A protein balancing act
RIKEN PLACES: Introducing the facilities that drive RIKEN’s world-class research

The Nishina Center for Accelerator-Based Science is shedding light on the creation of the Universe.
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When the French emperor Napoleon III offered funds for a new butter substitute to feed his army and subjects in 1866, he unknowingly spurred development of the margarine industry, now valued in the billions of dollars. The first margarine consisted of churned beef tallow and milk, but French and German chemists soon found a way to turn inexpensive liquid vegetable oils into solid saturated fats through a process called catalytic alkene hydrogenation—a reaction that turns carbon double bonds into single bonds with the help of hydrogen gas and a metal catalyst (Fig. 1).

Such hydrogenation processes also have wide applications beyond the food industry and are now one of the key technologies used in petrochemical refining, pharmaceutical synthesis and biofuel production.

The Achilles’ heel of hydrogenation chemistry remains its reliance on expensive platinum-series catalysts. The limited supply of these metals and their volatile pricing, as well as increasing awareness of their possible environmental toxicity, has prompted an intense search for alternative ways to add hydrogen to unsaturated chemical bonds. Yoichi Yamada and Yasuhiro Uozumi from the RIKEN Center for Sustainable Resource Science, in collaboration with researchers from McGill University in Canada and Japan’s Institute for Molecular Science, have now developed an efficient iron-based catalyst that promises to fundamentally change industrial hydrogenation processes.

Getting the rust out
Iron (Fe) is the second most abundant metal in the Earth’s crust. It has an essential role in a multitude of biological processes and is used on large scales as a catalyst for ammonia fertilizer production. Iron can also catalyze hydrogenation reactions—but only under the right conditions. Iron catalysts require far higher gas pressures in comparison to the conventional metal catalysts, notes Uozumi, and the iron must remain in its pure metallic Fe(0) state. In fact, any contact with water or air during hydrogenation causes the iron catalyst to oxidize into iron oxide, which is not catalytic.

Because such conditions run counter to the demands of large-scale food manufacturing and other industries, iron has generally been ruled out as a hydrogenation catalyst. However, new research into iron-based nanoparticles has prompted chemists to take a second look at the potential of iron catalysts and their economic and environmental advantages. Such nanoparticles expose a significantly higher proportion of the active iron surfaces to chemical reactants than other structures and can also be attached to permanent substrates to boost catalyst recycling. Unfortunately, iron nanoparticles remain highly susceptible to oxidation and corrosion.

**Figure 1:** Example of a simple metal-catalyzed alkene hydrogenation reaction. When an alkene group bonds to a suitable metal surface, the carbon double bond (C=C) becomes a single bond (C–C) and two hydrogen atoms from the catalyst’s surface are transferred to the group to create a saturated alkane molecule.
can even become explosive, necessitating special handling.

Yamada and Uozumi are specialists in the preparation of polymer resin coatings that allow metal particles to perform as catalysts in water. The pair met Chao-Jun Li and Audrey Moores, both experts in nanoscale catalysts, at the 2012 RIKEN-McGill University Scientific Workshop, and the group decided to combine their expertise to tackle the challenge of stabilizing iron nanoparticles with polymers.

According to Uozumi, optimal polymers for iron nanoparticles must be amphiphilic, containing both water-repelling and attracting components to protect the metal and at the same time ensure compatibility with aqueous mixtures. To achieve this goal, the researchers developed a synthetic procedure using polystyrene beads just 100 micrometers in diameter coated with polyethylene glycol. Heating an iron precursor in the presence of polystyrene beads coated with polyethylene glycol, which inhibits oxidation (Fig. 2), forms nanoparticles coated with polymers. This greatly lowers the risks associated with high-pressure reactions by exposing unsaturated molecules to high catalyst concentrations.

By studying a model organic hydrogenation reaction using their microreactor, the researchers discovered that the iron–polymer catalysts showed extraordinary tolerance toward water-based solvents. While other iron nanoparticles undergo significant activity loss in a 50:50 ethanol–water mixture, the hybrid iron–polymer materials achieved perfectly efficient hydrogenation in 99% pure water. Besides being one of the ‘greenest’ solvents for chemical transformations, water plays a critical role in enhancing the safety of iron nanoparticle catalysis by suppressing the possibility of explosive accidents, explains Uozumi.

The flow chemistry system also allowed the team to study multiple organic hydrogenation processes, which revealed that the new catalyst preferentially hydrogenates carbon bonds over other types of unsaturated chemical units. This behavior, which promises to be useful for industry, was put to the test by scaling up reactions to the multi-gram reactant conditions commonly seen in large reactors. The polymer-protected nanoparticles performed admirably and showed only a slight decrease in activity after many hours of use.

**Not your average cup of tea**

Intriguingly, the researchers also discovered that polyphenol compounds extracted from a cup of brewed black tea could also generate hybrid iron–polymer nanoparticles at room temperature. Although not as efficient as the thermal decomposition method, the team notes that small steps toward lowering costs and providing safer alternatives to conventional processes all help to make chemistry more sustainable. Currently, the researchers are investigating other reactions and can also overcome their dependence on costly metals with the help of amphiphilic polymers.

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**ABOUT THE RESEARCHER**

Yasuhiro Uozumi graduated from Hokkaido University in 1984 and received his Doctor of Pharmacy degree from the same university. In 1994, he joined Columbia University in the United States as a research associate but soon returned to Japan to take up the position of lecturer at Kyoto University. Since 2000, Uozumi has served as professor at the Institute for Molecular Science and The Graduate University for Advanced Studies. In 2007, Uozumi became team leader at RIKEN, first heading the former Chemical Process Engineering Team and later, in 2010, the Nanocatalysis Research Team at the RIKEN Advanced Science Institute in Wako, which has since been reorganized into new centers. His current research explores novel catalytic reactions to develop chemical processes that are safe, simple and green.
Enzymes called protein kinases modulate cellular activities in virtually every organism. They switch other proteins off or on by tacking on phosphate chemical groups—a process known as phosphorylation—to regulate the activity of downstream signaling pathways. Abscisic acid (ABA), a critical regulator hormone related to plant growth and survival, is one such protein that is modulated by protein kinases. Kazuo Shinozaki from the RIKEN Center for Sustainable Resource Science and colleagues have now obtained valuable insights into a family of kinases that link ABA signaling with another environmental stress pathway.

Shinozaki’s group has spent over a decade studying protein kinases that modulate ABA function. “We have been interested in ABA signal transduction pathways in order to understand plant responses to environmental stressors such as drought, high salinity, cold and heat,” says Shinozaki.

The researchers previously found that SNF1-related protein kinase 2 (SnRK2) proteins help switch on various downstream effectors of ABA, and identified a subset of ‘subclass III SnRK2s’ that also helps plants respond to dehydration via a distinct signaling pathway. In their most recent research, in collaboration with Taishi Umezawa from the Tokyo University of Agriculture and Technology, Shinozaki’s group devised a series of experiments to identify downstream targets of these kinases that are involved in mediating the response to osmotic stress or ABA signaling.

Shinozaki and colleagues identified phosphorylated proteins in thale cress plants treated with ABA or subjected to dehydration and compared the results with those for the srk2dei mutant strain, which is deficient in three key subclass III SnRK2 proteins. Their analysis revealed numerous differentially modified targets, with remarkably little overlap between the dehydrated and ABA-treated targets.

By analyzing the amino acid sequences that undergo modification by SnRK2 proteins under these conditions, the researchers gained valuable new insights into plant cellular signaling machinery. For example, they identified a link between the SnRK2 pathway and the mitogen-activated protein kinases, a family of signaling proteins with a critical role in virtually every cellular function. They also detected a novel protein termed SnRK2 substrate 1, which inhibits ABA-mediated growth restriction (Fig. 1) but is apparently unaffected by dehydration-induced signals.

Having demonstrated the power of this approach, Shinozaki hopes to delve deeper into how plants cope with environmental challenges—information that could assist the development of crops that can withstand drought and other crises. “We are interested in ‘osmosensors’ that can detect water deficit conditions,” he says, “and we will continue to analyze the roles of SnRK2 in osmotic-stress signaling.”

Figure 1: In wild-type plants (upper left, lower right), the SnRK2 substrate 1 protein inhibits abscisic acid signaling. In mutants lacking SnRK2 substrate 1 (upper right, lower left), the growth-limiting effects of abscisic acid are greatly amplified.

Lab-on-a-chip devices are microfluidic cells that incorporate pipes, reaction vessels, valves and a host of other implements typically found in laboratories. These components are typically carved into a flat plastic plate smaller than a credit card to enable efficient processing of microliter-volume samples. The use of plastics, however, has several drawbacks that could be remedied by using glass. Unfortunately, glass chips have proved difficult to fabricate due to their fragility. Yo Tanaka from the RIKEN Quantitative Biology Center has now developed a reliable and durable system for incorporating glass microfluidics into lab-on-a-chip devices.

Most lab-on-a-chip devices are formed from polydimethylsiloxane (PDMS), an inexpensive plastic that is easy to pattern with microfluidic elements. Valves, in particular, take advantage of the plastic’s elasticity—simply applying or releasing pressure can close or open a channel in the device, controlling fluid flow. Plastics, however, have several disadvantages, including degradation when exposed to reactive chemicals and a tendency to adsorb sample molecules before they can be analyzed. They can also interfere with analysis techniques that rely on shining a light through the device due to their imperfect transparency. Glass is an attractive alternative because it is chemically resistant, transparent to light and also capable of withstanding higher fluid pressures than PDMS. Producing flexible and durable glass valves, however, has proved difficult.

To allow glass to be used in these devices, Tanaka developed a Teflon frame to hold an ultrathin sheet of glass so that it could be handled without breaking and incorporated the frame into an all-glass lab-on-a-chip (Fig. 1).

Next, Tanaka used hydrogen fluoride to etch channels and chambers into a pair of glass slides, and covered these chambers with ultrathin glass sheets in a way that allowed fluid to be prevented from passing through the chamber by simply pressing down on the glass cover. He then fused the glass sheets together by heating them at 750 °C.

After trying various thicknesses of ultrathin glass sheets, Tanaka found that a 10 micrometer-thick glass film was ideal: strong enough to withstand more than 100 depressions yet able to deform by up to 126 micrometers—enough to completely close the valve. Tests using water containing small fluorescent polystyrene beads demonstrated that closing the valve using this method blocked fluid flow within 0.12 seconds.

Tanaka now plans to develop his all-glass device for applications such as highly sensitive biochemical analyses and cell studies.

Musicologists, psychologists and philosophers have long puzzled over the fact that, despite sadness being an emotion normally avoided, people often voluntarily listen to sad music. New research from Ai Kawakami, Kazuo Okanoya and colleagues from the Emotional Information Joint Research Laboratory at the RIKEN Brain Science Institute shows that we listen to sad music because the emotion it induces is vicarious and not born of events in our own daily life.

Kawakami and her fellow researchers thought that the ability for people to feel pleasure when listening to music they perceive as sad might have something to do with a difference between the perception of emotion in the music and the emotion it actually evoked. In earlier work, the group tested the response to dissonance and music in a minor key—musical structures that are associated with sadness. Although the musical stimuli they used were short—only one to four bars—they found that musicians recognised the music as sad and often found pleasure in it.

In this study, the researchers broadened their work by using existing music and testing two hypotheses—that the emotion felt did not necessarily correspond to perceived emotion, and that musicians would gain more pleasure from sad music than non-musicians. The researchers asked 17 musicians and 27 non-musicians to listen to one of 3 musical excerpts in a major and minor key. The participants then rated how they perceived the music and how it made them feel with respect to 62 emotion-laden words.

“The results revealed an ambivalent emotion when people listened to sad music. This emotional response did not correlate with musical training,” Kawakami says. “While listeners, including musicians and non-specialists, perceive the sad music as sad, their own emotional state at the time was less tragic.”

After considering the possible reasons for such emotional ambivalence induced by sad music, the researchers concluded that a new model was required to examine the emotions evoked by music. The model they proposed includes the possibility that what listeners experience when listening to music are vicarious emotions.

“On this basis we propose that while sadness experienced in daily life is a negative emotion, the sadness experienced by listening to music can be a positive emotion,” says Kawakami. The researchers further suggest that this capacity to experience positive emotions from negative perceived emotions could help people deal with negative emotions in daily life.

Collisions of atomic and subatomic particles at very high energies reveal important properties about the beginning of the Universe and the atomic forces, and how fundamental particles are formed and react with each other. Adam Bzdak from the RIKEN BNL Research Center and colleague Vladimir Skokov from Brookhaven National Laboratory in the United States have now proposed a scheme that allows for a better understanding of how light and subatomic particles react with each other during such high-energy collisions.

At the very early stages of the Universe, there were no atoms: energies were so high that atoms would have been torn apart. Instead, there was a mix of subatomic particles such as gluons and quarks. These make up the protons and neutrons inside atomic cores, but at very high energies they form a hot cloud known as a quark–gluon plasma. These plasmas can also be produced artificially by smashing heavy atoms together as is currently being performed by the PHENIX Collaboration at the Relativistic Heavy Ion Collider at Brookhaven.

In these experiments, it has been observed that light (photons) emanating from the collision zone varies in intensity depending on the direction of light emission (Fig. 1). This uneven distribution of photons is similar to the pattern expected for a quark–gluon plasma, which has surprised scientists. “Photons do not interact with the created matter and cannot be sensitive to the shape of the fireball,” says Bzdak. “This is a clear paradox and so far there is no compelling explanation. Clearly we do not understand something very basic.”

Although several theories, such as the role of magnetic fields, have been proposed that could explain this effect, a clear explanation has not been possible. Bzdak and Skokov have now proposed a scheme that aims to identify whether magnetic fields are indeed responsible or whether photons are simply produced non-uniformly during the collisions. Their theoretical study compares the emission patterns of photons for different shapes of the quark–gluon plasma and different numbers of particles creating magnetic fields. If the emission pattern of the photons follows that of the different quark–gluon plasma, it would verify the direct connection between the two phenomena.

The experimental verification of their proposal will be the next step, says Bzdak. “I believe our job is done and now the ball is in the experimentalists’ court. The implementation of our scheme is quite straightforward and is currently being studied by the PHENIX Collaboration.”

Lithium-ion batteries are now found everywhere in devices such as cellular phones and laptop computers, where they perform well. In automotive applications, however, engineers face the challenge of squeezing enough lithium-ion batteries into a vehicle to provide the desired power and range without introducing storage and weight issues. Hye Ryung Byon, Yu Zhao and Lina Wang from the RIKEN Byon Initiative Research Unit have now developed a lithium-iodine battery system with twice the energy density of conventional lithium-ion batteries.

Byon’s team is involved in alternative energy research and, specifically, improving the performance of lithium-based battery technologies. In their research, they turned to an ‘aqueous’ system in which the organic electrolyte in conventional lithium-ion cells is replaced with water. Such aqueous lithium battery technologies have gained attention among alternative energy researchers because of their greatly reduced fire risk and environmental hazard. Aqueous solutions also have other advantages, which include an inherently high ionic conductivity.

For their battery system, the researchers investigated an ‘aqueous cathode’ configuration (Fig. 1), which accelerates reduction and oxidation reactions to improve battery performance. Finding suitable reagents for the aqueous cathode, however, proved to be a tricky proposition. According to Byon, water solubility is the most important criterion for screening new materials, since this parameter determines the battery’s energy density. Furthermore, the redox reaction has to take place in a restricted voltage range in order to avoid water electrolysis. An extensive search led the researchers to produce the first-ever lithium battery involving aqueous iodine—an element with high water solubility and a pair of ions, known as the triiodide/iodide redox couple, that readily undergo aqueous electrochemical reactions.

The team constructed a prototype aqueous cathode device and found the energy density to be nearly double that of a conventional lithium-ion battery, thanks to the high solubility of the triiodide/iodide ions. Their battery had high and near-ideal power storage capacities and could be successfully recharged hundreds of times, avoiding a problem that plagues other alternative high-energy-density lithium-ion batteries. Microscopy analysis revealed that the cathode collector remained untouched after 100 charge/discharge cycles, with no observable corrosion or precipitate formation.

Byon and colleagues now plan to develop a three-dimensional microstructured current collector that could enhance the diffusion-controlled triiodide/iodide process and accelerate charge and discharge. They are also seeking to raise energy densities even further by using a flowing-electrode configuration that stores aqueous ‘fuel’ in an external reservoir—a modification that should make this low-cost heavy metal-free design more amenable to electric vehicle specifications.

A neural code for navigation

Neurons in the rat brain use a preexisting set of firing sequences to encode future navigational experiences

Specialized neurons called place cells, located in the hippocampus region of the brain, fire when an animal is in a particular location in its environment, and it is the linear sequence of their firing that encodes in the brain movement trajectories from one location to another. Building on previous work, George Dragoi and Susumu Tonegawa from the RIKEN–MIT Center for Neural Circuit Genetics have now shown that place cells have a preexisting inventory of firing sequences that they can use to encode multiple novel routes of exploration.

Specific sequences of place cells are known to encode spatial experiences, but it has been debated whether such sequences are formed during a new experience or preformed and adapted to specific experiences when required. Dragoi and Tonegawa recently showed that ‘future’ place cells fire in sequence while the animal is asleep, prior to experiencing a novel environment, and that animals use this preexisting neuronal firing pattern to rapidly learn how to navigate their surroundings.

To confirm and investigate this mechanism further, the researchers first recorded the neuronal activity of place cells in rats during one hour of sleep. Next, they monitored this activity during movement along a track that the rat had not previously explored, and later recorded it during movement along the same track with two additional lengths separated by right-angle turns. They then correlated the temporal pattern of place cell activity recorded during sleep with the spatial pattern of activity recorded while the animals were freely exploring the longer track.

The researchers found that the sequences of place cell activity were unique for each of the three lengths of the track and matched those recorded during sleep (Fig. 1). “We had observed the same sequences as independent clusters of correlated temporal sequences during the preceding sleep period,” explains Dragoi.

The results suggest that rapid encoding of particular trajectories within novel environments is achieved during exploration by selecting from a set of preexisting temporal sequences that fired during sleep. In other words, hippocampal place cells appear to be prearranged into sets of sequential firing cells that can be adapted rapidly to encode for multiple spatial trajectories that the animal could undertake in its surroundings. Based on their data, Dragoi and Tonegawa predict that the sets of hippocampal place cells could encode at least 15 unique future spatial experiences. In addition, their findings could explain the role that the hippocampus plays in humans in imagining future encounters within our own complex environment.

Early in development, the embryo establishes the various axes that determine the symmetry of the mature animal. For example, the patterning of dorsal and ventral surfaces governs formation of the organism’s back and belly. There are developmental mechanisms that regulate this patterning to ensure that the various body parts develop in proportion to each other but exactly how these mechanisms function remains uncertain. Yoshiki Sasai, Hidehiko Inomata and colleagues from the RIKEN Center for Developmental Biology have now clarified how dorsal-ventral (DV) scaling is maintained in the African clawed frog, *Xenopus laevis*.

A cluster of cells known as Spemann’s organizer establishes the ‘dorsality’ of the embryo by secreting the protein Chordin, which inhibits signals that would otherwise initiate development of ventral tissues. The effect of Chordin is known to be tightly constrained to the dorsal region. “If a *Xenopus* embryo is bisected into a dorsal and ventral half, the dorsal half will still give rise to a well-proportioned, half-size embryo,” explains Inomata. However, the mechanism responsible for localizing the effect of Chordin was previously unknown.

The researchers conducted a series of experiments to understand how *Xenopus* establishes this DV boundary. Chordin is naturally degraded by protease enzymes distributed throughout the early embryo. These proteases are selectively inhibited by another protein called Sizzled, and the researchers found that Chordin’s reach is determined by the range at which Sizzled can block protease activity.

Sizzled is primarily produced at the ventral pole of the embryo via the same ‘ventralizing’ signal that gets switched off by Chordin. This creates a critical feedback loop: Chordin only acts in cells where Sizzled is present, but Sizzled is only produced in cells where Chordin levels are low. The DV boundary is thus established in those cells where Chordin prevents continued production of Sizzled and where low levels of Sizzled prevent further diffusion of Chordin (Fig. 1). Regardless of embryo size, this boundary reliably scales with the distance of the organizer from the ventral pole. “Our results indicate that the dynamic state of Sizzled protein accumulation conveys body size information for scaling,” says Inomata.

While these findings resolve an important developmental puzzle, the frog embryo lacks elements of complexity found in other vertebrate species. “During early *Xenopus* development, the size of the embryo is nearly unchanged, but in many animals the embryo becomes larger and dynamically changes size,” says Inomata. “We’d like to examine whether our scaling model is applicable to this type of growing developmental field.”

Lab-on-a-chip technology gets a flexible upgrade

Electrically responsive polymers help make miniature systems for biomedical analysis even more compact

Microfluidic devices move liquids through tiny, hair-sized pathways carved into glass slides and have distinct advantages over traditional laboratories when it comes to medical diagnostics. At these reduced scales, fluid transport is enhanced by factors such as diffusion and high surface-to-volume ratios, making testing procedures much faster. By constructing parallel arrays of microfluidic pathways, researchers are working to produce lab-on-a-chip technologies that allow multiple biological tests to be performed using just a drop of blood or urine. In a development that promises to make lab-on-a-chip devices more portable and economic to construct, Yo Tanaka from the RIKEN Quantitative Biology Center and colleagues have now produced a new type of microfluidic control valve that takes up significantly less space on a microchip than existing approaches.

In the majority of today’s microfluidic devices, silicone pneumatic valves are used to manipulate liquid samples. Pneumatic valves, however, require noisy compressors and complicated air channel systems, which are often too bulky for practical lab-on-a-chip applications. Piezoelectric actuators—inorganic crystals that change shape when electrically stimulated—are feasible alternatives, but while piezoelectric materials are less obtrusive than pressurized air technology, they are excessively large when compared to the size of the microchip itself.

Tanaka and his colleagues instead investigated the remarkable properties of electroactive polymers. These materials are rubber-like organic compounds that expand and contract when exposed to an electric current. As electroactive polymers can exhibit large mechanical strain forces at small scales, the team deduced that creating membranes incorporating these materials could be a promising way to miniaturize microfluidic control valves.

After experimenting with many valve shapes, the researchers settled on a micrometer-sized, dome-shaped polymer diaphragm sandwiched between soft electrode sheets (Fig. 1). They tested its ability to stop flow by fabricating it on top of a small hole drilled into a microfluidic channel. By monitoring fluorescent polystyrene tracking beads using high-speed video cameras, the team saw that stimulating the electroactive polymer caused the diaphragm to expand and close off the microchannel at sub-second speeds, nearly identical to the response time of piezoelectric actuators but with an order-of-magnitude smaller form factor. Furthermore, the polymer structure strongly resisted leaks because of its resilient structure.

The researchers note that the improved size-scaling of their valve system should prove more efficient for the sorting of biological cells than current fluorescent technology. Other more mobile applications may also be on the horizon. “Many portable devices for personal diagnosis, environmental analysis, or fuel cells could benefit from these miniaturized valves,” says Tanaka.

Combining imaging forces to understand disease

Simultaneous imaging of bio-metal and molecular radiotracers in the body is now possible using a gamma-ray emission camera

Trace metals in living organisms play a variety of important roles in many processes, including gene expression and the development of diseases such as cancer. It is possible to image the behavior of these ‘bio-metals’ by following radioactive tracers injected into the body. However, analyzing the corresponding expression of metal-associated molecules in the body using technologies like positron emission tomography (PET) has so far been impossible due to differences in the kinds of radiotracers used by these techniques. Shuichi Enomoto, Shinji Motomura and colleagues at the RIKEN Center for Life Science Technologies have now developed a way to simultaneously image a wide range of bio-metal and PET radiotracers in the body through the use of a semiconductor Compton camera called GREI-II.

“Our first gamma-ray emission imaging camera was developed for the imaging of multiple tracers and enabled us to track bio-metals in living organisms,” explains Motomura. “We used it in the detailed exploration of molecules, which led us to the idea of integrating bio-metal analysis and PET technology.”

The gamma-ray emission imaging (GREI) system works by gathering information about gamma rays emitted by the radiotracers through measurement of the changes in direction and energy of the gamma-ray photons as they hit radiation sensors. By tracing the trails produced by this ‘Compton scattering’, the source of gamma rays can be located, and the different radioisotopes in each tracer can be distinguished by the gamma-ray wavelength (Fig.1).

“The half-lives of the molecular imaging agents for PET are very short,” explains Motomura. “To realize the simultaneous and efficient imaging of the PET agents and bio-metal tracers, we had to improve the sensitivity of the camera and the speed of data processing.”

Enomoto’s team tested their GREI-II technology by collecting images from cancer tumors in live mice using copper-64 and zinc-65 radiotracers. Results showed that copper was found in abundance at tumor sites, whereas zinc collected in greater quantities in the liver, pancreas and kidneys. Comparisons with images from the first GREI system showed that the new system had less background noise and greater clarity. GREI-II is also capable of imaging at more than ten times the speed.

“The GREI-II images cannot be obtained by other imaging systems because their gamma-ray energies are high and the energy range is too wide,” explains Motomura. “We expect GREI will be able to help us understand the intricate mechanisms of many diseases in the future.”

Proteins are typically encoded by linear strands of messenger RNA (mRNA). These mRNA molecules are translated into polypeptide chains by ribosomes, with each ribosomal read-through of the mRNA generating a single, discrete copy of the encoded protein. New work from Hiroshi Abe and Yoshihiro Ito of the RIKEN Nano Medical Engineering Laboratory now demonstrates the potential to turn a closed loop of mRNA into a steady assembly line for continuous protein production.

Scientists routinely employ a technique called ‘rolling-circle amplification’ to generate many continuous copies of a single circular DNA template, and Abe had become interested in devising an equivalent approach for protein production. “I came up with the idea of using circular RNAs for translation,” he says. Other researchers have attempted this in the past in the bacterium *Escherichia coli* but came to the conclusion that the process was considerably less efficient than for linear RNA templates.

Abe and Ito therefore set out to systematically test different constructs in order to identify factors that could boost or undermine the productivity of circular RNAs in *E. coli* cellular extracts. A typical protein-coding RNA begins with a ribosomal binding sequence and a ‘start codon’ and ends with a ‘stop codon’ that causes the ribosome to detach from the finished polypeptide chain. The researchers built four different circular RNAs of different lengths, featuring ‘infinite’ coding regions that lack stop codons, and found that all but the smallest of these could successfully generate continuous chains of tandem protein repeats (Fig. 1).

Subsequent experiments permitted a direct comparison of productivity between a 126-nucleotide circular RNA and its linear equivalent. Abe was surprised to observe that the circular molecule exhibited a far superior performance, generating 100-fold more protein than the linear template. He hypothesizes that this efficiency boost arises from the fact that linear templates require ribosome ‘recycling’, in which ribosomes released at the end of translation must subsequently seek out new binding sites, while closed templates keep the same ribosome locked in on a continuous circular path. “We have proved that the rate-limiting step of protein translation is ribosome turnover,” says Abe.

Circular RNAs encoding fluorescent proteins could offer useful ‘tags’ for labeling molecular targets in biochemical assays, but Abe notes that this rolling-circle protein production strategy might also provide an effective tool for churning out long strands of protein for biotechnology or materials applications. “It could be used for the production of spider silk, collagen or other proteins with repeated sequences,” he says.

**Figure 1**: As the ribosome (green and blue) translates the circular RNA template (red), it generates a continuous string of repeated protein sequences (purple).

Songbirds and humans both learn to vocalize by imitation and produce their respective sounds in much the same way, by arranging syllables into sequences. Very little is known, however, about how this ability arises during development. Kazuo Okanoya from the Laboratory for Biolinguistics at the RIKEN Brain Science Institute, as part of a collaboration with a research team from the City University of New York, have now shown that humans and songbirds also acquire their vocalization skills in the same way—by learning new combinations of syllables in a stepwise manner.

The research team, led by Ofer Tchernichovski and Dina Lipkind from the City University of New York, first compared the development of sound combinations in zebra finches through a series of experiments involving song training using slightly altered songs. In these experiments, the subject would hear and learn one song, then the original song would be altered slightly so that the birds would have to rearrange the order of syllables in the song or insert an entirely new syllable. The researchers then examined natural song development in Bengalese finches (Fig. 1) and ‘babbling’ development in humans.

Of the 17 zebra finches tested, 8 learned the new song successfully, on average after 17 days of training. The birds stopped singing the original song either at the same time as, or a few days before, starting to sing the new song.

Analysis of the songs revealed that the transition between the original and new songs occurred gradually, with new pairs of syllables being added in a series of intermediate steps. Birds would sing the intermediate songs several thousands of times, with an average gap of about six days between the appearance of each syllable change. Once the transition was complete, the birds suddenly switched to the new song and never sang the original song again. Birds that failed to completely adopt the new song learned in the same way, but ceased the transition to the new song prematurely, resulting in a song somewhere between the original song and the new song.

The researchers also found that Bengalese finches learn to sing their more complex songs in a similar manner and that the babbling of human babies develops in the same way too, with newly learned syllables first being repeated and then connected to other new syllables in gradual steps.

“We can now study neural mechanisms for sequencing in birds and apply the results to understand human speech production,” says Okanoya. “This might help to understand and provide cures for certain speech disorders.”

Based at the RIKEN Wako campus, the Nishina Center for Accelerator-Based Science (RNC) is a leading international center for nuclear physics research. Inaugurated in 2006 and continuing an 80-year history of ground-breaking accelerator-based research at RIKEN, the center is named after Yoshio Nishina who constructed Japan’s first cyclotron in 1937. The RNC is renowned for its study of the nuclei of heavy elements and how they first formed and also conducts pioneering accelerator-based research by applying radioisotopes to a range of fields—including agriculture and medicine.

**Supersized accelerator**
The RNC’s key facility is the Radioactive Isotope Beam Factory (RIBF), a world-leading heavy-ion research facility. The RIBF is a multistage accelerator complex whose final-stage accelerator, the Superconducting Ring Cyclotron, measures 18 meters in diameter and weighs 8,300 tons, making it the largest of its kind in existence. The integration of the RIBF’s five cyclotrons and superconducting radioactive isotope beam separator allows the facility to generate some of the most intense ion beams in the world. Through the utilization of superconductivity, the RIBF is considerably more energy-efficient than similar facilities that only use conventional accelerators.

Data collected at the RIBF was instrumental in achieving a major breakthrough at the RNC in 2012—the unambiguous identification of element 113. Early signs of the element were detected in 2004 and 2005 by researchers using the RIKEN Heavy-ion Linac, a linear particle accelerator, although a further seven years of painstaking experimentation at the RIBF were required to obtain a set of conclusive data. Currently under review by an international committee, the claim—if accepted—would mark the first time a predicted element has been synthesized by a country in Asia.

By using heavy-ion irradiation to induce mutations in flowers, rice and wheat, the RNC has gained international attention for creating new varieties of plants with shortened breeding cycles. Two new types of ornamental cherry tree that have larger flowers and bloom more frequently have also been developed. At the same time, the RNC is exploring the production of radioisotopes for pharmaceutical and environmental applications.

**Discovering the undiscovered**
The RNC currently employs 696 members of staff, including 112 women and 112 foreign researchers, and has a budget of over 3 billion yen for the 2013 fiscal year. Within the center, the RIBF Research Division leads two ambitious initiatives: the Euroball RIKEN Cluster Array (EURICA) Project that brings together 51 institutions from 16 countries to investigate nucleosynthesis in supernova explosions, and the SAMURAI (Superconducting Analyzer for Multi-particles from Radioisotope beams) study of stellar nucleosynthesis.

Looking ahead, the RNC hopes to utilize the capabilities of the RIBF to uncover at least 1,000 so-far-undiscovered atomic nuclei from a pool of over 10,000 predicted by theory. The center will also continue its quest to elucidate the formation of heavy elements in the Universe as well as contributing to applied research that aims to overcome food and energy shortages.

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A turning point for research into Arabidopsis

Arabidopsis thaliana, an annual of the Brassicaceae family of flowering plants, is a well-known experimental plant. It produces seeds within 2 months of seeding, reaches about 40 centimeters in height and can grow under fluorescent indoor lighting. These features make it attractive to experimentalists, and it is widely used in basic research to understand a variety of plant phenomena and functions. In December 2000, Arabidopsis became the first species of higher plant to have its genome completely sequenced.

In 2001, soon after the analysis of the Arabidopsis genome had been performed, the US National Science Foundation launched the Arabidopsis 2010 project. An international effort, the project set a goal of identifying the functions of all 27,000 postulated Arabidopsis genes by 2010. In the final year of the project, the RIKEN Plant Science Center in Yokohama and the RIKEN BioResource Center (BRC) in Tsukuba sponsored the 21st International Conference on Arabidopsis Research in Japan. Over 1,300 participants from 33 nations attended the conference.

“The year 2010 marked a major turning point for Arabidopsis research,” says Kobayashi. Under the theme ‘2010 and Beyond’, the conference participants engaged in lively discussions about research conducted in the decade since the entire Arabidopsis genome had been decoded, as well as future prospects. “I think that all of the participants were interested in the future research trends in Arabidopsis and plant science as a whole, following completion of the 10-year project,” explains Kobayashi. Five key areas for prospective research into Arabidopsis were identified at the conference. Most important for Kobayashi was the commitment to promote applied research under the motto ‘from laboratory to field’. “Basic research using Arabidopsis has yielded a great deal of important information. Now is the time to apply the results to crop research,” suggests Kobayashi. “Plant science cannot contribute to society unless the gap between basic research and applied research is bridged. And that requires a combination of technology, resources and information—in other words, creating the infrastructure.”
**Linking genetic information from many plants**

The Experimental Plant Division, led by Kobayashi and part of the BRC, recognized the need for a paradigm shift long before the 2010 conference. Since the BRC’s founding in 2001, the division has been conducting applied research as well as collecting, preserving and supplying seeds, genes, cell cultures and other resources for Arabidopsis research as part of Japan’s National BioResource Project. In addition to Arabidopsis genes, the division also stores genes from the moss Physcomitrella patens and other plants such as tobacco, poplar and cassava. “I have long been considering how we can make the best use of our resources,” explains Kobayashi.

One example of the Experimental Plant Division’s applied approach is offered by the Systematic Consolidation of Arabidopsis and other Botanical Resource (SABRE) database, jointly developed with the BRC’s Bioresource Information Division. SABRE links genetic information from a wide variety of plant species by searching multiple data sources in real time.

Entering a gene code name and keywords of interest into SABRE results in the listing of similar genes from plant species maintained by the BRC, such as Arabidopsis and tobacco, and other relevant information can also be obtained. SABRE is further linked to the Arabidopsis Information Resource database, which contains Arabidopsis information compiled by researchers worldwide. Using SABRE, the function of a gene discovered for the first time in a certain plant species can also be inferred, provided that a similar gene exists in Arabidopsis.

The SABRE database was first published in 2007 and the system was expanded in early 2013 to offer access to databases from research organizations other than RIKEN, with support from the Plant Genome DataBase Japan and the BRC’s Bioresource Information Division.

**Moving from Arabidopsis to Chinese cabbage**

Kobayashi soon realized that SABRE alone could not link the information it holds on Arabidopsis to applied research, mainly because the database is only used by scientists engaged in basic research. In an effort to make the information more broadly usable, he turned to Chinese cabbage, another plant of the family Brassicaceae. “An international project to decode the whole genome of Chinese cabbage was already underway,” says Kobayashi, explaining his choice. “In addition, Ibaraki prefecture, where the BRC is located, is a center of production of Chinese cabbage.”

In 2009, the Experimental Plant Division, together with the Research Institute for Biological Sciences at the Okayama Prefectural Technology Center for Agriculture, Forestry, and Fisheries and the NARO Institute of Vegetable and Tea Science, started a joint research program to create a library of full-length complementary DNAs (cDNAs) from Chinese cabbage. A cDNA is a piece of DNA synthesized using messenger RNA (mRNA)—a copy of the desired target gene from the genome—as a template. A full-length cDNA is a piece of DNA that contains all the necessary information to produce a certain protein. By using a full-length cDNA, it is possible to determine the complete structure of a gene and to synthesize and examine the functions of a protein. As part of the project, the researchers succeeded in creating about 10,000 different full-length cDNAs.

Concurrently, the Experimental Plant Division also cooperated with the Multinational Brassica Genome Project, a consortium of research institutes from...
The ABRANA database

Also in 2011, the Experimental Plant Division published the online Arabidopsis-Brassica Network Access (ABRANA) database—a compilation of information describing the expression of stress-related genes from Chinese cabbage developed through the many experiments conducted by Kobayashi and his team.

Kobayashi explains how he went about collecting the information for what later became the ABRANA database: “The goal of our experiments was to find useful genes of Chinese cabbage by making the best use of Arabidopsis information, and to use that information for plant breeding and other purposes, so as to enable the paradigm shift of ‘from laboratory to field’.”

Kobayashi took a strategic approach to information collation that had the most applicable value for agricultural purposes. “Our project on Arabidopsis set out to collect cDNAs of all genes and elucidate their functions for application in research across all biological phenomena. But when the purpose of a project is to apply the information to crops, it is not necessary to cover all biological phenomena. Rather, emphasis should be placed on streamlining the program to minimize labor, time and money. Hence, we focused on genes related to stress responses.”

Crops undergo a wide variety of stresses that affect their yield and quality, such as high and low temperatures, drought, salinity and pests. There is significant demand for the development of crops that are highly resistant to environmental stresses. Many stress-related genes have already been discovered and extensively studied in Arabidopsis.

Kobayashi began by selecting about 2,000 candidate genes with base sequences similar to those of Arabidopsis stress-related genes from the pool of Chinese cabbage cDNAs that had been created up to that point. Thereafter, Kobayashi developed a new microarray that would enable researchers to comprehensively explore conditions for the expression of individual genes of Chinese cabbage.

“ABRANA is a groundbreaking database that allows Arabidopsis and Chinese cabbage to be compared with each other—not only in terms of base sequence information but also gene expression information,” says Hiroshi Abe, the scientist who is leading the ABRANA project at the Experimental Plant Division. “I developed ABRANA to assist researchers in university faculties of agriculture, agricultural experiment stations and seed companies who want to find out about the various applications of plants.”

In Arabidopsis databases, it is common practice to enter all relevant information, including very specific details. The Chinese cabbage data within ABRANA, however, is much more accessible. “We designed the database system so as to make it easily understandable to the senses, sometimes even omitting detailed information. This makes users interested in actually using our database,” says Abe. “For example, gene expression information was initially displayed in the same way as in the Arabidopsis database, but some users complained that they were unable to understand how to read the data. Therefore, we changed the manner in which data was presented to a line graphic representation so that the information could be understood at a glance.”

Although no applied research has been conducted using the information available in the database as yet, Abe says that a number of plans have been announced. “I hope that a new variety of Chinese cabbage that is highly resistant to pests will be created by making the best use of ABRANA.”

Since the publication of ABRANA, Kobayashi has often been asked whether RIKEN also intends to start breeding Chinese cabbage, but he denies any such plans. “We do not intend to engage directly in breeding Chinese cabbage. Rather, we deal with Chinese cabbage as the key to linking Arabidopsis information to crops (Fig. 1). The family Brassicaceae includes a wide variety of crops such as cabbage, broccoli, radish and rapeseed. We are planning to make the best use of the information that has been compiled from Chinese cabbage to find applications for these crops.”

Brachypodium distachyon, a new model plant

At present, there are limits to the wider application of research on Arabidopsis and Chinese cabbage for crop development, explains Kobayashi. “While Arabidopsis is a dicotyledonous plant [whose seed typically has two embryonic leaves, or cotyledons], many cereal
crops—including rice and wheat—are monocotyledonous [and have only one such leaf]. Because they are phylogenetically distant from each other, they cannot be linked together as easily as Arabidopsis and Chinese cabbage.” Recognizing these limitations, Kobayashi has recently started research on a new plant species. “We are now working to establish the research infrastructure, focusing on Brachypodium distachyon as a model experimental monocotyledon,” says Kobayashi.

Brachypodium distachyon, also known as purple false brome, reaches up to 30 centimeters in height, produces seeds within about 3 months of seeding and, like Arabidopsis, can grow under fluorescent lighting in the laboratory (Fig. 2). The plant is already being used by some wheat researchers in Europe and the United States but was scarcely known in Japan until introduced by the RIKEN Biomass Engineering Program (BMEP), a cross-organizational research program launched in 2010. The Experimental Plant Division is a participating institution in the program, which aims to develop innovative technologies that contribute to the shift from a fossil fuel-based economy to a sustainable one that exploits the biomass produced by plants.

“Although biomass fuels are attracting attention as a substitute for fossil fuels, they must compete for space with food because they are produced from cereals such as wheat and corn. We aim to link the knowledge obtained through the study of Brachypodium distachyon to producing biomass from plants that are not food crops,” explains Kobayashi.

Without the development of useful techniques for handling genes and cells, Arabidopsis would not have gained such popularity as a model experimental plant. Similar techniques will be needed to establish the technology, resources and information infrastructure for Brachypodium distachyon, and Yasuyo Himuro of the BMEP’s Biomass Research Platform Team has been assigned to the Experimental Plant Division to achieve this. The only researcher with technical expertise in Brachypodium distachyon gene transfer, she has been tasked with the development of such methods. “Himuro previously studied a pasture crop. The knowledge and skills she gained are highly transferable to our current research,” says Kobayashi.

Himuro is now working to develop a gene transfer technology for Brachypodium distachyon that will be essential for analyzing genetic functions and crop breeding. When a gene is introduced to Arabidopsis, it is first integrated into the plasmid—the circular DNA outside the nucleus—of Agrobacterium, a microorganism that infects the leaf of Arabidopsis. However, this method cannot be used for Brachypodium distachyon because Agrobacterium is unlikely to infect its leaves. “In the case of Brachypodium distachyon, we first culture the embryo of an immature seed collected just after flowering to obtain a cell mass called a callus. The callus is then infected with Agrobacterium to introduce the target gene, and cells found to incorporate the gene are selected and propagated (Fig. 3). This is much more painstaking than the approach applied to Arabidopsis,” explains Himuro.

“When I saw Brachypodium distachyon for the first time, I did not believe it had much potential since I had previously conducted research on a pasture crop that grows to 2 meters in height. However, because Brachypodium distachyon can be grown in the laboratory and has a short generation cycle, we expect our research to progress much faster than my earlier approach of using a pasture crop,” says Himuro. “I was able to realize the power of the laboratory plant model. Now I strongly hope to establish a research infrastructure that will allow Brachypodium distachyon to serve as a model plant and to link research results to large herbaceous plants such as pasture and energy crops.”

With exciting new work on Brachypodium distachyon and the Experimental Plant Division’s many years of research into Arabidopsis and Chinese cabbage to lean on, Kobayashi looks set to achieve his aim of joining basic and applied research. “I want to raise public awareness of the potential of plant science research for making great contributions to society,” he says. “It is worthwhile research.”

ABOUT THE RESEARCHER

Masatomo Kobayashi was born in Tokyo, Japan, in 1957. He graduated from the University of Tokyo’s Faculty of Agriculture in 1981 and obtained his PhD from the same university. In 1985, he became a research scientist at RIKEN where he started to introduce knowledge of molecular biology into agricultural science. He received postdoctoral training from the former Department of Botany at the University of California Los Angeles in the United States from 1990 to 1992. In 2001, he was promoted to laboratory head of the Experimental Plant Division at the RIKEN BioResource Center. Since then, he has directed the BRC’s project on Arabidopsis—the most well-known model plant. His recent work focuses on the application of the resources, technologies and knowledge of model plants to crop research.
How did you join RIKEN?
During my PhD studies at Keio University, I developed a computational method that can rapidly simulate biological processes at the molecular scale. Koichi Takahashi, a collaborator of mine at Keio, encouraged me upon graduation to continue similar research in his laboratory. I accepted the invitation and in 2010 embarked on my postdoctoral career with a position at RIKEN. A year after I joined, the group was officially named the Laboratory for Biochemical Simulation at the newly established RIKEN Quantitative Biology Center (QBiC) in Osaka.

What is your current field of research?
At QBiC, particle simulations of biomolecules remain my primary focus. Currently, I am looking at the interactions between individual molecules that affect the behavior of the whole cell. I use Spatiocyte—the particle simulator that I developed during my PhD—to build upon the experimental measurements obtained by my collaborators. This approach allows us to gain insight into the basic biological principles that drive bacterial cell division, amoebal chemotaxis and neuronal polarization.

Why were you drawn to RIKEN?
RIKEN has a worldwide reputation for excellence, so naturally I was keen to be a part of it. And Dr. Takahashi, our laboratory team leader, is known for his generosity in giving members of his research group the freedom to pursue their own scientific goals—be they to seek out new collaborations or to develop new approaches to research.

What is the best thing about working at RIKEN?
Labs at RIKEN continually collaborate with one another, creating a stimulating environment for research and learning. At QBiC, we are encouraged to exchange ideas and expertise with other labs to develop new technologies, which allows us to work at the frontier of the ever-evolving world of biology. I am sure that the knowledge and skills I have gained through these collaborations will help me in the next stages of my career.

What has been the highlight of your time at RIKEN so far?
I was able to simulate some of the observed dynamic patterns arising from the self-organization of molecules in a cell. It was an amazing experience to uncover a few of nature’s complex, yet beautiful, processes. The results we obtained were also very useful for our collaborators in designing experiments to confirm the simulation’s predictions.

What would you say to other people considering joining RIKEN?
The many English-speaking staff at RIKEN make life for non-Japanese researchers much easier, and I feel very comfortable here. RIKEN will surpass the expectations of scientists looking for an exciting and rewarding career. Through schemes such as the International Program Associate (IPA) program and the Junior Research Associate program, RIKEN enables talented young researchers to pursue their interests in science and technology. Thanks to the IPA program in particular, I have had the opportunity to supervise and mentor a graduate student from Malaysia, my home country, who is conducting long-term research at RIKEN.
RIKEN’s K computer simulates brain activity

Researchers at the RIKEN HPCI Program for Computational Life Sciences, the Okinawa Institute of Science and Technology Graduate University and Forschungszentrum Jülich, an interdisciplinary research center based in Germany, have carried out the largest neuronal network simulation to date by exploiting the full computational power of the K computer—RIKEN’s supercomputer in Kobe.

The team led by RIKEN alumni Markus Diesmann and Abigail Morrison—both now based at Forschungszentrum Jülich’s Institute of Neuroscience and Medicine—used advanced data structures developed for the open-source Neural Simulation Technology (NEST) tool to replicate a network consisting of 1.73 billion nerve cells connected by 10.4 trillion synapses. To realize this feat, the program recruited 82,944 processors from the K computer.

While significant in size, the simulated network represents a mere 1% of the neuronal network in the human brain. Even with the K computer’s significant computational power, the process took 40 minutes to simulate 1 second’s worth of neuronal network activity. However, rather than providing new insight into the brain, the project’s primary objective was to test the limits of the simulation technology—as well as the capabilities of the K computer.

Through their efforts, the researchers were able to gather invaluable knowledge that will guide the construction of new simulation software. In addition, their achievement offers neuroscientists a glimpse of what can be achieved by using the next generation of computers—so-called exascale computing.

“If petascale computers like the K computer are capable of representing 1% of the network of a human brain today, then we know that simulating the whole brain at the level of the individual nerve cell and its synapses will be possible with exascale computers—hopefully available within the next decade,” said Diesmann.

RIKEN to attend BioJapan 2013

RIKEN researchers are set to present their work at BioJapan 2013, Asia’s top networking event for the global biotechnology industry. The annual meeting plays an important role in facilitating interactions between Japanese and international biotech companies and stimulating new opportunities for business. Company executives, as well as leading industry professionals in the areas of business development and licensing, alliance management and research and development, will gather in Yokohama to attend the three-day event, which runs from 9 to 11 October.

On the first day, Yukio Nakamura of the RIKEN BioResource Center will share his research into the in vitro production of red blood cells. The following day, Atsushi Miyawaki at the RIKEN Brain Science Institute will make a presentation on the development of new bioimaging tools, and Yuki Hasegawa from the RIKEN Center for Life Science Technologies (CLST) is expected to give a talk on the stable culturing and efficient differentiation of induced pluripotent stem (iPS) cells using a chemokine protein.

The final day will see presentations by Koji Ueda from the RIKEN Center for Integrative Medical Sciences and Tomotaka Shingaki from the CLST. Ueda will present research on high-throughput glycan structure profiling of protein drugs using Erexim (energy resolved oxonium ion monitoring) technology, and Shingaki will describe his contribution to the development of positron emission tomography (PET) molecular imaging technology for pharmacokinetics.

RIKEN will also be hosting a booth at the exhibition to introduce the RIKEN Program for Drug Discovery and Medical Technology Platforms, and to provide information on how the SPring-8 Angstrom Compact Free Electron Laser (SACLA) X-ray Free Electron Laser (XFEL) and K computer can be applied to elucidate various life sciences phenomena.

A number of RIKEN researchers will be attending and presenting at October’s BioJapan 2013 in Yokohama.