded to five metals in a distinctive symmetry known as trigonal bipyramidal. "The confirmation of a penta-coordinated hydrogen atom in this geometry is unprecedented," says Hou.

Step-by-step scrutiny

Hou and colleagues' experiments then demonstrated that their heterometallic clusters possessed critical hydrogen storage and release capabilities. Heating H_2 and Y_4WH_{11} to 80 °C caused an oxidative addition of the gas molecule to the cluster, which they could reverse through ultraviolet-light treatment. Despite the Y_4MoH_{11} molecule not responding to the same chemical tricks, the researchers discovered that applying a vacuum could suck H_2 from the cluster, giving a new Y_4MoH_9 complex. Exposing this compound to hydrogen gas at room temperature spontaneously regenerated the original molecule (Fig. 2).

According to Hou, the most striking aspect of this chemistry is that the hydrogen addition to the Y_4MoH_9 cluster can be followed from single crystal to single crystal—meaning that the starting material, the reaction intermediates, and the product all retain the same rigid morphology. "No metal hydrides have previously shown such excellent crystallinity," he notes.

After gingerly sealing a Y_4MoH_9 crystal into a thin, hydrogen-filled capillary tube, the researchers monitored the spontaneous addition reaction over 60 hours. As the cluster gradually took in hydrogen and changed color from black to red, they watched—with precision greater than one-millionth of a meter—yttrium and molybdenum atoms separate and shift within the crystal unit cell. By providing the first-ever atom-resolved views of active sites and bonding modes for hydrogen addition to an organometallic crystal, these findings should aid design of more sophisticated alloys in the future.

Theoretical calculations performed by the researchers indicated that combining two metals with starkly different electronic properties played a big role in giving the clusters their unique reactivity. With wide swaths of the periodic table available for exploring using this technique, breakthroughs in heterometallic hydride materials may have only just begun. ■

1. Shima, T., Luo, Y., Stewart, T., Bau, R., McIntyre, G.J., Mason, S.A. & Hou, Z. Molecular heterometallic hydride clusters composed of rare-earth and *d*-transition metals. *Nature Chemistry* **3**, 814–820 (2011).
2. Nishiura, M. & Hou, Z. Novel polymerization catalysts and hydride clusters from rare-earth metal dialkyls. *Nature Chemistry* **2**, 257–268 (2010).

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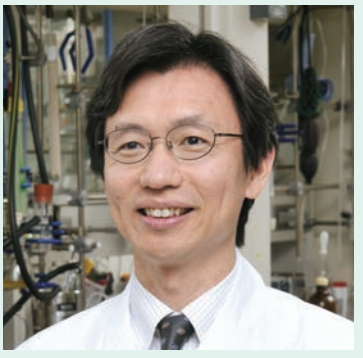

Zhaomin Hou was born in Shandong Province, China, in 1961. He graduated from the China University of Petroleum (Shandong, China) in 1982, and obtained his PhD in chemistry in 1989 from Kyushu University (Fukuoka, Japan). After postdoctoral training at RIKEN (1989–1991) and the University of Windsor (Windsor, Canada) (1991–1993), he joined RIKEN as a Research Scientist in 1993. He was promoted to Senior Scientist in 1997 and to Chief Scientist in 2002. Since then, he has been directing the Organometallic Chemistry Laboratory at RIKEN. His current research interests include the development of novel catalysts for more efficient, selective chemical transformations and the development of novel functional materials.

Biology

‘Mind-readers’ get a clearer view

A treatment that renders brain tissue transparent allows neuroscientists to visualize neural circuitry at once-unattainable depths

A recent breakthrough in biological sample preparation by scientists at the RIKEN Brain Science Institute in Wako may give Astro Boy’s ‘x-ray vision’ a run for its money. By treating tissue samples with an easy-to-prepare mix of chemicals, Atsushi Miyawaki, Hiroshi Hama and their colleagues can render the brain and other tissues as clear as glass, a reversible transformation that gives researchers an unobstructed view of fluorescently labeled cells residing within[1].

For decades, the limits of available technology have thwarted attempts to map the dizzying twists and turns of the brain. Neuroscientists have achieved some success in simpler organisms, such as the worm or fly, by using tiny blades to sequentially peel off ultrathin strips of tissue, which can then be imaged via electron microscopy and reassembled computationally. However, this approach is far too labor-intensive and time-consuming for the reconstruction of a system as complex as the mammalian nervous system.

More recent breakthroughs in optical microscopy technology and a rapidly growing arsenal of multi-colored fluorescent proteins have given researchers potent new tools for brain mapping. By restricting the expression of specific fluorescent labels to particular subsets of cells, one can clearly visualize neural circuits within their natural, three-dimensional context; however, the dense tissue of the brain tends to scatter light, limiting the depth to which such imaging strategies can penetrate.

Several research groups have developed ‘clearing agents’ that improve the transparency of biological samples, such as benzyl-alcohol/benzyl-benzoate (BABB) and a proprietary solution known as FocusClear, but each suffers important limitations. “BABB is an organic solvent that requires dehydration of samples to be cleared,” says Miyawaki. He adds that such treatment can greatly diminish overall sample fluorescence, “and FocusClear does not clear mouse brain samples [readily].” Accordingly, his team’s development of the reagent they call ‘Scale’ could open new frontiers in mouse brain imaging.

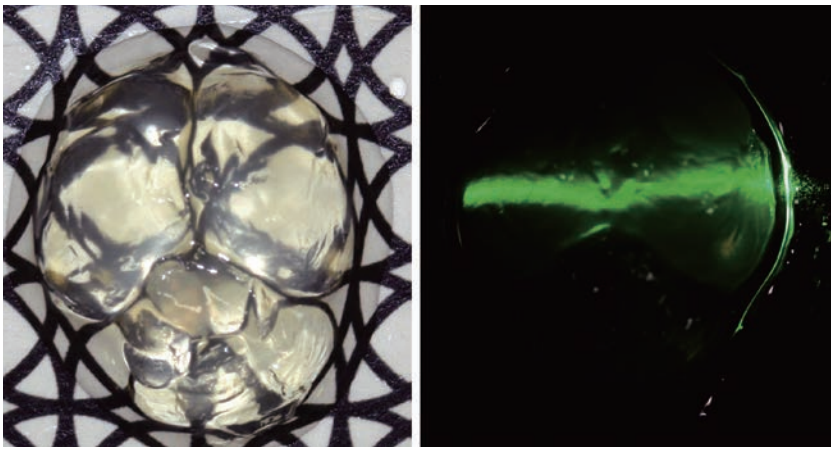

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Figure 1: After two weeks of treatment with ScaleA2, the mouse brain is transparent enough (left) to be readily traversed by the light from a laser beam (right).

Going deeper

Scale initially emerged from the unexpected, chance observation that membranes composed of the material polyvinylidene fluoride, which normally resemble sheets of white paper, become completely transparent when soaked in a high-concentration urea solution. By tinkering with this solution, Miyawaki and colleagues arrived at ScaleA2, a mixture that achieves the same feat with biological tissues.

ScaleA2 can render a mouse brain essentially transparent within two weeks (Fig. 1). This treatment also causes the tissue to swell as a result of water uptake, but the researchers determined that specimens maintain their overall shape and proportions, suggesting that this expansion does not significantly affect the arrangement of the cellular structures being imaged.

In an initial test of their imaging approach, Miyawaki and colleagues found that cells within ScaleA2-treated samples fully retained their fluorescent labels, while tissues treated with BABB yielded greatly diminished signal. More

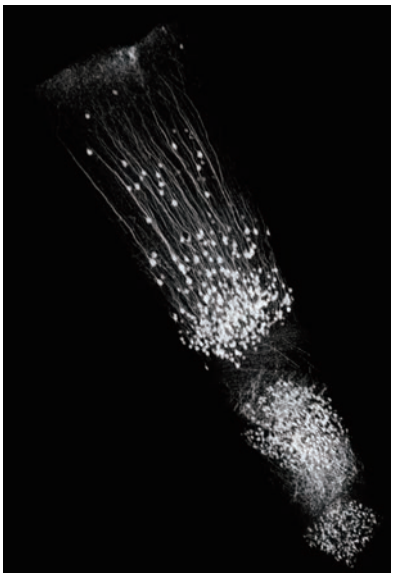

Figure 2: Visualization of fluorescently labeled neurons within a rectangular volume encompassing cells in the cerebral cortex and hippocampus. This image was captured with a specialized lens enabling penetration to a depth of 4 millimeters below the brain surface.

importantly, the transparency induced by Sca*l*eA2 allowed the researchers to visualize far deeper within the brain than before, even when using standard 'one-photon' microscopic approaches that are typically vulnerable to scattering and background image interference.

"Although the imaging depth limit of two-photon excitation fluorescence microscopy is usually around 0.7 millimeters in the brain, we were able to image fluorescent neurons with Sca*l*e down to a depth of 2 millimeters below the brain surface," says Miyawaki. By designing a specialized microscope lens, they were able to penetrate still farther, to an unprecedented working distance of 4 millimeters below the brain surface (Fig. 2). The level of detail obtained with Sca*l*eA2 proved sufficient for the researchers to map axonal connections between neurons in the corpus callosum, the bridge between the brain's hemispheres, and also allowed them to analyze the interaction between neural stem cells and the vasculature within the developing mouse brain.

Since not all specimens are created equal, Miyawaki and colleagues also experimented with alternative Sca*l*e formulations for specialized imaging applications. One of these, Sca*l*eU2, necessitates longer sample incubation, but results in less tissue expansion so may offer advantages for use with embryonic samples or other fragile tissues. In a preliminary experiment on 13.5-day-old mouse embryos, the researchers used Sca*l*eU2 to visualize regions of active cell division in the diencephalon—a part of the forebrain.

Importantly, the effects of Sca*l*e treatment proved fully reversible, and samples that had recovered from clearing proved indistinguishable from their uncleared counterparts, reaffirming the minimal impact of this treatment on tissue structure.

A clear view of the future

Some researchers have designed especially ambitious strategies for neural circuit mapping, such as the 'Brainbow' mouse developed at Harvard Medical School, which combines large numbers of different fluorescent proteins to turn the mouse brain into a dazzling light show in which virtually every neuron stands out clearly from its neighbors. Miyawaki believes Sca*l*e should prove highly complementary to such efforts. "All the fluorescent proteins we've tested so far are resistant to high concentrations of urea, and should be usable," he says, "and so this approach should be compatible with Brainbow."

His team is already engaged in collaborations to apply Sca*l*e to targeted investigations in mice. Although the work described to date has focused on genetically expressed fluorescent markers, this approach should also be compatible with other labeling methodologies. Once such techniques have been developed, Sca*l*e should prove effective for working with larger tissue samples obtained from species that are not readily amenable to genetic modification, such as primates.

The biggest limitation seen by Miyawaki at present is the need to work with 'dead' tissue, but he suggests that even this may change. "Sca*l*e is currently limited to fixed biological samples," he says, "but at some point in the future, there may be 'live Sca*l*e'." ■

1. Hama, H., Kurokawa, H., Kawano, H., Ando, R., Shimogori, T., Noda, H., Fukami, K., Sakaue-Sawano, A. & Miyawaki, A. Sca*l*e: a chemical approach for fluorescence imaging and reconstruction of transparent mouse brain. *Nature Neuroscience* **14**, 1481–1488 (2011).

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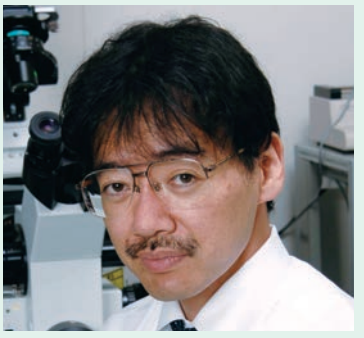

Atsushi Miyawaki obtained his PhD from Osaka University. He carried out postdoctoral studies on intracellular signal transduction at the University of Tokyo. In 1995, he initiated studies of fluorescent proteins and their application under Roger Y. Tsien at the University of California, San Diego, USA. Since 1999, his group at the Laboratory for Cell Function Dynamics has been interested in the development of new bioimaging technologies, principally using fluorescent proteins.

Hiroshi Hama obtained his PhD from the Graduate School of Medicine, Tsukuba University. He focused on cell biology of glial cells and muscle dystrophy in his postdoctoral studies at the Department of Pharmacology, Basic Medical Sciences, Tsukuba University and the Department of Cell Biology, National Institute of Neuroscience. Since 1999, he has been based at the Laboratory for Cell Function Dynamics as a research scientist and a specialist on the development of technologies for visualizing both the anatomy and brain function of mammalian brains. His interests include brain function repair by neural progenitors and the visualization of the neuronal/glial network.

Sharpening the focus of microscopes

Based on non-linear optical effects, imaging with light has reached atomic precision in the most precise microscope ever built

A new advanced imaging scheme—with a resolution ten times better than that of its counterparts to date—can resolve objects as small as atoms[1]. Previously, the maximum resolution of optical instruments, including cameras and microscopes, was fundamentally limited to a precision that corresponded to approximately half of the wavelength of incoming light.

The new scheme, developed by researchers from the RIKEN SPring-8 Center in Harima and Nagoya University, has a resolution up to 380 times better than the UV light used in the experiments. For microscopes using visible light, which means wavelengths of a few hundred nanometers, the best achievable resolution is around 100 nanometers, which fails to resolve the smallest structures on a computer chip. Imaging smaller nanostructures, or even atoms, requires light of much shorter wavelengths, such as x-rays that are difficult to handle, and which provide different types of images to those captured using visible light.

Led by Kenji Tamasaku of RIKEN, the researchers used a non-linear optical effect to achieve atomic resolution in diamond. Their process is based on the intrinsic interaction between the electrons of the material's crystal atoms and UV light that splits an incoming x-ray beam into a UV beam and a lower energy x-ray beam. The combined energy of these scattered beams is the same as that of the incoming beam. This process depends strongly on the activation of the UV beam, which occurs only in the vicinity of the electrons in the atoms, and only if the optical response of the electrons is a match to the incoming x-ray beam, Tamasaku explains.

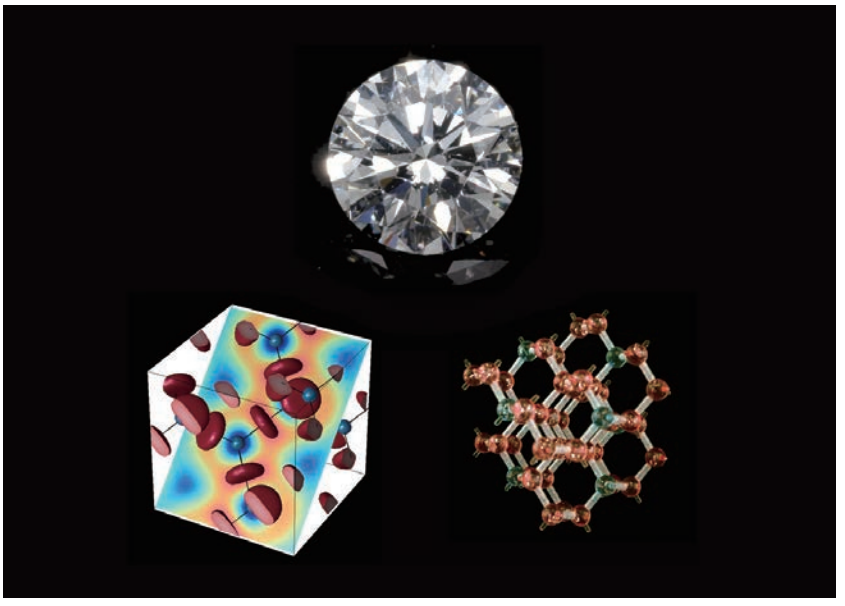

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Figure 1: The optical response of a diamond crystal (top) can now be analyzed at the atomic scale with extreme ultraviolet light (left). This technique can provide additional information to the crystal structure (right) typically obtained using x-rays.

Analyzing the scattered beams allowed a precise reconstruction of the motion of the electrons under UV illumination. Using a diamond crystal as an imaging object, the researchers demonstrated a resolution of 0.054 nanometers (Fig. 1). Because Tamasaku and colleagues used a non-linear optical effect, they obtained new information not only about how electrons move but also about atomic position.

There are many possibilities for using this new method, says Tamasaku. "This technique is very useful for the study of the physical properties of materials that couple to light." An example is the study of electronic materials, in which the sensitivity of the technique to the electron's electronic states can be used to probe electrical charges in materials such as high-temperature superconductors. Using the team's new approach, this will now be possible with atomic resolution. ■

1. Tamasaku, K., Sawada, K., Nishibori, E. & Ishikawa, T. Visualizing the local optical response to extreme-ultraviolet radiation with a resolution of λ/380. *Nature Physics* **7**, 705–708 (2011).

Green fixations for a cleaner future

Copper–carbene catalysts reveal the critical interactions needed to turn waste carbon dioxide into chemical feedstocks

Using fixation reactions to convert free carbon dioxide (CO_2) into different organic molecules is an attractive strategy to cut industrial greenhouse gas levels with marginal waste. Now, broadening the scope of CO_2 fixation is possible using a method developed by a research team in Japan led by Zhaomin Hou from the RIKEN Advanced Science Institute in Wako[1]. The method uses a 'green' catalyst system that transforms alkyl–boron molecules into carboxylic acids—an important ingredient for pharmaceutical production.

Organic boron compounds are attractive fixation substrates because they readily participate in carbon–carbon bond-forming reactions. Recently, chemists have used transition metal catalysts to activate hydrocarbons bonded to oxygenated boron esters; addition of CO_2 then splits off the activated group and generates a carboxylic acid derivative. However, attempts to reproduce this chemistry with alkylboranes—a widespread class of important synthetic reagents—have had limited success because the so-called 'catalytic transition metal alkyl' intermediates are usually unstable and decompose before reacting with CO_2.

Hou and colleagues turned to an innovative chemical system to resolve this instability. By combining electron-donating, bulky molecules called *N*-heterocyclic carbenes (NHCs) with copper atoms, they made metal alkyl complexes that can promote carbon–carbon bond formation with CO_2 under mild conditions and at lower cost than most precious metal catalysts—ideal characteristics for sustainably recycling CO_2 emissions.

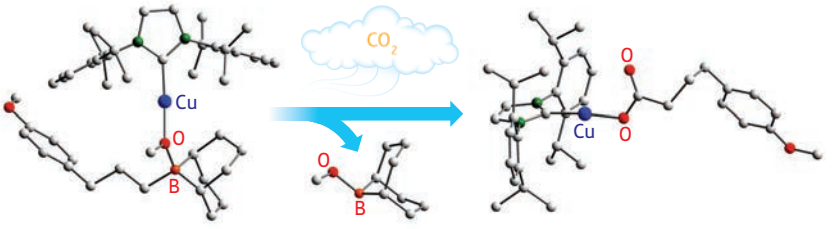

Figure 1: Isolation of a new copper–oxygen–boron complex (left) revealed that carbon dioxide (CO_2) can insert into its chemical framework (right) by eliminating the organo-boron component (middle panel, lower structure).

First, the researchers produced an easily activated alkylborane by connecting borabicyclononane (BBN)—a highly strained set of boron–hydrocarbon rings—to the terminal atom of a carbon–carbon double bond. In this approach, the target hydrocarbon for CO_2 addition is physically and electronically quite different from the two carbon–boron bonds of the BBN rings.

Hou and colleagues then mixed the alkylborane with the copper–NHC catalyst, a base, and CO_2 in a pressurized chamber. After one day at 70°C, they found that the target had transformed into a new carboxylic acid with near-quantitative yields. Diverse molecules bearing aromatic, halogenated, and bulky functional groups could all act as CO_2 fixation substrates using this technique.

The copper–NHC catalyst offered another advantage to the team: a unique chemical environment that enabled isolation of several catalytic intermediates as solid crystals. X-ray measurements of these structures provided the first hard evidence that bonding interactions between alkoxide base molecules, copper atoms, and alkylboranes are critical to enabling CO_2 addition (Fig. 1). "Fine-tuning the combination of central metals, bases, and supporting ligands will eventually lead to more efficient and selective catalysts," notes Hou. ■

1. Ohishi, T., Zhang, L., Nishiura, M. & Hou, Z. Carboxylation of alkylboranes by *N*-heterocyclic carbene copper catalysts: Synthesis of carboxylic acids from terminal alkenes and carbon dioxide. *Angewandte Chemie International Edition* **50**, 8114–8117 (2011).

Turning the spotlight on drug-resistant tumors

A newly developed group of fluorescent probes highlights cancer cells resistant to anticancer drugs

Molecular probes that can illuminate cancer cells are often invaluable tools in the fight against the disease. The latest addition to this group is a family of fluorescent probes that can highlight a particularly pernicious kind of tumor cell: those resistant to anticancer drugs. The international team of scientists behind the research, led by Hiroshi Abe at the RIKEN Advanced Science Institute in Wako, Japan, and Ralf Morgenstern at the Karolinska Institute in Stockholm, Sweden, say that their discovery could help expedite research efforts to defeat these drug-resistant tumors[1].

The probes work by targeting a family of enzymes called glutathione transferases (GSTs). These enzymes play a variety of roles in healthy cells, including protecting the cell from oxidative stress by labeling harmful molecules with a glutathione tag. This tag marks the molecule for rapid export out of the cell. But in certain cancer cells, GSTs are produced in elevated amounts, where they are able to tag many anticancer drugs in the same way. As a result, the drug is pumped away before it can kill the cell. Finding ways to block GSTs is therefore an active area of cancer research since a probe that can reveal the level of GST activity inside a living cell could prove a very useful tool in the search.

The team's fluorescent probes exploit the GSTs well-known molecular mode of action. The GST enzymes target molecules—such as anti-cancer drugs—by attacking an electron-poor point in its structure and attaching the glutathione label to it. Using a series of known fluorescent molecules, Abe, Morgenstern and colleagues added to them an electron-poor arylsulfonyl group ripe for attack by a GST enzyme. The arylsulfonyl group also has the effect of suppressing the fluorescence; but, as GST attacks, it breaks the probe in two which releases the fluorophore and instantly illuminates the cell.

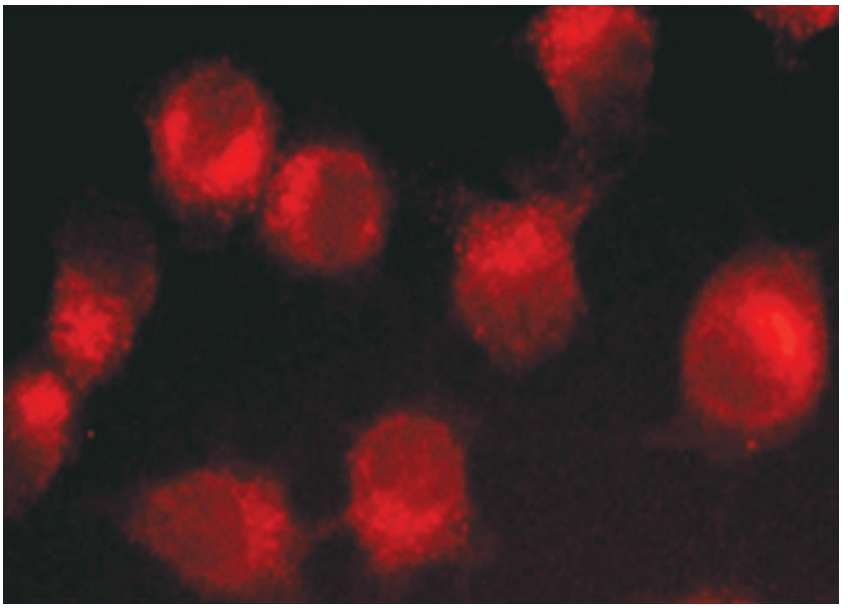

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Figure 1: Fluorescent probes light up living cancer cells to indicate the presence of glutathione transferase (GST), an enzyme associated with drug resistance.

The researchers demonstrated that they could use their probes to quantitatively measure very low levels of GST in cell extracts. Using a fluorophore known as cresyl violet, they were also able to image GST activity inside living cells (Fig. 1). According to Abe, the researchers already have plans to improve the performance of their probes, making them even more sensitive.

"By fine-tuning the chemical reactivity of the protecting arylsulfonyl group, we will improve the signal to background noise ratio for improved imaging," he says. "By re-designing the fluorescent compound, we can also achieve GST subtype selectivity," he adds. ■

1. Zhang, J., Shibata, A., Ito, M., Shuto, S., Ito, Y., Mannervik, B., Abe, H. & Morgenstern, R. Synthesis and characterization of a series of highly fluorogenic substrates for glutathione transferases, a general strategy. *Journal of the American Chemical Society* **133**, 14109–14119 (2011).

It takes a sugar to catch a sugar

A single sugar molecule plays an outsized role in helping a glucose-transporting protein respond promptly to insulin signals

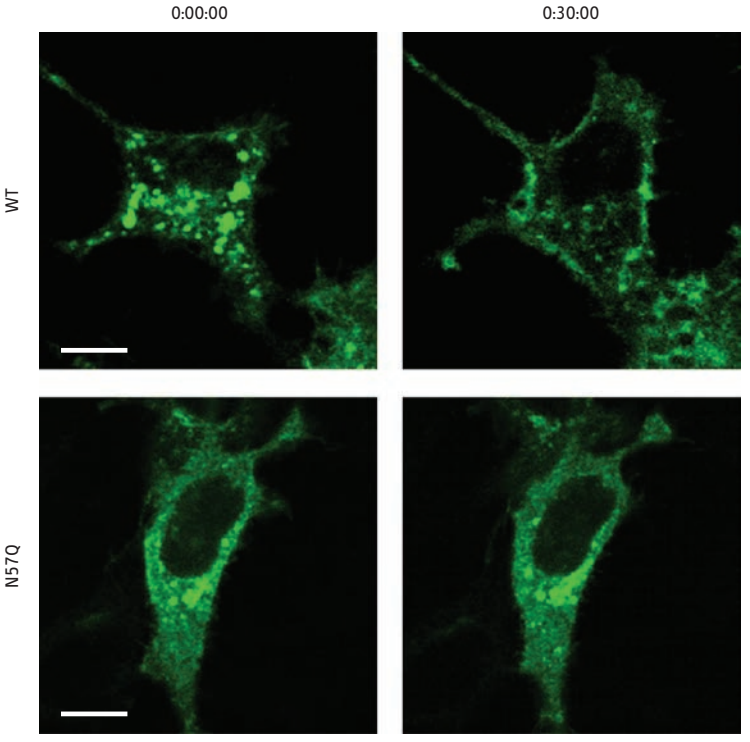

Figure 1: GLUT4 is normally retained within storage vesicles in the cytoplasm (top left), but is redistributed to the cell membrane within 30 minutes of insulin treatment (top right). However, the non-glycosylated N57Q mutant (bottom left) fails to respond to insulin and remains within cytoplasmic vesicles even after 30 minutes (bottom right) (scale bars, 10 µm).

After every meal, the hormone insulin is released into the bloodstream, issuing instructions to target cells to begin taking up excess sugar. In some situations, however, cells stop responding to these signals; and this insulin-resistant state is associated with onset of type 2 diabetes. Unexpected findings from Tadashi Suzuki's group at the RIKEN Advanced Science Institute in Wako have now revealed how a cellular malfunction may contribute to this insulin resistance[1].

Suzuki and postdoctoral fellow Yoshimi Haga had originally sought to develop imaging strategies to track localization of proteins modified with carbohydrate groups in a process known as glycosylation. They tested their method with the glucose-transporter protein GLUT4 and developed a mutant version of the protein that lacks a glycosylation site, but the results led their study in a new direction. "We accidentally found that behavior of our N57Q mutant and the wild-type protein was quite different," says Suzuki. "It was a completely serendipitous finding."

Insulin-responsive cells typically maintain reservoirs of GLUT4 in bubble-like vesicles within the cytoplasm; insulin signals induce the transport of GLUT 4 to the cell surface, where it begins pumping glucose into the cell. The N57Q mutant, on the other hand, was largely unresponsive to insulin (Fig. 1). Suzuki and colleagues determined that, instead of gathering within storage vesicles, this protein tended to accumulate either at the cell surface or within 'recycling vesicles' that continually shuttle to and away from the plasma membrane.

The N57Q mutant retains normal glucose-transporting capabilities, suggesting that this modification acts primarily as a trafficking signal rather than influencing protein function. Accordingly, the researchers observed the same insulin-insensitive behavior when they used a chemical treatment to alter the glycosylation of normal GLUT4. "These results clearly suggest that there must be a mechanism that detects subtle differences in glycan structure on this protein to sort it into specific GLUT4 vesicles," says Suzuki.

Several studies have found evidence that GLUT4 may be subject to altered glycosylation in a subset of patients with type 2 diabetes; and, these new findings provide a potential explanation for how malfunctions in this protein modification process could contribute to pathology. As a next step, Suzuki hopes to uncover more details about how this transport pathway intersects with the insulin response. "We believe that glycan-recognition molecules known as lectins should be involved in this process," he says, "and we will try to identify these lectins and other players involved in the fine-tuning of intracellular trafficking of GLUT4." ■

1. Haga, Y., Ishii, K. & Suzuki, T. N-glycosylation is critical for the stability and intracellular trafficking of GLUT4 glucose transporter. *The Journal of Biological Chemistry* published online 14 July 2011, (doi: 10.1074/jbc.M111.253955).

Finding the sweet spot

Modifications to chromosomal proteins help ensure that brain-specific sugars are produced only in the appropriate tissues

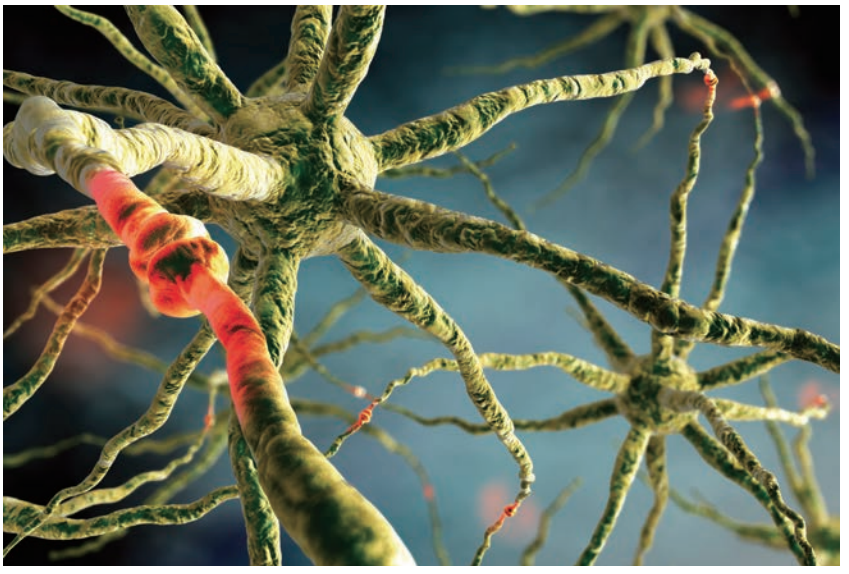

Figure 1: Neural cell-specific modifications to chromosomal proteins govern the production of Gnt-IX and thereby ensure that branched O-mannose glycan production is restricted to these cells.

Many proteins are adorned with carbohydrate chains called glycans that can dramatically alter their stability, localization or function. These diverse sugars are assembled and modified by a variety of glycosylating enzymes, with some glycans exclusively manufactured within specific organs or tissues.

The β1,6-branched O-mannosyl glycan appears only in the mammalian brain. Naoyuki Taniguchi's team at the RIKEN Advanced Science Institute in Wako recently characterized the enzyme, N-acetylglucosaminyltransferase IX (GnT-IX, also called GnT-Vb) that produces this particular glycan variant[1] (Fig. 1). "We knew that some glycan-synthesizing enzymes are expressed in restricted tissues, but did not know how they are expressed," says Yasuhiko Kizuka, a researcher in Taniguchi's laboratory. "This led us to investigate how GnT-IX is specifically expressed in the brain."

Many genes are regulated by so-called 'epigenetic mechanisms', in which gene expression is modulated via modification of the histone protein scaffold that supports chromosomal DNA, and the researchers began by examining this possibility. When histone proteins undergo a modification known as acetylation, nearby genes are typically activated; conversely, removal of this acetylation has an inhibitory effect.

Taniguchi and colleagues determined that the gene encoding GnT-IX is typically maintained in an inactive, non-acetylated state in 3T3-L1, a cell line derived from the fibroblasts that form connective tissue. However, when the researchers treated these cells with a drug that promotes histone acetylation, they strongly expressed GnT-IX. The brain tumor-derived Neuro2A cell line, however, naturally expresses high levels of GnT-IX. The researchers found that these cells normally maintain the chromatin near this gene in a state that stimulates activation.

In subsequent experiments, Kizuka and Taniguchi not only identified specific DNA sequences that directly regulate GnT-IX activity, but also two proteins that bind to these sites to drive expression. They found one of these factors, CTCF, in both 3T3-L1 and Neuro2A cells, but its recruitment to the GnT-IX gene was far stronger under the favorable histone modification conditions found in the latter cells.

Intriguingly, a preliminary screen of four other glycosylation enzymes suggested that similar mechanisms govern their tissue-specificity. "Our work suggests that expression of many other glyco-genes could be regulated epigenetically," says Kizuka.

In future studies, the researchers intend to explore how this regulatory mechanism plays into the bigger picture of glycan function. "Our group has been trying to elucidate the 'glycan cycle'—how glycans are dynamically synthesized, play diverse roles and are degraded—using a systems biology approach," says Kizuka. "This work tells us that epigenetic regulation is a part of this cycle." ■

1. Kizuka, Y., Kitazume, S., Yoshida, M. & Taniguchi, N. Brain-specific expression of N-acetylglucosaminyltransferase IX (GnT-IX) is regulated by epigenetic histone modifications. *The Journal of Biological Chemistry* published online, 19 July 2011 (doi: 10.1074/jbc/M111.251173).

Conducting how neurons fire

Research on the mechanism that controls neuron firing in the rat brain reveals a surprise for neuroscientists

Figure 1: Changing a neuron's membrane conductance of ions can switch its response to a neurotransmitter from excitation to inhibition.

Contrary to expectations that the neurotransmitter GABA only inhibited neuronal firing in the adult brain, RIKEN-led research has shown that it can also excite interneurons in the hippocampus of the rat brain by changing the conductance of ions across the membranes of these cells[1].

According to conventional wisdom, activation of the $GABA_A$ receptor subtype at the communication junction between neurons—the synapse—strongly increases membrane conductance of ions, triggering a process called shunting, which inhibits neuronal firing. Led by Alexey Semyanov of the RIKEN Brain Science Institute in Wako, the team demonstrated that activation of these receptors outside of synaptic junctions, so-called 'extrasynaptic receptors' can also excite the neurons.

Further activation of these extrasynaptic receptors by application of higher concentrations of GABA turn excitation into inhibition (Fig. 1). "To our knowledge, this is the first demonstration that changes in membrane conductance can switch the action of a neurotransmitter from excitation to inhibition," Semyanov says.

Semyanov and colleagues treated slices of the mouse hippocampus with low or high concentrations of GABA and compared the effects. They showed that the more GABA they added, the more they could detect an increase in the conductance of the membranes of hippocampus cells called CA1 interneurons. The increased conductance was mediated through extrasynaptic $GABA_A$ receptors.

The CA1 interneurons could spontaneously fire action potentials—electrical impulses that transfer signals in the network of interconnected neurons. Adding low concentrations of GABA increased the rate of action potential firing, while high concentrations of GABA reduced action potential firing in the cells. Because the concentration of GABA that slowed neuronal firing had also enhanced membrane conductance, the researchers argue that increasing this conductance by activating extrasynaptic $GABA_A$ receptors can result in inhibition via shunting along the membrane, which would cause a decrease in action potential generation in the neurons.

The hippocampus plays a key role in learning and memory, and GABA concentrations are known to increase in this part of the brain during exploratory behavior in rats. The findings therefore raise the intriguing possibility that changes in GABA concentration in the brain during some behavioral tasks could bidirectionally change neuronal excitability; this could be a characteristic of the hippocampal neuronal network that may be required for some behavioral tasks in animals.

"Many clinically used drugs, such as sedatives or anti-epileptics, target GABA receptors," notes Semyanov. "Our findings could potentially explain their therapeutic action as well as some of their unwanted side effects." ■

1. Song, I., Savtchenko, L. & Semyanov, A. Tonic excitation or inhibition is set by $GABA_A$ conductance in hippocampal interneurons. *Nature Communications* **2**, 376 (2011).

Characterizing a toxic offender

Clarification of the role of a specific protein fragment that forms toxic clumps and damages the brain could lead to therapeutics for Alzheimer's disease

The brains of individuals with Alzheimer's disease contain protein aggregates called plaques and tangles, which interfere with normal communication between nerve cells and cause progressive learning and memory deficits. Now, a research team led by Takaomi Saido from the RIKEN Brain Science Institute in Wako has identified a particular fragment of the amyloid precursor protein (APP) that contributes to the formation of plaques in the brain[1].

Enzymes cut APP to form shorter protein fragments and, in Alzheimer's patients, these sticky fragments clump together to form amyloid plaques. Most current research on this disease focuses on a 42 amino acid-long fragment called $A\beta_{42}$, in part because other researchers had shown that *APP* mutations that increase $A\beta_{42}$ cause Alzheimer's disease in some families. Other APP fragments are also found in the brain of individuals with Alzheimer's disease, but their role in disease was unclear.

Saido and colleagues studied a 43 amino acid-long fragment called $A\beta_{43}$ because other groups have shown that it can form aggregates as readily as $A\beta_{42}$ (Fig. 1). The researchers generated mice that have a mutation in the *presenilin-1* gene that contributes to the cleavage of APP, and showed that it led to increased formation of $A\beta_{43}$ in cell culture experiments.

The research team then mated these *presenilin-1* mutant mice to *APP* mutant mice, which display many symptoms of Alzheimer's disease, such as deposition of plaques in the brain and a gradual loss of memory. *APP* mutant mice generally exhibit plaque formation at one year of age. However, with the increase in $A\beta_{43}$ caused by the presence of the *presenilin-1* mutation, these so-called 'double-mutant mice' had plaques in their brain six months earlier than usual. The double-mutant mice also seemed to show memory deficits at an even earlier age than *APP* mutant mice. Furthermore, the research team showed that $A\beta_{43}$ is even more prone to aggregate and to cause neuronal damage than is $A\beta_{42}$.

The findings therefore suggest that $A\beta_{43}$ plays a role in accelerating Alzheimer's disease. Saido and colleagues argue that therapies that specifically prevent $A\beta_{43}$ accumulation, such as by enhancing the cleavage of $A\beta_{43}$ into shorter Aβ fragments, or by stimulating the immune system to clear $A\beta_{43}$, could therefore be beneficial in slowing the progression of Alzheimer's disease.

"$A\beta_{43}$ could also be a diagnostic marker for Alzheimer's disease," explains Takashi Saito, the first author of the study. "We would now like to develop it along these lines." ■

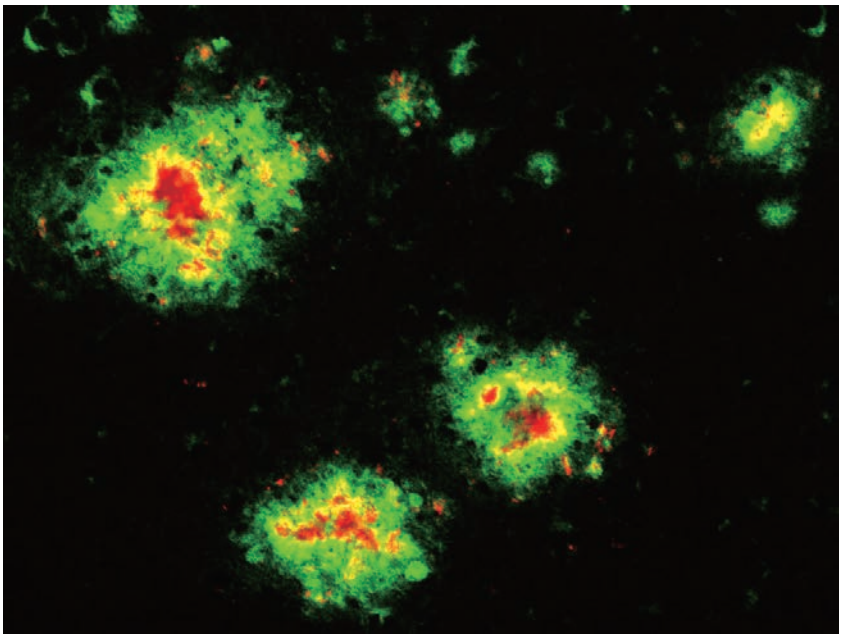

Figure 1: Amyloid plaque in the brain of a human suffering from Alzheimer's disease in which $A\beta_{43}$ forms the plaque core. Green shows the total Aβ peptide. Red shows the $A\beta_{43}$ peptide and yellow shows these colors merged.

1. Saito, T., Suemoto, T., Brouwers, N., Sleegers, K., Funamoto, S., Mihira, N., Matsuba, Y., Yamada, K., Nilsson, P., Takano, J. *et al*. Potent amyloidogenicity and pathogenicity of $A\beta_{43}$. *Nature Neuroscience* **14**, 1023–1032 (2011).

Pinpointing asthma susceptibility in Japanese adults

Identification of genetic variations that increase the risk to adult asthma will lead to better understanding of this disease

A team of geneticists has identified five specific gene regions associated with asthma susceptibility among Japanese adults[1]. Mayumi Tamari of the RIKEN Center for Genomic Medicine, Yokohama, led the research.

Asthma is a chronic inflammatory disease affecting the airways and lung (Fig. 1). With an estimated 300 million sufferers worldwide, symptoms include recurrent wheezing and coughing, and shortness of breath. Although controllable in most cases, serious asthma attacks can be fatal.

"Asthma is caused by a combination of genetic and environmental factors," explains Tamari. "By identifying asthma genes and studying their function, biologists hope to elucidate mechanisms underlying asthma development and progression, leading to more effective treatments."

Other researchers have identified regions of the human genome containing genes associated with asthma. For example, one recent large-scale, consortium-based study identified nine of these so-called susceptibly loci in European populations. "Little is known about genetic differences contributing to asthma susceptibility among other ethnic groups such as Asians," says Tamari.

With this in mind, Tamari and her colleagues conducted a genome-wide association study involving a total of more than 7,000 adult Japanese asthma sufferers and around 28,000 unaffected individuals from the same population. By statistically analyzing nearly half a million genetic markers called single nucleotide polymorphisms (SNPs) distributed across the human genome, they identified five susceptibility loci in the Japanese population.

Figure 1: The respiratory disease asthma, although controllable, affects millions of people worldwide and can be fatal.

Two loci identified by the researchers were reported previously in Europeans. One was found in the vicinity of the major histocompatibility complex (MHC) on chromosome 6, which contains genes encoding molecules important for the functioning of the immune system. The other previously reported locus spans a region containing two genes, *TSLP* and *WDR36*. *TSLP* is already known to play a role in allergies.

In addition, Tamari and colleagues found three chromosomal regions significantly associated with asthma susceptibility in the Japanese population. They identified a locus on chromosome 10, a gene-rich region on chromosome 12, and a region on chromosome 4 spanning two known genes, *USP38* and *GAB1*. These two genes encode, respectively, an enzyme called ubiquitin-specific peptidase 38, the biological function of which is unknown, and a protein involved in signaling within the immune system. Interestingly, they found that the strongest association with asthma among Japanese adults was close to a SNP in a gene called *AGER* within the MHC previously shown to be important for lung function.

"Our findings contribute to a better understanding of the genetic contribution to asthma susceptibility, opening the way to further functional studies," concludes Tamari. ■

1. Hirota, T., Takahashi, A., Kubo, M., Tsunoda, T., Tomita, K., Doi, S., Fujita, K., Miyatake, A., Enomoto, T., Miyagawa, T., *et al*. Genome-wide association study identifies three new susceptibility loci for adult asthma in the Japanese population. *Nature Genetics* **43**, 893–896 (2011).

ICHIRO TANIUCHI

Team Leader
Laboratory for Transcriptional Regulation
RIKEN Research Center for Allergy and Immunology

Exploring the mechanism behind the differentiation of immune cells

When a virus or bacterium enters the body, the foreign 'antigen' is detected and attacked by the body's immune system—a remarkable defense mechanism that has evolved on all living organisms. Immunity is mediated by helper T cells, which serve as the lynchpins in signaling and regulatory functions, and also by killer T cells, which directly attack antigen-infected cells. Ichiro Taniuchi, team leader of the Laboratory for Transcriptional Regulation at the RIKEN Research Center for Allergy and Immunology, is carrying out research to understand the mysteries of organism evolution by investigating the mechanism behind the differentiation of these immune cells.

Roles of T cells in the immune system

All living organisms possess an immune system that senses the entry of antigens, such as viruses and bacteria, and attacks these foreign invaders. This immune response is acted out by immune cells in leukocytes, commonly known as white blood cells. Immune cells are diverse, ranging from dendritic cells to macrophages, T cells (T lymphocytes) and B cells (B lymphocytes). These cells circulate throughout the body in the blood and lymph systems, and upon detection of an antigen, act cooperatively in defense (Fig. 1).

"Lymphocytes are the major player in the immune system in higher animals such as humans," says Taniuchi. "According to their roles, lymphocytes can be classified into B cells and T cells, and T cells can be further classified into helper T cells and killer T cells. Upon entry of pathogenic bacteria or a virus into the body, cells that sense antigens, such as dendritic cells and macrophages, first work to transmit signals about the foreign matter to helper T cells. On receiving the signal, the helper T cells issue directives to killer T cells and B cells. The killer T cells attack virus-infected or cancerous cells directly, whereas B cells release antibodies to attack pathogenic bacteria. The immune system functions are realized through the various roles of the different types of immune cells."

Th-POK, the master transcription factor for helper T cells

Although they have varied functions, all types of immune cells have their origins in hematopoietic stem cells in the bone marrow. However, while most immune cells are produced in the bone marrow, T cells are produced in the thymus, an organ located near the heart. Migrating from the bone marrow to the thymus, T-cell progenitor cells become involved in the processes of forming diverse T-cell clones that can recognize a wide variety of antigens and distinguish the body's own cells from the invaders. Following this course of 'positive' selection, double positive (DP) thymocytes are prompted to determine their own fate and differentiate into either helper or killer T cells.

"I am working to elucidate the mechanism by which T cells determine their own fate in differentiating into helpers or killers," says Taniuchi. "To understand the mechanism behind the determination of T-cell fate and to differentiate into the two types with different roles is very important for controlling graft rejection

in organ transplantation, as well as having a role in allergies, autoimmune diseases and cancerous cells. Artificially creating helper T cells and killer T cells could lead to applications for regenerative medicine and immune therapy."

The two types of T cells are easily distinguishable by the expression pattern for the glycoproteins CD4 and CD8 on the cells' surfaces. Helper T cells only express CD4, whereas killer T cells only express CD8. DP thymocytes, the undifferentiated precursor of these two cell types, express both CD4 and CD8 at the same time.

Most cells in the body contain an antigen-presenting molecule called the 'major histocompatibility complex' (MHC), which allows cells to present fragments of foreign invaders on the cell surface. When a T cell encounters an antigen-presenting MHC, the 'T-cell antigen receptor' (TCR) on the T cell's surface reacts with it and antigen information is passed from the MHC to the TCR.

This is not the full story, however: there are two types of MHCs, referred to as class I and class II. Class I MHCs are possessed by most cells, including virus-infected cells, whereas class II MHCs are possessed only by specific antigen-presenting cells. The TCR on T cells is only reactive with one of these two types of MHC, a phenomenon known as 'MHC restriction.' CD4 in helper T cells only binds to class II MHCs and aids the reaction with the TCR, whereas CD8, only expressed in killer T cells, binds only to class I MHCs. The differentiation of T cells into killers and helpers is therefore directly coupled with this MHC restriction. "It was presumed that signaling from the TCR by MHC restriction may be involved in determining the fate of T cells as helper versus killer T cells, but the precise mechanism remains elusive," says Taniuchi.

To solve this mystery, research at the gene level is needed. "In multicellular organisms, all the somatic cells of the same individual carry exactly the same genome," says Taniuchi. "For example, somatic cells cannot differentiate into skin cells unless only the gene that is necessary for differentiation into skin cells is transcribed from all the genetic information embedded on the DNA. Transcription of genes is regulated by proteins known as transcription factors, which bind to the regulatory regions in the genes. Among these transcription factors, the one that turns on the switch of the developmental program for a particular type of cell is called the 'master transcription factor.'"

In 2005, a groundbreaking achievement concerning the determination of the differentiation fate of DP thymocytes was published by two research groups from the US. They discovered the master transcription factor for helper T cells, called Th-POK. It was found that in mice bearing artificially expressed Th-POK, all DP thymocytes differentiated into helper T cells, and that conversely in

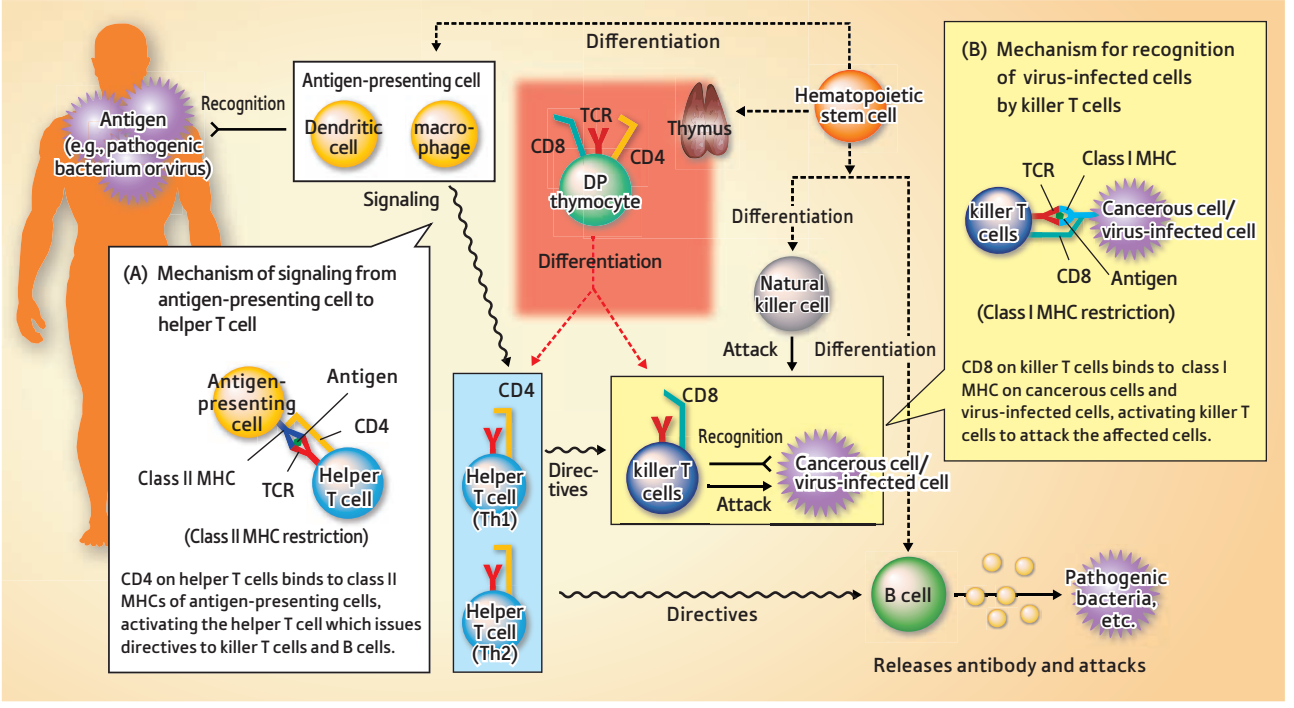

Figure 1: The immune system and the process of T-cell differentiation

Pathogenic bacteria and viruses are sensed by antigen-presenting cells, such as dendritic cells, and the signal is transmitted to helper T (Th) cells. Th1 cells direct killer T cells to attack virus-infected cells and cancerous cells, while Th2 cells direct B cells to produce antibodies to attack pathogens. Most immune cells differentiate from hematopoietic stem cells in the bone marrow. T cells, however, differentiate in the thymus, where T-cell progenitor cells from the bone marrow differentiate into DP thymocytes, which further differentiate into either helper or killer T cells. CD4, CD8 and TCR, are expressed on the surfaces of DP thymocytes; CD8 disappears with differentiation into helper T cells and CD4 disappears with differentiation into killer T cells.

mice deprived of the Th-POK function, all DP thymocytes differentiated into killer T cells. "Hence, the key to lineage fate determination for DP thymocytes depends on whether Th-POK is expressed or not. The next problem to solve is how the helper-lineage specific expression of the Th-POK gene is regulated."

Runx, the transcription factor for killer T-cell differentiation

In February 2008, Taniuchi and his colleagues succeeded in clarifying the mechanism behind the expression of Th-POK. Taking note of the transcription factor Runx, which had been assumed to play a key role in the differentiation of immune cells, he identified Runx to be a factor that binds to a 'transcriptional silencer' on the *Th-POK* gene to suppress its expression (Fig. 2). In the DNA sequence of a gene, there is a regulatory domain for controlling the gene's expression, in addition to a domain where the structure of the protein generated on the basis of the genetic information is written. A transcriptional silencer is a regulatory region that suppresses the expression of a gene when a particular transcription factor binds to it. "We discovered in 2002 that the transcription factor that binds to the silencer of the *CD4* gene is Runx. We then thought that Runx may also play some roles in determining the developmental fate of DP thymocytes."

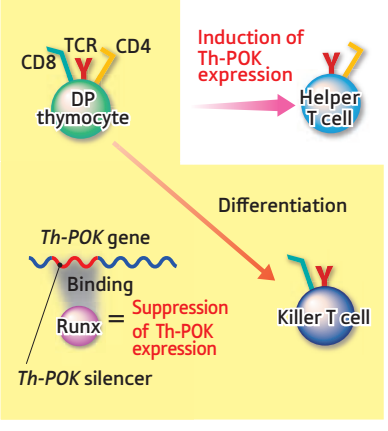

Figure 2: Suppression of Th-POK expression by Runx

For the differentiation of DP thymocytes into killer T cells, it is essential that Runx binds to the Th-POK silencer to suppress the expression of Th-POK. Without normal suppression of Th-POK expression, DP thymocytes do not differentiate into killer T cells. Differentiation into helper T cells requires the mechanism behind the suppression of Th-POK expression to be cancelled.

Taniuchi and his colleagues then bred mice lacking the Runx function in DP thymocytes, and carried out some experiments. "First, we examined the mice and found, surprisingly, that almost all killer T cells had disappeared. Due to the lack of Runx, even the cells that were otherwise destined to differentiate into killer T cells differentiated into helper T cells." Next, they examined whether the non-Runx mice exhibited any abnormality in the expression of Th-POK. They found that Th-POK, which would normally have negligible expression in DP thymocytes, was expressed in amounts more than 40 times greater in the Runx-deficient DP thymocytes. "This finding demonstrates that the expression of Th-POK is aggressively suppressed in DP thymocytes by Runx."

While working to elucidate the mechanism behind suppressing the expression of Th-POK by Runx, Taniuchi discovered a silencer region where Runx binds to the Th-POK gene. This Th-POK silencer proved to be indispensable for the differentiation of killer T cells because it suppresses the expression of Th-POK, the master transcription factor for helper T-cell differentiation.

"We bred mice deprived of the Th-POK silencer and found that Th-POK was abnormally expressed and all DP thymocytes differentiated into helper T cells. These experiments revealed the mechanism by which Runx binds to the Th-POK silencer to suppress the expression of Th-POK. Hence, Runx is essential for the differentiation of DP thymocytes into killer cells."

Increased expression of Th-POK gene essential for helper T-cell differentiation

After announcing their achievement that Runx suppresses the expression of the *Th-POK* gene, Taniuchi and his colleagues proceeded to elucidate the mechanism behind control of the expression of the *Th-POK* gene in further detail. In September 2008, they clarified the molecular mechanism by which *Th-POK* binds directly to the silencer of the *Th-POK* gene to increase its expression (Fig. 3).

"The first step we took was to extensively examine the level to which the *Th-POK* gene was expressed in the thymus. We found that the *Th-POK* gene, which is essential for differentiation into helper T cells, was expressed in the process of the initial differentiation of a small but measurable number of killer T cells. This means that even cells expressing the *Th-POK* gene retain their potential for differentiating into killer T cells," says Taniuchi. Next, they examined the differentiation potential of a population of cells that had a moderate reduction in the expression of CD8 in the midst of their differentiation process of DP thymocytes. "A reduction in the expression level of CD8 should be indicative of an ongoing process for differentiation into helper T cells. It was known, however, that the potential for differentiating into killer T cells still persists at the stage of a moderate reduction in the expression level. We conducted further experiments to quantify the actual residual potential of these cells for differentiating into killer T cells. As a result, about half were found to retain the potential for differentiating into killer T cells. This suggests that a further increase in the expression of the *Th-POK* gene is necessary for differentiation into helper T cells."

Next, Taniuchi bred mice deprived of one of two enhancer regions in the *Th-POK* gene. An enhancer region is a control region that promotes the expression of a gene—effectively the opposite number to the silencer region. "We discovered two enhancer regions in the *Th-POK* gene. We had presumed that the enhancer region located downstream was important for maintaining as well as amplifying the expression of the *Th-POK* gene, and when we bred mice actually deprived of this enhancer region, the enhancer region was found to be necessary for the elevation and maintenance of the expression of the *Th-POK* gene."

Further experimentation revealed that Th-POK promotes differentiation into helper T cells and cancels the potential for differentiation into killer T cells. Also, it appears that a sufficient elevation of the expression of the *Th-POK* gene

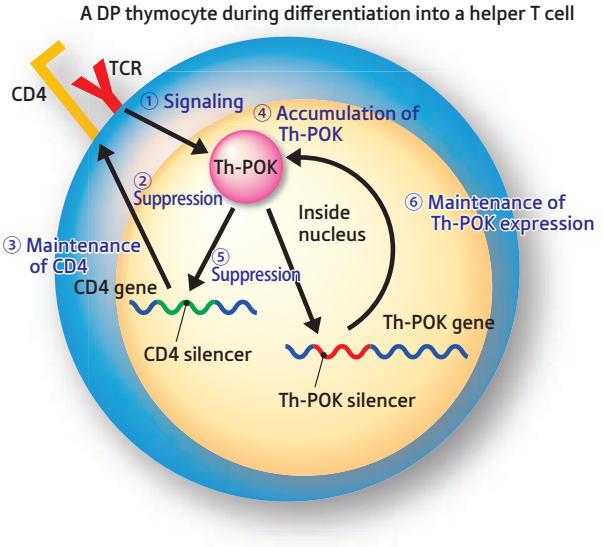

Figure 3: Mechanism by which silencer functioning is stopped to promote differentiation into helper T cells

is critical for the complete differentiation of helper T cells.

Mechanism behind the elevated expression of *Th-POK*

"Our investigations have shown that in the process of differentiation into killer T cells, the expressions of the *CD4* and *Th-POK* genes are repressed by Runx as it binds to the respective silencers, that is, as the silencers work. Therefore, conversely, it can be considered that in the process of differentiation into helper T cells, *CD4* and *Th-POK* can be expressed because these silencers are not working. We examined this mechanism using mice and found that the expression of CD4 was maintained as Th-POK suppressed the functioning of the CD4 silencer. It was also found that the expression of the *Th-POK* gene was maintained as Th-POK binds directly to the Th-POK silencer within its own gene to suppress the function of the silencer."

Figure 3 shows the mechanism that determines the differentiation of helper T cells, deduced from these experimental results. When a DP thymocyte receives an initial external signal for differentiation into a helper T cell via TCR, expression of the *Th-POK* gene is induced. The expressed Th-POK binds to the CD4 silencer to make the silencer no longer functional, allowing CD4 to be expressed on the cell surface. Signaling from the TCR continues and expression of the *Th-POK* gene persists, leading to the accumulation of Th-POK. Meanwhile, Th-POK binds not only to the CD4 silencer but also to the Th-POK silencer in its own gene to inactivate the silencer, which allows the expression of the *Th-POK* gene to continue and Th-POK to accumulate further. At this stage, the expression of *Th-POK* is maintained, even without signaling from the TCR, so that helper T cells are able to remain as they are even after leaving the thymus and entering the body.

"The process of differentiation into helper T cells is now understood up to this point," says Taniuchi. "However, how the expression of *Th-POK* is induced initially remains a mystery. I think probably the point resides in the cancellation of the function of the Th-POK silencer due to signaling from the TCR bound to class II MHC, although the mechanism is still unclear. I feel, however, that the main players are at last lined up as research has progressed with a focus on Th-POK."

Discussing biological evolution from the viewpoint of immunity

"The traces of biological evolution can be found in the mechanism behind the differentiation of immune cells," states Taniuchi. Although killer T cells work as attackers, their defensive measures are rudimentary. Meanwhile, helper T cells are highly functional in that they fight alongside the B cells as a team. "Hence, we have hypothesized that T cells initially emerged as killer T cells, and that helper T cells evolved later. If this is true, we can conclude that the immune system originally existed as an assembly of attackers, such as killer T cells and phagocytes, but later became unable to cope with the invaders merely through such one-on-one battles and hence created helper T cells to build an organized system for immunity."

The human body is cohabited by a diverse range of organisms, including enteric bacteria. The immune system ignores these, but selectively attacks those that must be eliminated. The immune system can therefore be described as the only 'sensory organ' for distinguishing among organisms in the body. "Clarifying the processes of emergence and evolution of the immune system will lead to an understanding of the biological strategies that have been developed by organisms to enable them to cohabit with the wide variety of other organisms on Earth," concludes Taniuchi. ■

ABOUT THE RESEARCHER

Ichiro Taniuchi was born in Osaka, Japan, in 1964. He graduated from the Graduate School of Medicine of Osaka University in 1989, and obtained his PhD in 1996 in Immunology from Kyushu University. After five years' postdoctoral training at the Skirball Institute of Biomolecular Medicine of the NYU Medical Center in New York, USA, he returned to Japan as an assistant professor at Kyushu University. He joined RIKEN in 2004 as a team leader in the RIKEN Research Center for Allergy and Immunology (RCAI), and has since acted as principal investigator of his own research group. His research focuses on transcriptional regulation of immune cell development.

Wonkyung Kang

Coordinator
Corporate and International Relations Division
Advanced Science Institute
(Ms Kang has been working for the Global Relations Office
as Deputy Manager since 1 November.)

Cultivating careers in corporate and international research

What do you do at RIKEN?

I work as a coordinator in the Corporate and International Relations Division of the Advanced Science Institute.

How and when did you join RIKEN?

After graduating from my university in South Korea, I came to Japan to study at graduate school. Once I obtained my Master's and PhD, I moved to Imperial College London where I carried out research on insect viruses. Around that time I found out that a key researcher in this field, the late Susumu Maeda, was setting up a research laboratory in RIKEN, which is one of the research centers that I had admired during my student days. RIKEN also seemed attractive because there was a strong possibility to further develop my research there. I got in contact with Maeda and eventually joined RIKEN through its Special Postdoctoral Researchers (SPDR) Program, became a researcher and stayed.

How was the transition to life at RIKEN?

Laboratories at RIKEN are not only equipped with a wide range of facilities, but their staff also provides fully comprehensive technical support. My first position at RIKEN was as a researcher in the SPDR Program, which came with research funding, so I was able to focus on my research free of worry.

Please tell us about your research or other work at RIKEN.

My job currently involves providing support to research activities while making use of researchers' backgrounds. The merits of the Advanced Science Institute as a multidisciplinary laboratory for natural sciences are utilized to promote cooperation not only within RIKEN, but also to drive various kinds of research that goes beyond the realm of regular collaborative research. I also help with such collaborative activities, but my specific responsibilities are multifold because I also coordinate with foreign institutions, prepare written agreements, coordinate with various in-house departments and support personnel exchange.

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

I think it is the fact that I have taken a step forward from being a student to a researcher. When I worked as a postdoc in England, my only job was to conduct experiments, and so I somehow felt that I could rely on my laboratory boss to look after everything else. However, various things have happened since I started to work in my current post, such as my boss suddenly passing away, which pushed me to do things that researchers normally do, such as completing theses, peer reviewing, obtaining research funds, and teaching students. This has helped me improve as a researcher.

What is the best thing about working at RIKEN?

I have been able to secure a great environment where I can devote myself to research. There is an increased range and depth in research here, not only because of excellent personnel and facilities as well as ample research funding, but also because of the complete range of staff and departments that support research. RIKEN's reputation as a well-known research institute is also a major advantage when carrying out collaborative research in- and out-of-house. It is a huge benefit for researchers, particularly female researchers who have children, that RIKEN is very understanding about maternity leave and childcare, and there is a generous support system for working mothers.

CONTACT INFORMATION

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An Expert Panel Discusses the Future of RIKEN

Chair of the RIKEN Advisory Council (RAC), Rita R. Colwell (third from left), leads a series of meetings to discuss the future direction of RIKEN.

A high-level panel of experts from around the world gathered in Tokyo for a three-day series of meetings on the future of RIKEN, held from 26 October. This body, known as the RIKEN Advisory Council (RAC), was established in 1993 with the goal of transforming RIKEN into an internationalized and world-class research institute. At this meeting—the eighth so far—RIKEN was evaluated by a group of 18 people, including six Japanese, headed by Rita R. Colwell, Distinguished University Professor of University of Maryland and former director of the National Science Foundation in the United States.

Executive Director Maki Kawai (left) and RIKEN President Ryoji Noyori (right) at the RAC meeting in Tokyo.

The RAC meeting was the culmination of a process which began earlier this year with evaluations of the individual RIKEN research centers as well as an evaluation of the administrative structure by the Administrative Advisory Council, a newly established committee of experts. RIKEN is in the fourth year of its current five-year plan, so the RAC members were charged with evaluating its responses to the previous recommendations, made in 2009, and to offer recommendations for the framework for the next five-year plan, which will begin in fiscal 2013.

The meeting began with presentations from RIKEN's top management on the direction and current status of RIKEN's operations. The center directors then gave presentations on the activities of their own centers and institutes, their advisory council meetings, and their future plans. The members of the RAC then met in a series of private sessions to formulate a set of initial recommendations.

On the final day, the chair presented an initial set of recommendations to RIKEN President Ryoji Noyori. Due to be presented in about two months' time, the final recommendations will provide a framework for the future operations of RIKEN. ■

David Baltimore appointed as RIKEN Honorary Fellow

David Baltimore, the President Emeritus and Millikan Professor of Biology at the California Institute of Technology (Caltech), was inaugurated as a RIKEN Honorary fellow on 6 October 2011 at a ceremony held at the RIKEN Yokohama Institute. The fellowship was established to promote RIKEN's activities and internationalization, and is conferred upon persons without a RIKEN affiliation who have achieved eminence on a global scale. RIKEN has previously conferred the title upon Leo Esaki, winner of the 1973 Nobel Prize in Physics, former Malaysian Prime Minister Mahathir bin Mohamad, and Yuan T. Lee, winner of the 1986 Nobel Prize in Chemistry.

Baltimore is a 1975 Nobel laureate in Physiology or Medicine for his discovery, at the age of 37, of the enzyme responsible for reverse transcription, through which viruses insert their RNA into a cell's DNA. This discovery was the key to understanding the reproduction of retroviruses, such as HIV. From 1997 to 2006, he worked to promote advances in scientific research as President of Caltech. In 1999, he was awarded the National Medal of Science by President Bill Clinton.

Over 200 participants attended the ceremony and listened to Baltimore's special lecture, "Control of the Inflammatory Process," and afterwards engaged in active discussions. Baltimore also toured the RIKEN Yokohama Institute and Wako campus, where he praised RIKEN's cutting-edge research facilities and high-quality research. His wife, Alice Huang, who is President of the American Association for the Advancement of Science, also visited Japan, and presented the lecture "Diversity and Diplomacy in Science: A Necessity for Future Success" at Sophia University in Tokyo. ■

Nobel laureate and President Emeritus of Caltech, David Baltimore (center), accompanied by his wife, the President of the American Association for the Advancement of Science, Alice Huang (left), is appointed RIKEN Honorary Fellow by RIKEN President Ryoji Noyori (right).

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