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Taking the pulse of X rays

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X-rays diagnosed

How to measure the beam characteristics of the world's first x-ray free electron laser

RIKEN and the Japan Synchrotron Radiation Research Institute (JASRI) are currently building an x-ray free electron laser (XFEL) at the SPring-8 synchrotron facility in Harima (Fig. 1). XFEL is scheduled to be completed by 2010 and will be the first such laser in the world that produces short, intense pulses of x-ray laser radiation.

The laser will be used for biomedical and materials research to study atomic structures and molecules. For example, to determine the structure of a protein or other complex molecules, conventional x-ray sources require the molecule to be in an ordered crystalline state. Normally, this is difficult to achieve and may not reflect the true state of the protein in its natural biological environment. The laser radiation produced by XFEL, on the other hand, will be so strong and powerful that it will be able to image a single molecule without the need to obtain a whole crystal. Furthermore, the short laser pulses from XFEL will allow the study of the temporal evolution of single molecules as well as their interaction with other molecules. The results of such studies could lead to significant advances in our understanding of biological processes.

Figure 1: The SPring-8 facility in Harima. This aerial view shows the existing synchrotron. The future XFEL buildings are superimposed within the red line.

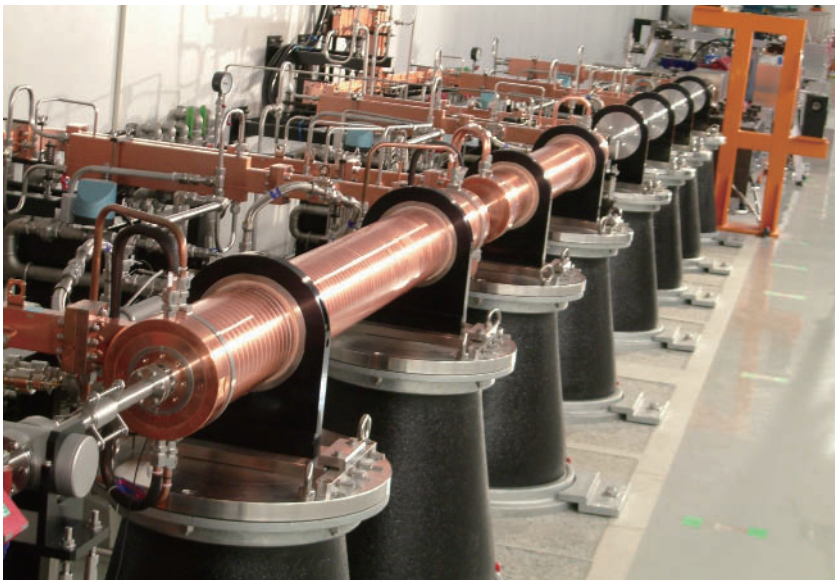


Figure 2: The so-called 'C-band' accelerator of the XFEL prototype. The device accelerates electrons to high energies before they reach the actual laser section where strong magnets force them on a curved path so that they emit x-ray laser radiation.

Conceptually, the XFEL design is the same as that for conventional low-energy free electron lasers, only on a much grander scale. Electrons will be accelerated to high energies in an 800 m long tunnel (Fig. 2) and, by virtue of very strong magnets, forced on a tightly curved path that causes them to produce short, intense pulses of x-ray laser radiation.

Color snapshots of XFEL

According to Makina Yabashi, a physicist from the joint RIKEN/JASRI team at SPring-8, knowledge of the precise laser beam properties for each pulse is critical for the successful operation of XFEL. "This is because the spectrum of the laser changes in every shot and the average spectrum completely differs from the single-shot spectrum," he explains.

As the XFEL specifications are very broad and pulses of different durations can be generated, designing a suitable spectrometer to measure the XFEL beam characteristics is a challenging task. In particular, it is difficult to directly measure the pulse length. The problem is that the time delay between the different pulses varies slightly, which makes precise measurements impossible.

Fortunately, it is relatively easy to measure the energy spectrum of the laser pulses. As it turns out, such a measurement already provides sufficient information, owing to one of the fundamental principles of nature that directly relates the energy spectrum to the duration and shape of the laser pulses. The shorter a laser pulse, the broader its energy spectrum and vice versa. Therefore, knowledge of one property allows the derivation of the other. However, as the pulse lengths of the laser can vary across several orders of magnitude, the resolution of the spectrometer needs to be extremely high in order to resolve the fastest, as well as

the slowest, pulses. A prototype of a suitable single-shot spectrometer has now been tested successfully (Fig. 2) and the results have been published in the journal *Physical Review Letters*[1].

Precision, precision, precision

A key component of the spectrometer is its single-crystal silicon mirror. Laser light from the XFEL is focused onto the silicon mirror at the heart of the spectrometer. In silicon crystals, the periodic arrangement of the atoms is comparable to the wavelength of the x-ray radiation. This leads to diffraction of the laser light away from the crystal and the angle at which the light is reflected is determined by the wavelength—similar to the way a prism disperses sunlight into colored rays. A conventional photo detector then picks up the reflected rays and a complete energy spectrum of the beam is obtained in a single laser shot.

The attainable energy resolution of the spectrometer is determined by the minimum difference of laser beam wavelengths the detector array can distinguish. This, in turn, is influenced crucially by the quality of the silicon mirror. It is the perfection of the mirror that distinguishes the RIKEN spectrometer from previous, less successful designs.

Although silicon crystals can be grown with high purity and extremely smooth surfaces, the specifications required for the spectrometer go even beyond that of the microelectronics industry. "Fortunately, we can utilize a very good mirror with an atomically smooth surface that was developed by our partners at Osaka University," Yabashi points out. In a laborious polishing process carefully controlled by a monitoring system, any remaining imperfections on the surface are removed individually.

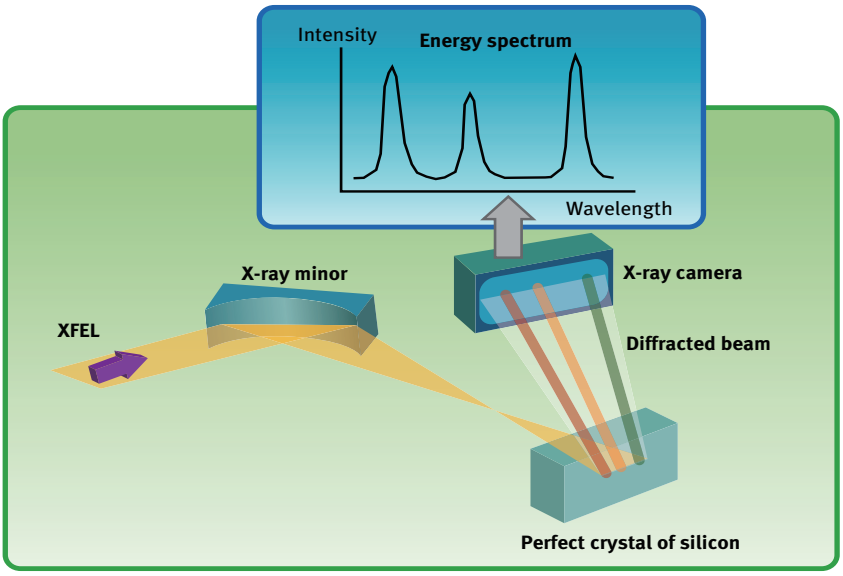


Figure 3: The spectrometer design. The incident XFEL beam is diffracted by the perfect silicon crystal. The profile of the diffracted beam is recorded using an x-ray camera.

All systems ready to go

In their first test runs, the team achieved an energy resolution of about a thousandth of the beam energy, which is sufficiently small to cover most of the region of interest for XFEL operation. In fact, the resolution achieved beats that of conventional instruments by two orders of magnitude. Therefore, the spectra provide valuable feedback on the parameters for operating XFEL that allows continuous optimization of the shape and length of the laser pulses.

The RIKEN/JASRI team will now develop the spectrometer for the actual XFEL. "As the proof-of-principle stage is finished, the next step is to develop the spectrometer into a convenient and practical system for the real diagnostics," says Yabashi.

Based on such impressive scientific and engineering advances, XFEL is expected to outperform its international competitors, not only in terms of performance, but also cost. The high-resolution spectrometer will ensure that XFEL runs at maximum performance and produces new and exciting scientific discoveries. ■

1. Yabashi, M., Hastings, M., Zolotorev, M., Mimura, H., Yumoto, H., Matsuyama, S., Yamauchi, K. & Ishikawa, T. Single-shot spectrometry for x-ray free-electron lasers. *Physical Review Letters* **97,** 084802 (2006).

About the researcher

Makina Yabashi was born in 1971 in Gifu, Japan. He received his BSc degree in 1994 and MSc in 1996 from the University of Tokyo. In 1996 he joined the Japan Synchrotron Radiation Research Institute (JASRI). In 2003 Yabashi earned his PhD from the same university for work on x-ray intensity interferometry. Since 2005 he has been a member of a joint team of RIKEN and JASRI researchers involved in developing the x-ray Free Electron Laser (XFEL) optics and beamlines at SPring-8 in Harima. In 2004, he received the Japanese Society for Synchrotron Radiation Research Award for Young Scientists.

Building bridges with nickel

Linking together nickel atoms with organic sulfur compounds leads to molecular conductors

Complexes between metal ions and organic ligands have a wide range of chemical, optical and electronic properties and find application as catalysts, dyes and sensors. Although the organic ligands play a crucial role in orchestrating the assembly of such complexes, often the characteristics of the metal ions dominate the behavior of these molecules.

Some well-defined single-core metal complexes show promising conducting properties, and compounds containing more than one metal are of particular interest. "Multimetallic complexes exhibit peculiar properties not observed in monometallic complexes," points out Kazuya Kubo from RIKEN's Discovery Research Institute in Wako.

Kubo and co-workers have made the first well-defined trimetallic conducting complexes and report their work in the *Journal of the American Chemical Society*[1]. Using an organic ligand containing four sulfur atoms, known as tetrathiooxalate, the team linked two or three nickel atoms together in a molecular chain.

Each of the opposite faces of the ligand bind to one nickel atom through two sulfur atoms forming a chain where the metals are bridged by these organic ligands. The nickel atoms at the ends of the molecule are capped on the outside with ligands that only contain two sulfur atoms and so cannot form bridges with other metals. According to Kubo, his synthetic method is, "simple and versatile and can be applied to a series of similar compounds".

The molecular structure of the trimetallic nickel complexes (Fig. 1) was determined using x-ray crystallography. These crystals were shown to be moderate conductors of electricity over a range of temperatures. Computational studies also indicate that there is a small energy gap between the filled and empty electron states, suggesting that they are good candidates for the formation of a conducting material—a so-called single-component molecular metal.

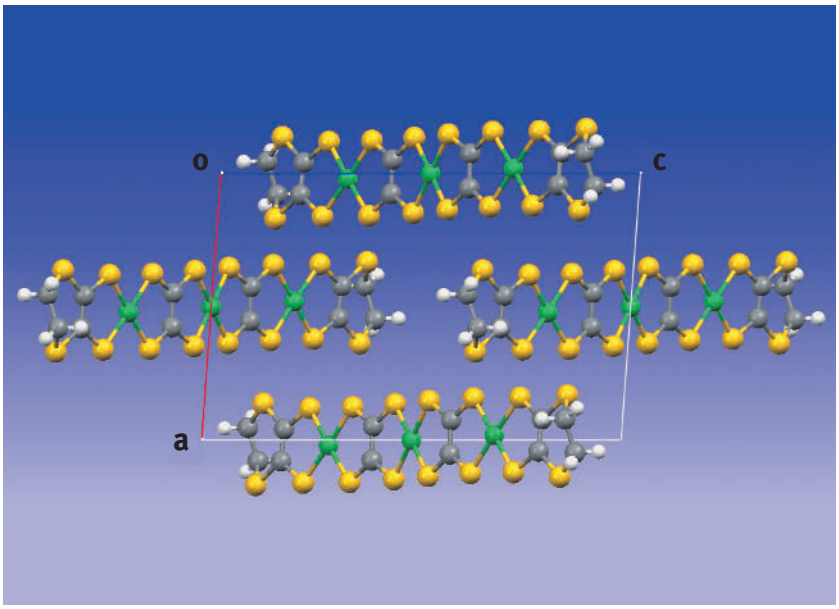


Figure 1: The x-ray crystal structure of a conducting trimetallic nickel-dithiolene complex showing how the molecules pack together in the solid state. The atoms are colored as follows: nickel (green), carbon (grey), sulfur (yellow) and hydrogen (white). The cations of the salt are omitted for clarity.

Interestingly, a single-component molecular metal may appear to have no free electrons that can contribute to electrical conduction; however, the crystal shows metallic behavior. Only one example of a single-component molecular metal has been reported previously[2], and this was based on a compound with a single metal core.

Kubo's nickel complexes would pack together in the solid state through a multidimensional network of intermolecular interactions. This arrangement of the molecules, in addition to their extended π-electron systems, would give rise to the observed metallic behavior. Although he considers this work to be, "basic science", Kubo is thinking long term, "in the future, multimetallic complexes may be used to build nanoscale devices". ■

1. Kubo, K., Nakao, A., Yamamoto, H. M. & Kato, R. Preparation and characterization of conducting trimetallic nickel-dithiolene complexes with bridging tetrathiooxalate ligands. *Journal of the American Chemical Society* **128,** 12358–12359 (2006).
2. Tanaka, H., Okano, Y., Kobayashi, H., Suzuki, W. & Kobayashi, A. A three-dimensional synthetic metal crystal composed of single-component molecules. *Science* **291,** 285–287 (2001).

Frustrated crystal chills out

Cooling allows chemical compound to rearrange itself in a spin Peierls transition

When electrons start to move in mysterious ways, it's usually a sign that the material around them could possess exotic electrical or magnetic properties. Recent research into how electrons rearrange themselves inside a new chemical compound as it cools down could therefore help to deliver new insights into phenomena such as high-temperature superconductivity.

Masafumi Tamura and colleagues from RIKEN's Discovery Research Institute in Wako have been investigating a crystalline material based on building blocks called $Pd(dmit)_2$—this formula denotes a palladium atom attached to two molecules of sulfurous dimercaptoisotrithione[1].

These flat units lie parallel to each other in the crystals, forming pairs—or 'dimers'—that share a single, unpaired electron (Fig. 1).

As the negatively-charged electron spins, it generates a magnetic field that can either point 'up' or 'down'. The electron shared by each dimer will generally try to adopt the opposite spin orientation to its neighbor (in the next dimer) to avoid bringing two north poles into close contact.

If these dimers were all arranged in a straight line, it could produce a stable system with a series of alternating electrons spins. But this crystal has a triangular network of dimers, spread over two-dimensional sheets—so the electron in each dimer has six neighbors to contend with. This means that it's impossible for them all to take opposing spins, and the system is, as they say in the business, 'frustrated'.

The team found that below -248 °C the structure of the crystal changes to reduce this frustration. Neighboring dimers containing electrons with opposing spins edge slightly closer to each other, sharing their electrons to form a new 'valence bond' between them. "Unlike the stick-like bond between ball-like atoms, as found in chemistry textbooks, the intermolecular valence bond is like glue spread between two plates," explains Tamura.

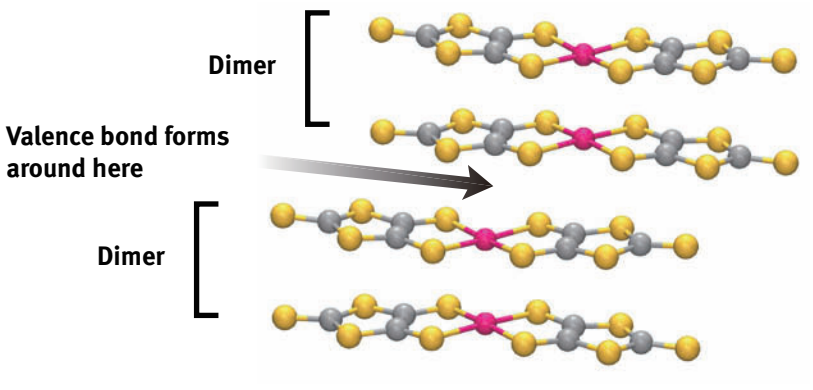


Figure 1: Molecular dimers can pair up to ease frustration with their neighbors.

When seen in a one-dimensional case, this molecular rearrangement is called a spin Peierls transition (named after theoretical physicist Rudolf Peierls). This is the first time it has been observed in a two-dimensional system, the team says. The discovery could help to understand why certain superconducting materials are able to carry electrical current with no resistance.

"High temperature superconductors are also two dimensional materials," says Tamura. "It is widely believed that fluctuating valence bonds may have a significant role in creating superconducting electron pairs."

However, it's difficult to observe valence bond formation directly in these materials, adds Tamura. "So our finding contributes to understanding the valence bond physics in 2D materials because it presents a real experimental system." ■

1. Tamura, M., Nakao, A. & Kato, R. Frustration-induced valence bond ordering in a new quantum triangular antiferromagnet based on $[Pd(dmit)_2]$. *Journal of the Physical Society of Japan* **75,** 093701 (2006).

Seeds of change

Use of heavy-ion bombardment allows the development of new plant strains

A research group at the RIKEN Nishina Center for Accelerator-Based Science in Wako, with colleagues at the National Institute of Vegetable and Tea Science in Mie, has used RIKEN's ring cyclotron to bombard dry seeds of sweet peppers (*Capsicum annuum* L.) with accelerated heavy ions to develop commercially useful mutants.

Introducing mutations in plants is desirable because it quickens development of new commercial plant types, while allowing the clarification of their basic physiology through genetics. Several mutagenic techniques have been used, including chemicals, gamma radiation, and fast neutron bombardment. Heavy-ion irradiation (HII) has also been used to introduce mutations into ornamental plants, many of which are available commercially. Now, a team led by Tomoko Abe from RIKEN has applied HII to vegetable plants, as described in a recent report in *Euphytica*[1].

The team developed three mutants of sweet peppers: two dwarf plants and a yellow pepper (Fig. 1). These mutant appearances (or phenotypes) are recessive and due to changes in single genes (or monogenic). Discovery of recessive, monogenic mutations causing dwarfism in sweet peppers is important because genes controlling growth in this plant have yet to be identified, and this type of mutation allows easier identification of these genes. Dwarf plants are also desirable commercially because they are easier to maintain and can grow in limited spaces.

Likewise, the yellow phenotype of the third mutant is also desirable scientifically and commercially because it may help in understanding the process of chlorophyll development, while offering an alternative color to pepper enthusiasts.

The researchers also showed that the mutant phenotypes appeared in the plants grown directly from the irradiated seeds, rather than from the progeny of these plants. In previous studies using other mutagenic techniques, identification of desirable mutations required laborious breeding over two or three generations.

"The HII technique allows isolation of unique mutants that have never been obtained by conventional methods," says Abe. Despite limited time available to use the accelerator, another advantage of HII includes a high mutation rate at relatively low doses of irradiation without severe growth inhibition. "We irradiate for only a few seconds or minutes," she explains. HII also has potential for use in plants such as wheat, taro and chrysanthemum. Mutants are difficult to isolate in these plants because they have six sets of chromosomes.

In the immediate future, Abe and team plan to use the HII technique to cultivate a seedless sweet pepper, thus avoiding the bitterness and spiciness of the seeds. ■

1. Honda, I., Kikuchi, K., Matsuo, S., Fukuda, M., Saito, H., Ryuto, H., Fukunishi, N. & Abe, T. Heavy-ion-induced mutants in sweet pepper isolated by M1 plant selection. *Euphytica* [online] (2006) (doi: 10.1007/s10681-006-9177-5).

Dwarf | Wild type | Semi-dwarf | Wild type | Yellow fruit

Figure 1: Three mutant sweet pepper plants derived from heavy-ion irradiation.

Constructing the drugs of tomorrow

RIKEN researchers clear a roadblock to manufacturing bio-active compounds

Japanese researchers have developed a chemical technique that solves a long-standing problem in building certain important, biologically active, short chains of sugar molecules in the laboratory. Their new process does not demand special reaction conditions, and can take place in a single reaction vessel. It may lead to automated synthesis of some important pharmaceutical compounds.

Chains of sugar molecules from three to 10 units long—known as oligosaccharides—are linked to many biologically active proteins and lipids in the human body. The particular three-dimensional conformation of each of the sugars with respect to one another, and to the protein or lipid molecule to which they are linked, can be crucial to how the whole complex functions.

The sugars are added by means of a reaction known as glycosylation. Until now, this important reaction typically demanded special, highly controlled, reaction conditions including temperature and concentrations of solvents and reagents used. In this reaction, two sugars are added in which the carbon backbone carries functional molecular groups on the same side as each other—the 1,2 *cis*- configuration.

New chemical reaction schemes for glycosylation developed by synthetic chemists from RIKEN and the Japanese Science and Technology Agency, were reported recently in the *Journal of the American Chemical Society*[1]. They use the compound *N*-benzyl-2,3-oxazolidinone carrying sugar as a donor. The reaction does not demand severe low temperatures, and the two sugars can be added within a single reaction chamber at good yield. As such, the research team thinks it is a good candidate for automation.

SPL/PPS

Figure 1: The bacterium, *Helicobacter pylori*.

"Our process is eco-friendly," says project leader Shino Manabe of the RIKEN Discovery Research Institute in Wako. "We are now trying to demonstrate its application by making bioactive oligosaccharides."

For instance, using their new reaction, the researchers are working at synthesizing the carbohydrate anti-blood clotting agent, heparin; a family of antibiotics known as the amino glycosides; and an oligosaccharide that can destroy the bacterium, *Helicobacter pylori* (Fig.1). This organism has been implicated as a cause of gastric ulcers and stomach cancer. ■

1. Manabe, S., Ishii, K. & Ito, Y. *N*-Benzyl-2,3-oxazolidinone as a glycosyl donor for selective α-glycosylation and one-pot oligosaccharide synthesis involving 1,2-*cis*-glycosylation. *Journal of the American Chemical Society* **128,** 10666–10667 (2006).

Helping mitochondria maintain their integrity

By marking other proteins for destruction, a newly discovered protein helps regulate the behavior of an essential organelle

Among other important functions, mitochondria act as the 'power-stations' of the cell, performing the metabolic reactions that generate ATP—the biochemical fuel that drives numerous cellular processes. Appropriately enough, the organelles themselves also lead dynamic lifestyles, routinely migrating and dividing and merging, although the processes by which these activities are regulated remains poorly understood.

New insights into these activities may soon arise thanks to the recent discovery of MITOL, a mitochondrial membrane protein with a surprising functional role (Fig. 1). MITOL was identified through the efforts of Satoshi Ishido of the RIKEN Research Center for Allergy and Immunology in Yokohama, Shigeru Yanagi of the Tokyo University of Pharmacy and Life Science, and their colleagues. Their findings are reported in *The EMBO Journal*[1].

These researchers were seeking new members of a class of proteins known as E3 ubiquitin ligases, whose function is to modify target proteins by tagging them with the small protein ubiquitin. This process marks them for subsequent destruction by proteolytic enzymes.

MITOL contains a distinctive domain, known as PHD, which is common to other E3 ubiquitin ligases. Like other members of this enzyme family, MITOL also appears to be capable of targeting itself for degradation, resulting in rapid and regular protein turnover. Mutation of this PHD domain led to swelling and aggregation of the mitochondria, suggesting that proteins intended for degradation were instead accumulating to excess within the organelle.

Ishido, Yanagi and colleagues also found that the targeted reduction of MITOL levels in cultured human cells led to a dramatic increase in mitochondrial fragmentation, suggesting that MITOL normally inhibits the fission process. This model was supported by data showing that MITOL interacts with—and appears to regulate the degradation of—multiple proteins known to be involved in mitochondrial fission.

According to Ishido, both the discovery of this protein and the determination of its involvement in the control of mitochondrial dynamics came as a pleasant surprise—with potentially important biological implications. "MITOL is the first example of a mitochondrial E3 ubiquitin ligase and interestingly contributes to the maintenance of mitochondrial morphology," he says. "We believe MITOL will provide new insights into mitochondrial biology." He adds that he and his colleagues have subsequently generated mice that lack MITOL, obtaining promising preliminary results that should further clarify this protein's biological importance. ■

1. Yonashiro, R., Ishido, S., Kyo, S., Fukuda, T., Goto, E., Matsuki, Y., Ohmura-Hoshino, M., Sada, K., Hotta, H., Yamamura, H., Inatome, R. & Yanagi, S. A novel mitochondrial ubiquitin ligase plays a critical role in mitochondrial dynamics. *The EMBO Journal* **25,** 3618–3626 (2006).

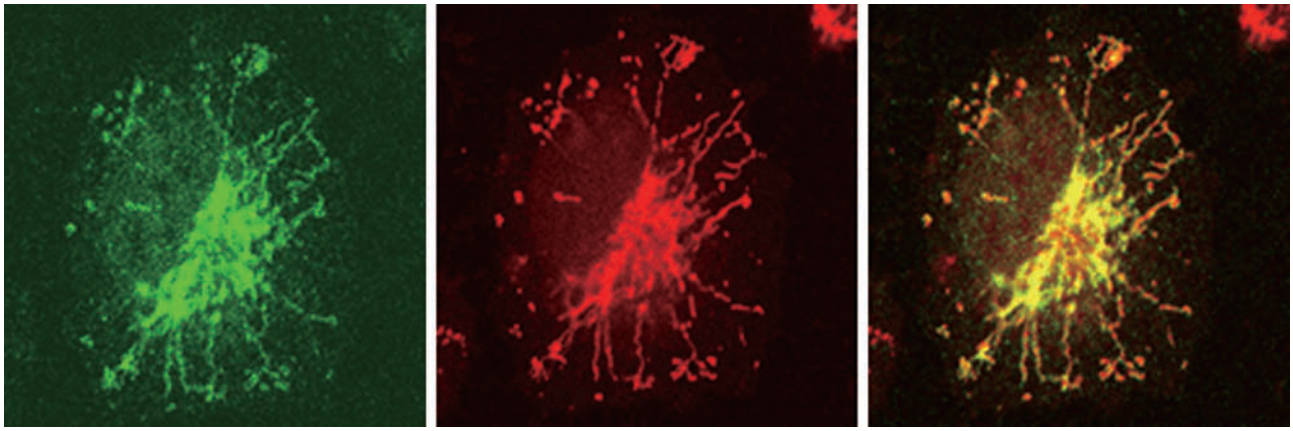
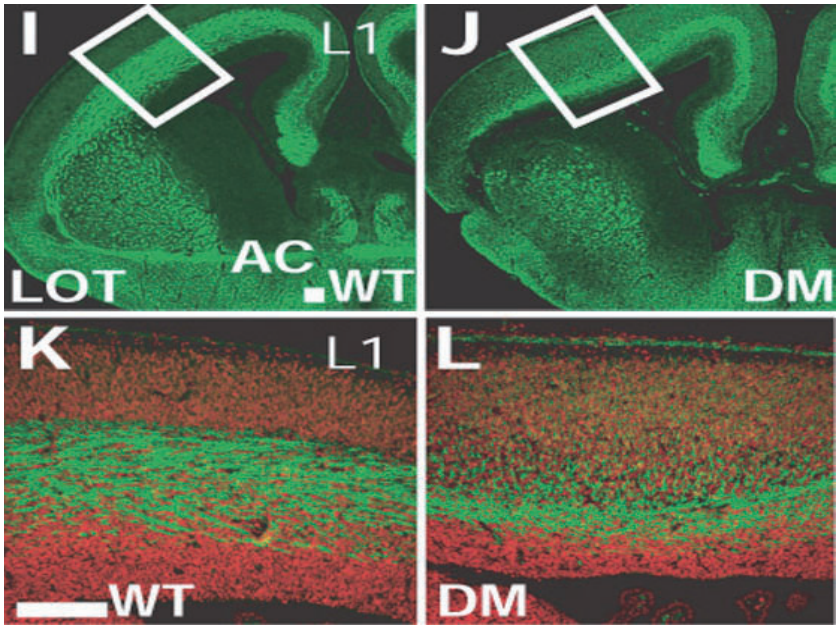


Figure 1: Localization of MITOL (left) and mitochondria (middle) in cultured cells. Merged image of the left and middle panels (right).

Too much of a good thing impairs brain development

Regulating brain glutamate concentration prevents developmental defects



PNAS/National Academy of Sciences

Figure 1: Brain sections of normal mice (I and K) and mice that lack the glutamate transporters GLAST and GLT (J and L). The green stain shows brain defects in the mice that have too much glutamate (J and L). The boxed areas in I and J are enlarged in K and L. The nuclei of cells are stained in red (right two slides). WT = wild type; DM = mice without GLAST and GLT; LOT = lateral olfactory tract; AC = anterior commissure; L1 = a cell adhesion molecule in the nervous system (stained green) used to visualize gross brain structures; scale bars (I, L) = 100 µm

Neuronal activity, important in refining neural connections in the developing brain, is mediated by neurotransmitters such as glutamate, GABA and acetylcholine.

In a recent issue of *Proceedings of the National Academy of Sciences USA*[1], a diverse team of Japanese neurologists and brain scientists led by Tsutomu Hashikawa and Kohichi Tanaka, from the RIKEN Brain Science Institute in Wako and Tokyo Medical and Dental University, respectively, evaluated how buildup of glutamate affects brain development in mice.

Studies by other scientists have evaluated the consequences for brain development by deleting genes in mice required for glutamate signaling. Surprisingly, those mice had normal brain development, which makes sense if one of the other neurotransmitters functions in place of glutamate.

But what if too much glutamate were present during brain development? Hashikawa and colleagues addressed that question by genetically deleting genes in mice required for glutamate transport in and out of cells.

In the adult brain, one consequence of too much glutamate signaling is neuronal death. Hashikawa's team found that when the glutamate transporters GLAST and GLT1 are absent during development, glutamate levels are high, multiple brain defects occur and the mice die before birth.

Generally, the brain defects in the altered mice include reduced proliferation, migration and differentiation of neurons, resulting in defective 'layering' of brain cells (Fig. 1). These changes are likely consequences of too much activation of glutamate receptors.

To demonstrate that increased activation of glutamate receptors was responsible for the defects, the team treated the mice with an inhibitor of glutamate signaling and noted a reduction in the brain defects.

The study by Hashikawa and colleagues is the first to demonstrate the critical importance of the GLAST and GLT transporters for regulating glutamate concentrations in brain development. "We understand now a new aspect of the mechanisms required for brain development: a major neurotransmitter (glutamate) has an important role in shaping the early development of the brain in addition to its known functions, that is, refinement of axonal projections and synaptic connections in developing brain," explains Hashikawa.

Clinically, these new findings could help plan future research to understand the consequences of a type of brain damage that occurs around the time of birth, known as perinatal hypoxia-ischemia injury, in which excess glutamate is implicated.

The mouse model of excessive glutamate may be useful for characterizing the mechanisms responsible for hypoxia-ischemia brain injury, and thus for developing strategies to reduce such damage therapeutically. ■

1. Matsugami, T., Tanemura, K., Mieda, M., Nakatomi, R., Yamada, K., Kondo, T., Ogawa, M., Obata, K., Watanabe, M., Hashikawa, T. & Tanaka, K. Indispensability of the glutamate transporters GLAST and GLT to brain development. *Proceedings of the National Academy of Sciences USA* **103,** 12161–12166 (2006).

Probing the secrets of a neuron's pulse

Scientists seek limits of information coded into electrical signals

A mathematical model describing the way that information is transmitted between neurons could help scientists to better understand how our brains manage huge amounts of data, RIKEN researchers say.

Neurons are the cells that make up our nervous systems—there are about 100 billion of them in your brain, and they are ultimately responsible for our ability to perceive the world around us and make decisions based on that information.

But how do neurons handle this information? "In the brain, neurons have contacts at synapses and communicate with each other through electric pulses," explains Taro Toyoizumi, a neuroscientist at the RIKEN Brain Science Institute in Wako.

Those electrical pulses can be measured, but any signal that carries useful information is always degraded by the presence of 'noise'—random electrical activity that carries no information of interest. Making an extensive series of measurements can help to pin down exactly what part of the pulse is noise, but there will always be a limit to the accuracy of scientists' assessment of the information carried by the neurons' signals. That limit can be calculated statistically, and is referred to as the Fisher information (after British statistician Sir Ronald Fisher).

Toyoizumi, along with RIKEN colleague Shun-ichi Amari and Kazuyuki Aihara of the University of Tokyo, have now calculated the Fisher information associated with a group of computer-generated neurons[1].

Previously, researchers have shown that the frequency of a neuron's pulses—say, three electrical 'spikes' per second—

carries information about whatever sensory input is stimulating it. But extra information can be encoded in the exact timing of these spikes—for example, whether the neuron generates a pulse at a certain number of milliseconds after being presented with a stimulus.

Toyoizumi and colleagues' analysis goes further than previous work by assessing the Fisher information for both of these factors. In this model, which has strong connections between neurons, "we found that a significant amount of information is in spike-timing," Toyoizumi says.

The teams' calculations could also give neuroscientists clues about where to look for the origins of particular kinds of neuronal stimulation, and the sources of electrical signals that wash through the brain. "Based on the spike-based Fisher information, we predict in a simple case what kind of recurrent connections are optimal for estimating the location of a stimulus or the onset of a stimulus," says Toyoizumi. "Those kinds of optimal connections might be discovered by future experiments with new imaging techniques," he suggests. ■

1. Toyoizumi, T., Aihara, K. & Amari, S. Fisher information for spike-based population decoding. *Physical Review Letters* **97,** 098102 (2006).

Mapping calcium highways within the cell

Clever experimentation reveals a secluded space through which calcium ions travel

Researchers from institutes in Japan and the US report direct evidence for differential calcium ion regulation within a confined region of the cytoplasm of specialized cells. This region is bordered by the plasma membrane (PM) and the sarcoplasmic/endoplasmic reticulum (S/ER)—which is the intracellular membrane network involved in the synthesis, modification and transport of cellular materials, and is a store for calcium.

Calcium ions 'transmit' signals within cells such as astrocytes, which surround and support nerve cells in the brain and spinal cord. When these cells are stimulated, calcium ions are often released from storage within the S/ER, and spread throughout the cytoplasm. However, cells 'sense' store depletion and respond by opening PM channels, allowing extracellular calcium to flow into the cytoplasm and restock empty stores. This emptying and refilling generates 'spikes' of cytoplasmic calcium during cellular activation.

The researchers, including Junichi Nakai from RIKEN's Brain Science Institute in Wako, set out to determine whether incoming calcium ions spread diffusely throughout the cytoplasm or move through a defined area between the PM and the S/ER. Their work is reported in the *Proceedings of the National Academy of Sciences*[1].

To track calcium ion movement within astrocytes, the researchers fused a protein, GCaMP2, which emits fluorescent light upon binding calcium ions to sodium pump subunits known to spread diffusely throughout the PM (α1) or localize exclusively within PM domains abutting the S/ER (α2).

Prior to stimulation, astrocytes were treated with the chemical EGTA, which acts as a sponge, soaking up background calcium ions and preventing their spread throughout the cell, and with cyclopiazonic acid, which unlocks and depletes S/ER calcium stores. These 'pre-treatments' allowed subsequent focus specifically on the site of extracellular calcium ion entry, and on the few nanometers between the PM and S/ER.

Astrocytes expressing the 'localized' GCaMP2-α2, but not the 'diffuse' GCaMP2-α1, fusion proteins 'glowed' after stimulation. In GCaMP2-α2-expressing astrocytes, fluorescence was confined to the narrow space between the PM and adjacent S/ER. Consistently, experiments with a low-affinity calcium probe called furaptra confirmed that S/ER depletion and refilling did actually occur in GCaMP2-α1-expressing astrocytes.

These data demonstrate that calcium ions streaming into astrocytes travel along a direct path towards the S/ER. "The possibility of differential calcium regulation within the intracellular space bordered by the PM and S/ER has long been discussed. Here, we present direct evidence for such specific regulation, as well as a technique that may be useful for studying localized calcium signals in other cell types," says Nakai. ■

1. Lee M.Y., Song, H., Nakai, J., Ohkura, M., Kotlikoff M.I., Kinsey, S.P., Golovina V.A. & Blaustein M.P. Local subplasma membrane Ca^{2+} signals detected by a thethered Ca^{2+} sensor. *Proceedings of the National Academy of Sciences USA* **103,** 13232–13237 (2006).

CRISTIAN TEODOSIU

Design for making manufacturing software more compatible

A veteran Romanian researcher at RIKEN plays a key role in an ambitious software development project that could revolutionize the manufacturing scene

For the past 45 years, Cristian Teodosiu has devoted his life to investigating complex physical and engineering problems. To this end, the native Romanian, who is now a French citizen, has modeled the properties of materials mathematically, simulated their behavior using computers, and developed a number of software programs. These have helped automobile and materials manufacturers to develop higher quality products.

It was Teodosiu's expertise in both engineering sciences and applied mathematics that attracted RIKEN when it appointed him in 2002 as the leader of the Manufacturing Process Simulation Team at the VCAD System Research Program in Wako. Since then, Teodosiu has been a powerful part of RIKEN's ambitious project to create a next-generation software system called Volume Computer-Aided Design (VCAD). The idea is to give a big boost to the efficiency of manufacturing—from casting a car body to processing plastics for mobile phones—by making it easy to control data at each step of the workflow.

Emigration to France

Teodosiu originally planned to become an engineer and earned his master's degree in civil engineering at the Institute of Civil and Industrial Engineering, in the Romanian capital of Bucharest, in 1958. Soon after, he was employed as a civil engineer for three years, working on the state's technical projects.

Teodosiu also had a strong interest in mathematics, and at the same time he was studying at the University of Bucharest, from 1955 to 1960, where he earned his second master's degree—in mathematics and physics.

Then, Teodosiu entered the Institute of Applied Mechanics of the Romanian Academy, and worked mainly on nonlinear random vibrations, applying his knowledge to optimize car suspensions and shock absorbers. In 1966, he obtained his PhD in mathematics from the Institute of Mathematics of the Romanian Academy.

In the early days of his professional life in the late 1960s, Teodosiu benefited from Romania's communist government liberalizing contact with other countries. Thanks to this more open policy, Teodosiu was able to do research and teach in Germany and France.

But the situation deteriorated in the mid-1970s following the establishment of a dictatorship. The government closed the country off and even banned imports of main scientific journals and drastically restricted the international scientific contacts. Teodosiu and his wife decided to emigrate, and in 1984 he gained a position as a visiting professor at Max Planck Institute for Metals Research in West Germany. In 1985, he was hired as Director of Research by France's Centre National de la Recherche Scientifique (CNRS; the National Center for Scientific Research) and began to work at the Laboratory for Physical and Mechanical Engineering of Materials at the National Polytechnic Institute of Grenoble.

An unexpected friendship

Teodosiu's new life in France came at around the same time as the start of his long-term commitment to Japan—quite by chance. A colleague of his had worked for six months at a Japanese laboratory run by Akitake Makinouchi, a mechanical engineering researcher at RIKEN, who is now the program director of the VCAD project. Teodosiu's colleague invited Makinouchi to France in return, but he moved away shortly before the Japanese researcher arrived. "I was told to take care of our guest," Teodosiu says.

And so one day in 1986, Makinouchi arrived in Grenoble, thinking he had no research partner, but found Teodosiu waiting for him at the city's small train station. "After talking for a while, we found many common interests in research, and felt there was a chemistry between us," says Makinouchi, who later learned that Teodosiu had a global reputation in materials science and continuum mechanics. "I was also impressed with his personality—he's a real gentleman." On Makinouchi's invitation, Teodosiu made his first visit to Japan in 1989. Since then, he has spent some time at RIKEN almost every year.

Teodosiu says one of his biggest achievements in France occurred in 1990, just before he moved to Paris to head a CNRS laboratory. Then, in collaboration with Makinouchi, Teodosiu developed a software to stimulate the processes involved in forming the large deformations of materials, such

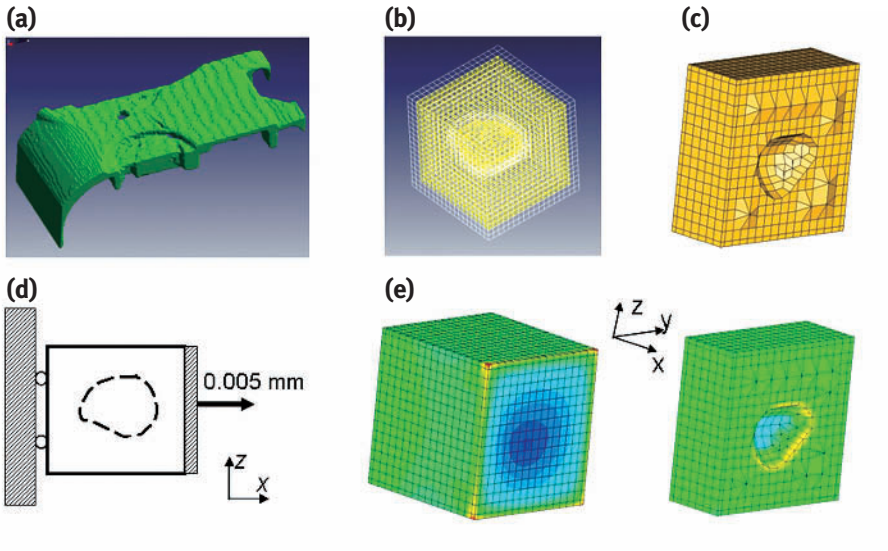


Figure 1:
Linear elastic analysis of the stress concentration around a pore in a cast plate of a magnesium alloy: (a) image obtained from x-ray computer tomography and V-CAT software; (b) VCAD grid of a representative volume element around a pore; (c) transverse section of the DHE mesh; (d) displacement boundary conditions (uniaxial extension); (e) distribution of the von Mises equivalent stress, max. value 80 megapascals.

as press stamping car parts. They also established a large-scale international research program with 22 companies and academic partners from Japan and Europe, which successfully contributed to increase the accuracy of such simulations.

When he reached the age of 65 in 2002, Teodosiu became Emeritus Research Director at CNRS. Seeing a good opportunity, Makinouchi called him to head one of the laboratories in the VCAD project. "I thought it was a very challenging and extensive research program," Teodosiu says. He now spends less than five months per year in Japan and the rest in France, where he lives with his family, and continues his research on modeling the behavior of very high-strength steels.

The VCAD project was launched by Makinouchi in 2001 to ease one of engineering's major problems. Engineers have always had to deal with each production step—from design and prototype development to measurement—with different software, so exchanging data between steps is difficult and time-consuming. "At the moment, it's quicker to make an actual product than to make the data compatible with all software," Makinouchi says. He wants to make the same data work in any software, drastically reducing not just human labor, but also cost and the time it takes to get a product to market.

To utilize the VCAD system, engineers design an object's shape with existing computer-aided design (CAD) software, and transfer the data to VCAD's platform software. Then, they use other VCAD-dedicated programs that assist upstream production processes such as analysis and measurement.

Looking below the surface

Aside from building a common platform, it's also vital for engineers to measure the complex and heterogeneous state inside the materials, in order to predict and prevent physical defects, such as micropores occurring in a cast magnesium part (Fig. 1). That's where Teodosiu's wisdom is needed. "Such pores will influence the behavior of materials and can eventually produce some premature failure," Teodosiu explains. "When you produce a part, you need to think not only about its shape, but also about the material's in-service properties."

The same logic is applicable to biology, where VCAD could also help in modeling cell behavior. In July 2006, RIKEN released nine open-source VCAD programs to seek advice from outside users and improve the quality (see *RIKEN RESEARCH*, Vol. 1, No. 3, pp. 13–16).

Teodosiu's six-member team has been working on forming processes like casting—simulating how molten metal flows, solidifies and shrinks at lower temperatures—and on the complex analysis of engineering structures. "For the first three years, it was hard to make the project take off," Teodosiu recalls. "Now, things are getting more and more interesting and exciting. At the beginning, it was not so rapid." Teodosiu's team has so far produced four types of simulation software, and now is striving to improve it and make it work within the VCAD environment.

The project is likely to require Teodosiu to stay in Japan longer than he originally thought. That is good for young researchers like Kenichi Ohura, one of his team members, who can learn more from him. Ohura says Teodosiu keeps throwing out ideas and sharp questions, even about details, but does not impose his own thought and always respects others' stance. "What impresses me is the way he is always asking 'Why?' and 'What's your opinion?'" Ohura says. "I think that reflects his passion and generosity."

Background reading

Teodosiu, C. *Elastic Models of Crystal Defects*, Springer-Verlag, Berlin-Heidelberg-New York, 1982.

About the researcher

Cristian Teodosiu was born in 1937 in Bucharest, Romania. He earned his first masters, in civil engineering, in 1958. Two years later, he obtained his second masters, in mathematics and physics, and in 1967 his PhD in mathematics. He worked as a researcher in Romania, and in 1977 was appointed as Director of Research at the Institute of the Physics and Technology of Materials in Bucharest. In 1984, he became a visiting professor at the Max Planck Institute for Metals Research in Germany, before moving in 1985 to the Laboratory of Physical and Mechanical Engineering of Materials in Grenoble, France, as a CNRS Director of Research. In 1991 he moved to Paris to lead the CNRS Laboratory for the Mechanical and Thermodynamic Properties of Materials. Since September 2002 he has been at RIKEN, managing the Manufacturing Process Simulation Team of the VCAD System Research Program.

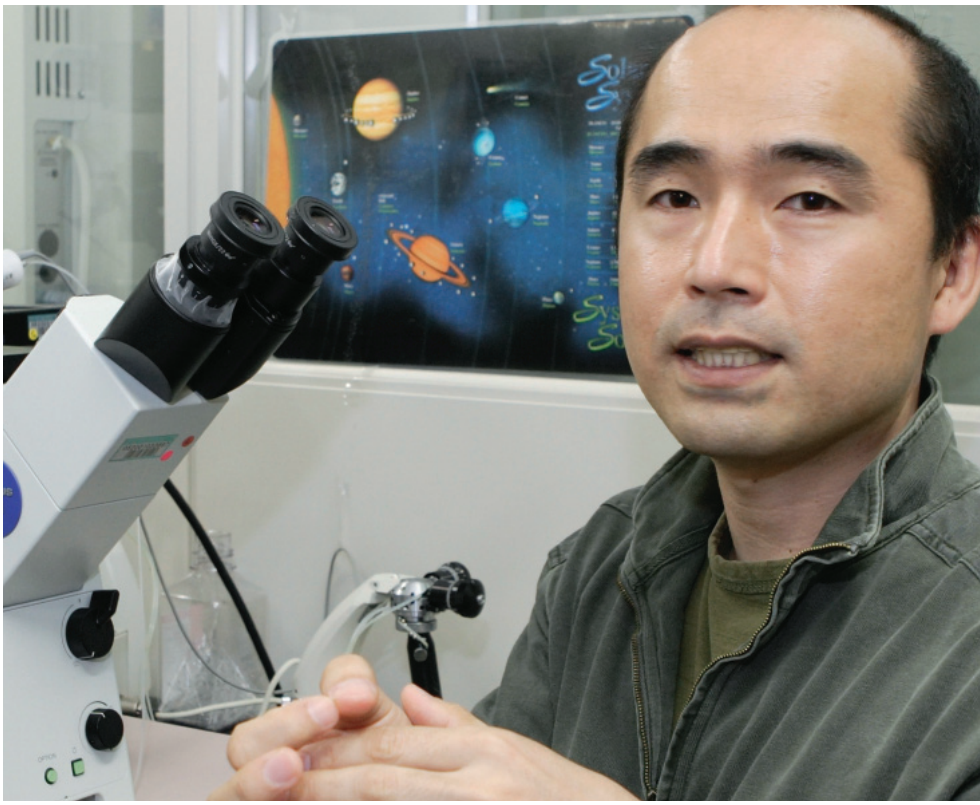
Further information on VCAD is available at:
http://www.riken.jp/engn/r-world/research/lab/vcad/index.html

Deft hands enable cloning

Getting closer to solving the mystery of genomic reprogramming

Teruhiko Wakayama

Team Leader
Laboratory for Genomic Reprogramming
The RIKEN Center for Developmental Biology
Kobe



In 1998, Teruhiko Wakayama, Team Leader of RIKEN's Laboratory for Genomic Reprogramming, surprised the world by achieving what had been considered impossible—cloning a mouse from somatic cells, or cells not involved naturally in reproduction. To date, only about 10 species of somatic cell clones have been produced. The success rate of cloning is low for all animal species, and animals that are born have abnormalities. This is considered attributable to imperfect programming of the nuclei of the cells transferred to egg cells. Since cloning technology is expected to be applied to regeneration medicine, elucidating the mechanism of genomic reprogramming is considered a big obstacle to overcome. The Laboratory for Genomic Reprogramming is now making full use of nuclear cell transfer technology with a micromanipulator, and is testing various conditions that potentially affect genomic reprogramming.

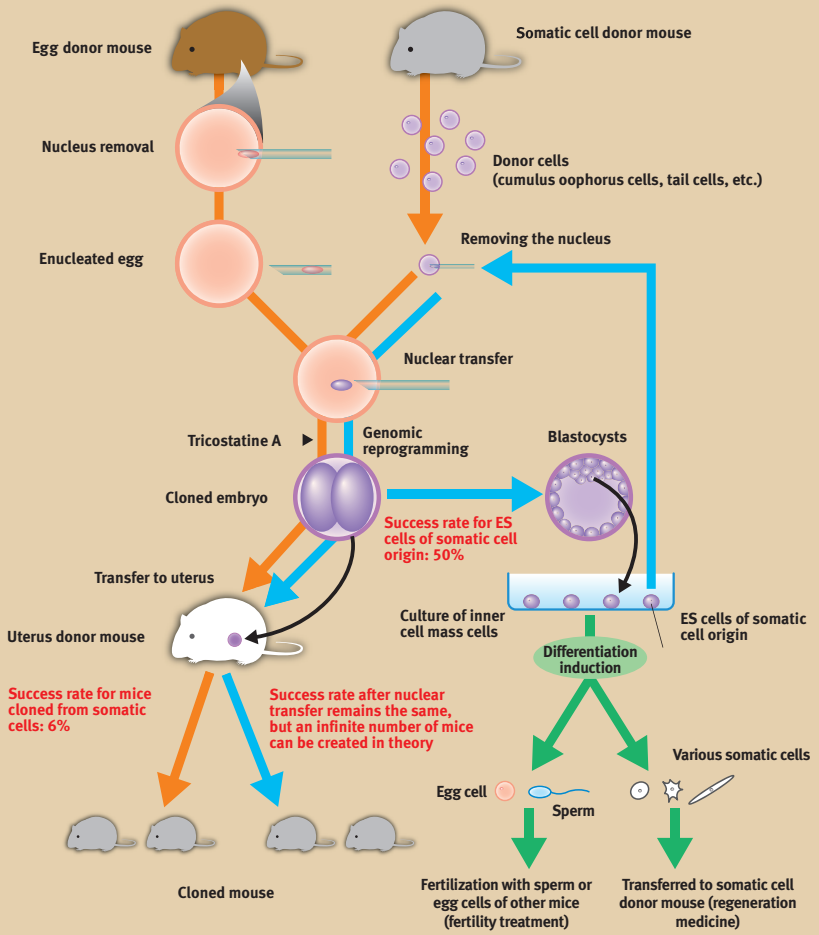
Birth of the world's first somatic cell cloned mouse

Wakayama headed the program that successfully led to the first birth of a mouse cloned from a somatic cell. It was in 1998 when he was studying abroad in Ryuzo Yanagimachi's Laboratory at the University of Hawaii. In the previous year, Dolly, the world's first sheep cloned from somatic mammalian cells was born.

"The birth of Dolly was shocking to me because everybody including myself took it for granted that it was impossible to create cloned mammals from somatic cells," reflects Wakayama. The technology that enabled the birth of Dolly had been used for cloning fertilized eggs. Thus Dolly's birth was not a technical breakthrough, but a breakthrough in achieving what was previously thought impossible. Wakayama therefore thought he might be able to clone mice from somatic cells, which had also been considered impossible.

A clone is an organism that has the same genetic information as another organism or organisms. Clones are either 'fertilized egg clones' or 'somatic cell clones' depending on how they were produced. To create a fertilized egg clone a fertilized egg is taken from a female fallopian tube. After the fertilized egg has divided into eight cells, these cells are separated into eight individual cells. Then, the nuclei of the fertilized eggs are transferred to eggs that have had their nuclei removed. Finally the cloned eggs are returned to the womb. The first successful fertilized egg clone was a frog in 1952. Fertilized egg cloning technology has been applied

Figure 1: How to create somatic cell cloned mice and ES cells of somatic cell origin.



practically in the animal industry since 1986 when scientists succeeded in cloning Dolly the sheep.

In contrast, somatic cell clones are produced by transferring nuclei obtained from various somatic cell tissues such as skin, milk gland, and cumulus oophorus to eggs that have had their nuclei removed in advance (Fig. 1).

"No technical breakthroughs can be found in our somatic cell cloning. I guess we were just lucky," says Wakayama. "I have been using a technique that uses a micromanipulator to remove the nucleus of an egg cell since when I was in Japan. At the University of Hawaii, I was engaged in micro-insemination with a micromanipulator, which is a technique to fertilize an egg by injecting a sperm into an egg under a microscope." In the nuclear transfer procedure of somatic cell cloning, the nucleus of an egg is just replaced with the sperm used for micro-insemination. "Honestly, I just tried to combine a technique I already had and the piezo-drive technique, which happened to lead to successful somatic cell cloning," he explains.

A micromanipulator is a device controlled by a joystick that enables accurate control of movement, often to an accuracy of less than 1 μm (Fig. 2). A piezo-drive is a driving mechanism with a pipette mounted on the tip of a piezoelectric element, which can be vibrated at a high speed by electricity. When a pipette is stuck into a cell, the cell usually collapses. However, using the piezo-drive, you can instantly make a hole through the cell membrane, and efficiently remove or inject a nucleus without collapsing the cell (Fig. 3). Of course, operating the micromanipulator, or the piezo-drive in particular, is not easy, and requires well-practiced skills.

Successful somatic cell cloning with mice has great significance. It was viewed as a supplementary experiment to sheep cloning, because doubt had been cast on whether or not Dolly was a somatic cell clone. Further, since mice are common experimental animals, it allowed cloning research to start in earnest.

Success in improving the success rate of mouse cloning

In 2002, Wakayama established the Laboratory for Genomic Reprogramming at RIKEN Center for Developmental Biology. "Our biggest challenge is to improve the success rate of mouse cloning from somatic cells," says Wakayama.

The success rate of somatic cell cloning is very low. For example, in the first experiment with sheep cloning, 227 nuclei were transferred to egg cells, but only Dolly was born. Thus, the success rate was about 0.4%. In Wakayama's experiments with mice, the rate improved to around 2%. Cows and goats cloned from somatic cells were also produced using this technique, but the success rate was in the range of 2–10%. Unfortunately, each individual had some kind of abnormality, such as obesity or a breathing disorder, and some died young. The key to understanding the cause of these problems is a process known as 'reprogramming'.

Reprogramming is the process of regenerating the nucleus of a cell that is differentiating into various cells. That is, scientists return the nucleus to its anaplastic state at fertilization. "Eggs and sperm are specialized cells, in which differentiation has developed. However, when an egg is fertilized, the nucleus is reprogrammed so that the fertilized egg can form into a new individual," explains Wakayama. Imperfect regeneration of the nucleus transferred to an egg is considered to be the cause of the low success rate of somatic cell cloning and the presence of abnormalities. "If the nucleus were perfectly reprogrammed, the abnormalities would disappear," he notes. "However, we do not understand at all how the nuclei of egg or sperm cells are reprogrammed."

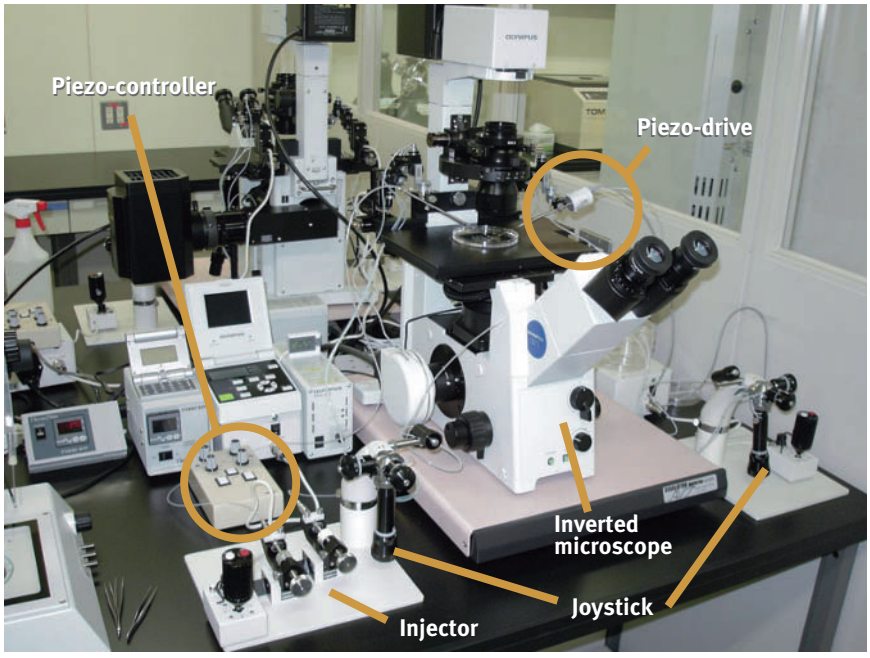


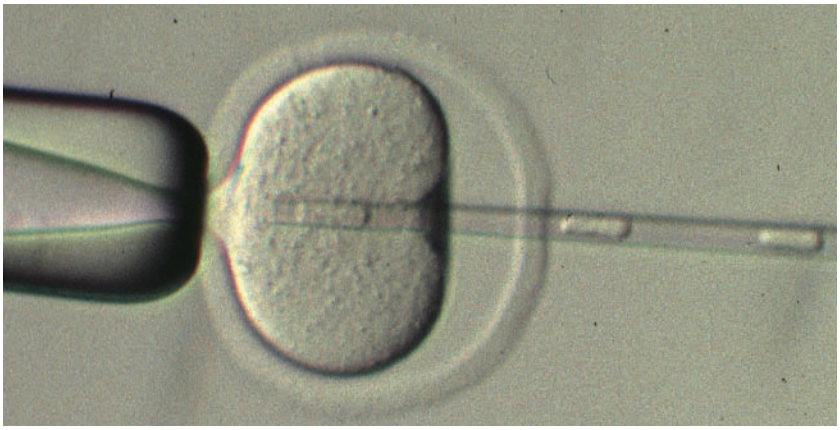
Figure 2: Micromanipulator system.
Enucleation and nuclear cell transfer (Fig. 3) are conducted by operating the joystick of the micromanipulator and the injector by hand and the switch of the piezo-drive by foot, and confirming the images observed through the microscope and listening to the changes in the operating sounds produced by the piezo-drive. Operating this system requires extreme concentration and well-practiced operational skills.

Scientists all over the world are conducting research into the reprogramming mechanism of nuclei. Although many scientists are adopting molecular biological approaches used for investigating the expression of genes and functions of proteins, Wakayama is taking a completely different approach. His team has the world-leading micromanipulating technique so his plan is to take advantage of this technique, and produce many somatic cell clones. Conducting experiments under various conditions and investigating the changes in the success rate may lead to the discovery of key factors related to genomic reprogramming he says.

In 2005, the Laboratory for Genomic Reprogramming succeeded in improving the success rate of cloning mice from 2% to 6%. Wakayama explains that the condition the team varied was the addition of a chemical called trichostatin A to the culture solution of cloned embryos—and it served to enhance the genomic reprogramming. "This was the first example that clearly improved the success rate since the research into somatic cell cloning started, and it was expected to add a fresh dimension to our understanding of the mechanism of genomic reprogramming."

The Laboratory for Genomic Reprogramming was successful in achieving this breakthrough due

Figure 3: **Transfer of a nucleus to an enucleated egg.**



to Satoshi Kishigami, a research scientist at Wakayama's laboratory, who determined the optimum conditions for genomic programming in which condensation and processing time are the two most important factors. "In the search for factors related to genomic reprogramming, we need to tenaciously try to test various conditions," says Wakayama. Thus, a large number of nuclear-transferred egg cells are needed. His team is capable of transferring about 1000 nuclei a day. "This number is amazing because no such capabilities in laboratories have so far been reported in the world," says Wakayama firmly. There are only four laboratories in the world (including Wakayama's) in which somatic cell cloned mice can be produced.

Expectations for ES cells of somatic cell origin

The team has further improved the success rate of cloning mice from somatic cells. Wakayama looked to ES cells, or embryonic stem cells of somatic cell origin to reliably create cloned mice. Instead of returning cloned embryos to the uterus, the team cultured them until they developed into blastocysts, thus creating ES cells (Fig. 1). Although the success rate for somatic cell clones was 6%, the rate for ES cell-based clones increased to around 50%. "Once created, ES cells can increase in large numbers," explains Wakayama. "Thus, in theory, we can create an infinite number of clones by using their nuclei."

With a degree in agriculture, Wakayama is interested in applications to the animal industry. "How nice it would be, if the research into mouse cloning which I am conducting now could benefit society, and as a result I could have the chance to enjoy delicious Kobe beef at a reasonable price," he muses.

Wakayama also acknowledges potential medical applications of his work since ES cells can differentiate into any type of cell. If ES cells are created from somatic cells taken from a patient, and returned to the patient's body after they have differentiated into necessary cells or tissues, the patient can receive medical treatment without any immunologic rejection reaction. Fertility treatment will also be available if egg or sperm cells are created from ES cells. "In our laboratory, we created ES cells from the tail cells of a sterile mouse that could not create sperm cells, and made the ES cells differentiate into sperm, which were then used to fertilize egg cells, thus successfully creating a cloned mouse."

Challenging the limits

Whenever possible, Wakayama welcomes people who want to learn nuclear transfer technology. "I myself learned various techniques from others," he says. "Thus it is very natural for me to teach this newly developed technique to others. Furthermore, if someone other than me fails to reproduce what I have done, that person will consider it to be a lie. I think an engineer finds it nice to see the technology he developed popularized."

To explain why he has an illustration of the solar system next to his own micromanipulator, Wakayama says: "I wanted to be an astronaut. I once applied for the astronaut program; I flunked the English test, though. From my childhood, I was a big fan of science fiction and I always dreamt of going into space. I remember I was very excited to see the word 'clone' in a science fiction story. My dream of going into space did not come true, but I have the feeling that I have been doing what I love to do and so, as it turned out, I find myself here."

And, his dream for the future? "Developing new technology that can make a significant difference even in what seemed impossible. I would like to challenge the limits in the field of technology." ■

Background information

Japanese Patent, pending No. 2006-071193
Japanese Patent, pending No. 2005-287351
Japanese Patent, pending No. 2005-042661

About the researcher

Teruhiko Wakayama was born in Kanagawa in 1967. He received his BSc in animal science in 1990 and MSc in reproduction in 1992, both from Ibaraki University. In 1996, he earned his PhD in reproductive biology from the University of Tokyo. From 1996 to 1998, Wakayama worked as a postdoctoral fellow at the University of Hawaii's Medical School, before being appointed as an assistant professor at that university in 1998. In 1999, he became a research assistant professor at the Rockefeller University, New York, and then moved to California in 2001 to work as a researcher at Advanced Cell Technology. In 2001, Wakayama returned to Japan and was appointed as the head of the Laboratory for Genomic Reprogramming at the RIKEN Center for Developmental Biology. He also teaches at Shiga Medical University as an adjunct professor and at Kyoto University and Kwansei Gakuin University as an adjunct assistant professor.

GSC starts monitoring NMR usage

The Genomic Sciences Center in Yokohama has implemented a monitoring system for its nuclear magnetic resonance facilities, in preparation for making them available to external users.

GSC owns forty high-performance NMR machines, more than any other organization in the world. These machines are currently used for analyzing the 3D structures and functions of proteins. This analysis requires judging whether a target protein is suitable for NMR, creating a stable isotope sample, taking multi-dimensional NMR measurements, and determining the 3D structure of the protein. At GSC all these stages can be carried out in a single process.

Since 2002, GSC has been carrying out a large part of Japan's National Project on Protein Structural and Functional Analyses, and analyzing about 300 proteins each year using the NMR machines. GSC's protein analysis facilities are among the most efficient in the world.

However, this national project will end next spring. After that, the NMR machines will be opened up for use by life science researchers from other organizations. Staff have begun monitoring how the machines are used to decide how many users should be allowed at a time, and to predict what problems might arise when the machines are made available to external researchers. ■

GSC and the University of Edinburgh sign an agreement on collaboration in systems biology

The RIKEN Genomic Sciences Center has concluded a collaboration agreement with the University of Edinburgh on research in systems biology that is expected to lead to new ways of treating diseases.

Last year GSC set up the RTK Networks Consortium, to coordinate research on the signaling pathways used by receptor tyrosine kinase and their connections with cancer and other disorders. Edinburgh's Centre for Bioinformatics, which focuses in particular on breast cancer, has also been participating in this consortium. The two institutes decided to make this new agreement to expand their collaboration further. Data and discoveries produced by GSC will be used in Edinburgh for systems biology research on new medical treatments. ■

The first X-Ray Free Electron Laser symposium

The X-Ray Free Electron Laser (XFEL) is a major national project and will be the first X-ray laser in the world. RIKEN is constructing the XFEL at its SPring-8 site together with the Japan Synchrotron Radiation Research Institute (JASRI), and the researchers are hoping that it will enter operation in 2010. RIKEN and JASRI already run SPring-8, the world's strongest X-ray light source. Like SPring-8, XFEL will create radiation beams by accelerating electrons. But XFEL will produce far brighter beams than SPring-8. It is expected to enable major steps forward in protein engineering, material science, nanotechnology, and medicine.

At the beginning of November the project team for the X-Ray Free Electron Laser held a large symposium, attended not just by scientists but also by many local residents and government employees. XFEL staff reported on the ideas behind the XFEL, the beam performance diagnosis that was carried out on the prototype machine in June, and the overall progress of work (see 'Highlight of the month' on pages 1–2). There was a discussion about the direction of the research in life and materials science that will use the XFEL.

Researchers from industry explained that the prototype machine will be extremely useful as an EUV light source.Members of the public asked how experiments using radiation affect the human body, and several scientists asked technical questions about the performance and specifications of the new laser. ■

Japan–Korea–Taiwan Symposium on Strongly Correlated Electron Systems

The seventh Japan–Korea–Taiwan Symposium on Strongly Correlated Electron Systems was held at the Harima Institute at the end of October. 'Strongly correlated' means that the effective Coulomb interactions between the electrons are especially strong. Many transition metals have this characteristic.

This annual series of symposiums was started by a group of material physicists from Japan, Korea, and Taiwan, to exchange information and learn about each other's research. The organizers have been encouraging young scientists to enter this field, and as a result the symposiums have been getting bigger each year.

In this year's presentations, Hidenori Takagi from RIKEN discussed iridium and rhodium oxides, and C.H. Chen from Taiwan explained the special characteristics of their silicon compounds. D.F. Feng, from China, talked about his photo-analysis of layered transition-metal compounds and his interpretation of the results. From Korea, J.G. Park explained his ideas about the interesting physical properties of the rubidium compound $Tl_2Tu_2O_7$ and the manganese compound $RMnO_3$. The symposium closed with a lively question-and-answer session. ■

BSI retreat in autumn

This autumn, researchers at BSI took their annual retreat in the countryside of Wako. Over two hundred people convened for three days to discuss neuroscience. BSI's researchers were joined by researchers from RIKEN MIT in Cambridge, MA and a special guest from University of California, San Francisco, Stephen G. Lisberger.

There were more than four hundred posters plus seventeen research presentations and two special lectures from Lisberger and Susumu Tonegawa. The sessions covered many diverse aspects of brain science: from single neuron investigations and local circuit models, to molecular studies on synapse formation and memory, to robotics and economic studies of behavior and perception.

On the second day, four discussion sessions covered the broader concerns. Two sessions shared stories on scientific inspiration and tips for better work-life balance in and out of the lab. The other two panel discussions examined the scientific basis of emotion and the role of long-term potentiation in the cerebellum.

The BSI retreat is an annual ritual for its researchers. Getting out of the lab encourages researchers from different labs to interact and learn more about the work on-going at BSI. Newly opened labs are introduced, hot-topics shared, and camaraderie between the laboratories increases. ■

The challenge to harness photosynthetic energy

By accepting the challenge to develop a new solar energy source, RIKEN researchers also created an effective model for conducting large, international research collaborations

For the two decades prior to 1999, researchers at RIKEN investigated the mechanism of photosynthesis in a bid to create a new energy source. Their work was supported by two big international projects with colleagues in the US and Europe. Various problems emerged during project negotiations and arrangements, but the researchers' efforts resulted in a model for international collaborations for RIKEN and other Japanese research institutes.

Research into photosynthesis—a process by which plants make energy from light, carbon oxide and water and produce oxygen as a byproduct—came into the spotlight in the 1970s amid concern over the shortage of fossil fuels due to rapid economic growth. Coupled with the first oil shock that occurred in 1973, many countries, including Japan, started seriously considering solar power as an alternative energy source.

In 1969, researchers at RIKEN began studying photosynthesis as a basic theme of plant physiology. They discovered that plants have a photosynthetic reaction center, a type of membrane protein, which converts solar energy into chemical energy. The researchers thought they could apply this research to alleviate the energy issue, so they developed a plan and won the budget for it in 1976.

At a summit meeting in 1978, Japan's then Prime Minister Takeo Fukuda proposed to his US counterpart, Jimmy Carter, that the two countries conduct joint research into photosynthesis with a view to biotically converting solar to chemical energy. The proposal was based on advice from RIKEN researchers, including plant physiologist Yorinao Inoue.

After the US accepted the proposal, the researchers spent much time with government and funding agencies deciding the proportion of the research budget to be shouldered by each country, and the rules to hold international seminars and exchange researchers. An agreement was finalized in 1979. RIKEN then set up a new research group focusing on biotic solar energy science in 1980. In 1983, Japan also signed an agreement with Europe on joint photosynthesis research.

From 1979 to 1988, RIKEN researchers strived mainly to reveal the structure of the photosynthesis reaction center so they could devise a way to use solar energy to dissolve water and yield hydrogen (Fig. 1).

But producing hydrogen in this way was so difficult the researchers quickly shifted their focus to fundamental research on the process of the photosynthetic reaction. To this end, RIKEN's biologists developed a method to effectively measure the generation of thermoluminescence in chloroplasts. This method has since been adopted widely in the US and Europe.

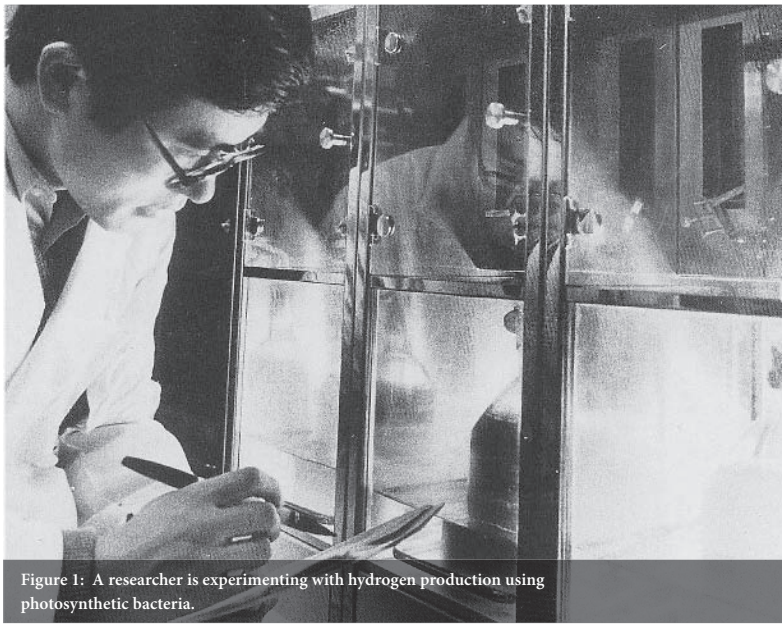


Figure 1: A researcher is experimenting with hydrogen production using photosynthetic bacteria.

For the next ten years from 1989, due to growing concern about increasing concentrations of atmospheric carbon dioxide, RIKEN researchers focused more on a chemical and physical approach with an aim to contain global warming. For example, they commenced research using electrodes to convert carbon dioxide into a useful resource.

Meanwhile, to compensate for the unsuccessful hydrogen from water project, Inoue was planning a new challenge: determining the mechanism of the manganese cluster, which is a catalyst plants use to dissolve water and produce hydrogen. After Inoue retired in 1999, other researchers continued his work, and from determining its crystal structure, suggested a way to artificially create the catalyst's molecular structure in 2002.

RIKEN's photosynthesis project ended in 1999, following the conclusion of the Japan—US collaboration. Currently, photosynthesis is studied at only a few RIKEN laboratories. But lessons learned from the earlier international collaborations were used to establish the foundation for RIKEN's international frontier research system. As such, researchers from around the world are now invited to RIKEN to participate in fixed-term projects.

Hydrogen energy is also regaining attention as an alternative energy source—particularly for fuel cells. RIKEN's researchers hope that their past achievements will continue to contribute to society in the future. ■

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