RIKEN RESEARCH

AUGUST
2007 Volume 2 Number 8

Big bang secrets told with gold

www.rikenresearch.riken.jp

RIKEN

Artificial lymph nodes induce strong immune response

Researchers open the way to new therapy for infection and cancer

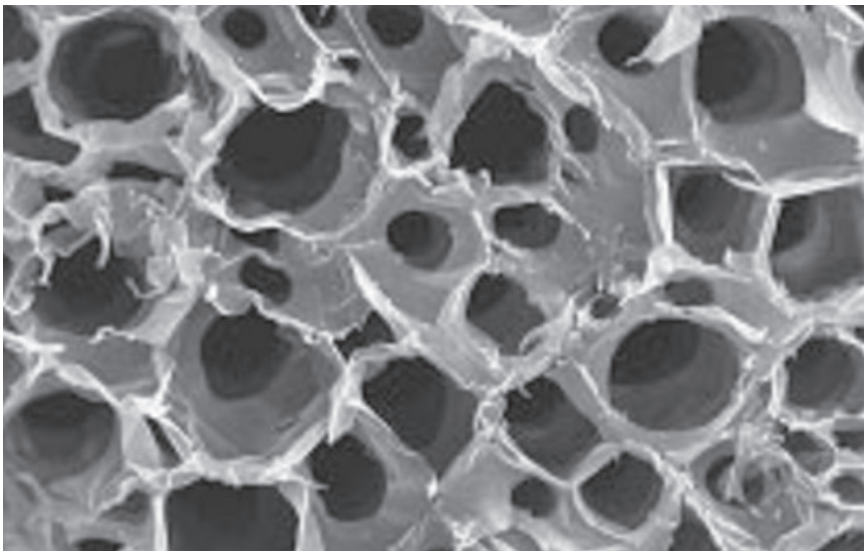


Figure 1: Micrograph of a section of the collagen scaffold used to construct the artificial lymph nodes.

A RIKEN research team, which developed functioning artificial lymph nodes (aLNs), has now shown their nodes can generate immune cells and strong immunological responses when transplanted into mice lacking a working immune system[1].

The research is a significant step towards being able to strengthen or perhaps even replace human immune systems. It has particular relevance to the fight against AIDS, cancer, and intractable infectious diseases, and also to treating allergies. "We want to make a prototype human model within two or three years," says project leader Takeshi Watanabe.

The researchers, from RIKEN's Research Center for Allergy and Immunology in Yokohama, constructed their mouse aLNs by impregnating two- to three-millimeter-diameter scaffolds of the fibrous structural protein collagen with dendritic cells and stromal cells extracted from the thymuses of newborn mice (Fig. 1).

Earlier work suggested that it is the stromal cells that are responsible for organizing the structure of lymph nodes, which house immune system cells involved in screening the body for pathogens and toxins. The researchers tested this hypothesis by implanting aLN scaffolds into mice transgenic for green fluorescent protein, so the cells of the recipient mouse were easily distinguishable from those which had been introduced. They found that most of the immune cells in the developing aLNs derived from the recipient mouse, suggesting the role of the implanted tissue was not to generate immune cells, but to organize those already present into an operational unit.

Testing the artificial lymph nodes

The researchers then checked how well their aLNs functioned. For this work, the aLNs were implanted initially into mice with a normal, healthy immune system, which had previously been injected with a harmless antigen compound to trigger an immune response. So the aLNs became populated with immune system T-cells and B-cells which specifically recognized and countered microbes or cancer cells expressing the injected antigen (Fig. 2).

These primed aLNs were then transplanted into two sets of mice—a group with a normal immune system which had never been exposed to the antigen, and a group in which the immune system did not function. When later exposed to the antigen both groups responded immediately by making the appropriate protective antibodies—and the response to the antigen lasted for longer than four weeks, which suggests that specific immune cells had been generated which retained the 'memory' of the antigen (Fig. 3).

Tracking the migration of immune cells

Further investigation using aLN tissue tagged with green fluorescent protein showed that in immunodeficient mice, T- and B-cells from the aLNs migrated to repopulate the spleens and bone marrows which lacked such immune cells. Once in these organs, the immune cells generated large numbers of antigen-specific antibody-forming cells. When tagged aLNs were transplanted into mice with normally functioning immune systems, however, the antibody-forming cells did not migrate, but remained in the aLNs. So the migration

of immune cells to take up residence in the spleen and bone marrow only occurred in mice which were immune-cell free.

The researchers then studied the migration process further. Previous work had suggested that the movements of migrating immune cells are directed by the signals generated by groups of receptors associated with guanine-nucleotide-binding or G proteins. The research team was able to test this idea using a bacterial toxin which blocks the action of G proteins. Culturing aLNs in this toxin before implantation dramatically decreased the numbers of immune cells which migrated to the spleen and bone marrow in immunodeficient mice.

The team undertook a similar experiment using a compound which blocks one specific type of G-protein-associated receptor based around sphingosine-1-phosphate. Again this action inhibited migration of immune cells from the implanted aLNs to the spleen and bone marrow of immunodeficient mice.

The successful development of the aLNs in mice opens the way to producing customized lymph nodes impregnated with antibody-forming cells and other compounds specifically geared to treating certain conditions. "That is our purpose," says Watanabe, "not necessarily to make replacements for natural lymph nodes, but rather more functional organs applicable to particular diseases and allergies." ■

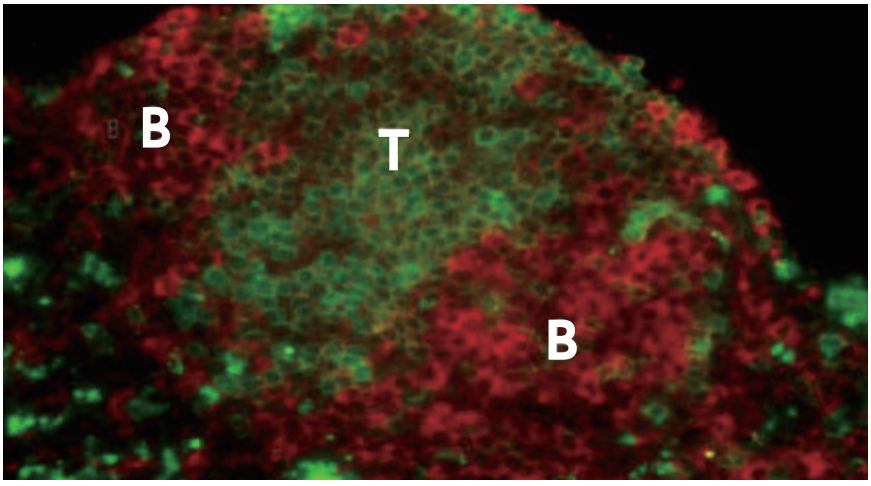


Figure 2: A section through the tissue of a developed artificial lymph node showing domains of T-cells and B-cells (labelled).

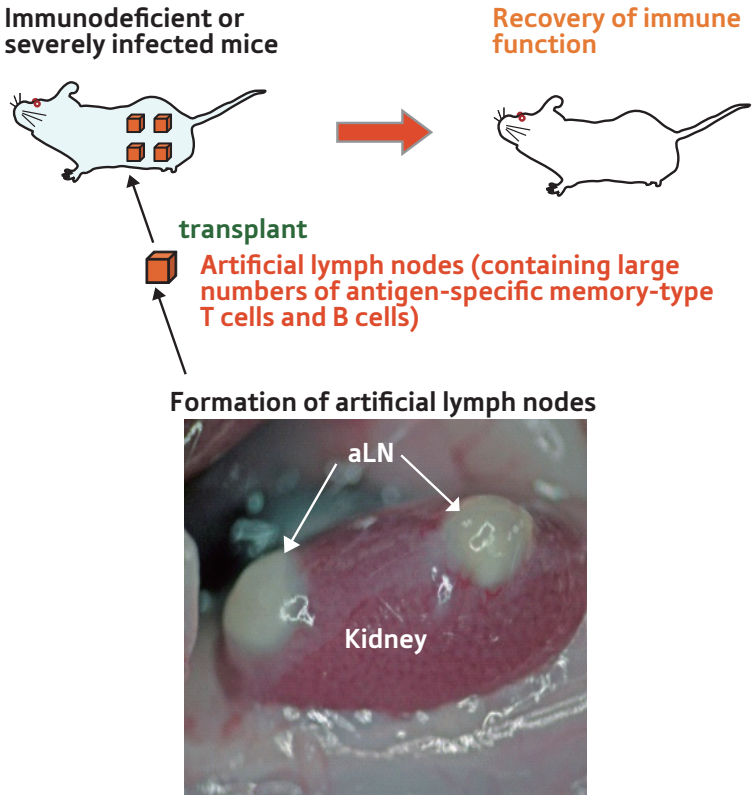


Figure 3: Artificial lymph nodes could be used in therapy to target specific disease conditions.

1. Okamoto, N., Chihara, R., Shimizu, C., Nishimoto, S. & Watanabe, T. Artificial lymph nodes induce potent secondary immune responses in naïve and immunodeficient mice. *The Journal of Clinical Investigation* **117**, 997–1007 (2007).

About the researcher

Takeshi Watanabe was born in Osaka, Japan, in 1940. He graduated from Osaka University's School of Medicine in 1965 before obtaining his MD and PhD degrees from the same university in 1966. He then worked as a physician at the university until 1969, before he left to undertake post-doctoral training at Roswell Cancer Institute in Buffalo, US. In 1972, Watanabe returned to the Osaka University School of Medicine and served as an assistant professor until 1980. During that time, he also worked as a researcher at Base Institute for Immunology in Basel, Switzerland for two years. In 1980, he attained his full professorship at Saga Medical School, Japan. From 1985 to 2004, he worked as a professor at the Medical Institute of Bioregulation, Kyushu University in Fukuoka, Japan, where he concurrently served as the director from 2001. Watanabe is currently a unit leader at the RIKEN Research Center for Allergy and Immunology.

Molecular double act in crystals

Conducting and magnetic electrons co-exist in crystals containing two differently arranged layers of the same metal complex

The properties of a material not only depend on what it is made from, but also on how its atomic (or molecular) building-blocks are arranged. For example, diamond and graphite are two very different substances, but each is made only from carbon atoms. The different organization of the atoms in each material have profound implications for their properties—diamond is an electrical insulator and one of the hardest substances known, whereas graphite is a very soft material that conducts electricity.

Although he uses more exotic building-blocks than carbon atoms, Reizo Kato from RIKEN's Discovery Research Institute in Wako is studying similar effects in molecular crystals. "The electronic properties of these materials can be strongly influenced by their structure," says Kato, "a molecular assembly can be conducting or insulating, depending on how the individual molecules are organized."

In their most recent work in this area[1], Kato and colleagues investigate how the arrangement of a nickel-containing metal complex—widely used in conducting or magnetic materials—can be controlled in a crystal. These molecules have sulfur atoms at both ends, which are known to form intermolecular interactions—known as 'halogen bonds'—with iodine atoms.

In what Kato describes as a breakthrough, a graduate student in his laboratory, Yosuke Kosaka, made a salt from the negatively charged nickel complex and a positively charged molecule containing two iodine atoms. The structure of this compound was investigated using x-ray crystallography, which revealed that the iodine–sulfur interactions direct the nickel complexes to form two kinds layers—with very different molecular arrangements—that alternate (Fig. 1) throughout the crystal.

In one layer, the nickel complexes are stacked on top of one another and group together in strongly associated pairs called dimers. This layer acts as an electrical insulator and electron spins on neighboring dimers show paramagnetic behavior with a tendency to align in opposite directions—described as antiferromagnetic interaction.

In the other layer, however, the nickel complexes form a non-columnar structure in which some molecules overlap with more than one neighbor and instead span across two. This layer exhibits two-dimensional metallic conduction down to a temperature of 4.2 K, resulting in an overall crystal that contains alternating insulating and conducting layers.

These two layers are composed of the same molecule, yet their physical properties are remarkably different because of the dissimilar molecular arrangements. "This is a molecular version of Dr Jekyll and Mr Hyde," says Kato, "and is the first example of such behavior in molecular crystals." ■

1. Kosaka, Y., Yamamoto, H. M., Nakao, A., Tamura, M. & Kato, R. Coexistence of conducting and magnetic electrons based on molecular π-electrons in the supramolecular conductor (Me-3,5-DIP)[Ni(dmit)$_2$]$_2$. *Journal of the American Chemical Society* **129**, 3054–3055 (2007).

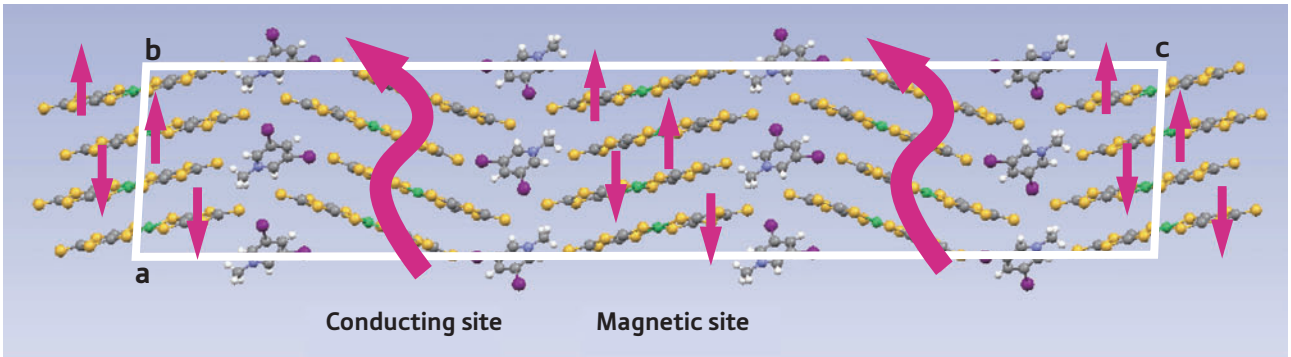


Figure 1: The structure of a 'Jekyll and Hyde' molecular crystal in which alternating layers of the same nickel complex (shown in yellow, green and grey) have different magnetic and electrical properties. The iodine atoms of the cationic 'glue' that holds the layers together are shown in purple.

The long and short of nanowire

New findings on growing and removing molecular wires at different temperatures could be used in nanoscale electronics

A study looking at growing and erasing simple nanowires on a silicon surface has provided insight into their potential use in molecular electronics at differing temperatures. Molecular electronic components attract much attention; however, the key to using them successfully depends on being able to join them, using nanowires, forming circuits.

Recently, Md. Zakir Hossain and colleagues at the RIKEN Discovery Research Institute, Wako, succeeded in growing interconnected nanowires—an important step towards the development of nanocircuits[1]. It is important that these circuits can withstand heat so that they are useful for electronics applications.

Hossain and colleagues investigated these simple nanowires using scanning tunnelling microscopy (STM) to reveal their thermal stability and to gain understanding of how best to form nanowires. The team used a technique known as 'dangling bond initiated chain reaction' to create their molecular wires. This technique uses a silicon surface coated with hydrogen, where the surface appears as rows of hydrogen and silicon (H-Si-Si-H) units.

By removing one hydrogen atom a radical is left behind on the silicon. This radical reacts with a building-block for the molecular wire that contains a carbon-carbon double bond. As a result, the building-block then possesses a free radical which further reacts to remove hydrogen from an adjacent silicon atom.

The end result is one molecule of building-block added to the surface and another silicon radical is generated, a so-called dangling bond. The dangling bond can then continue the cycle adding further molecules of building-block forming a molecular line, or wire (Fig. 1).

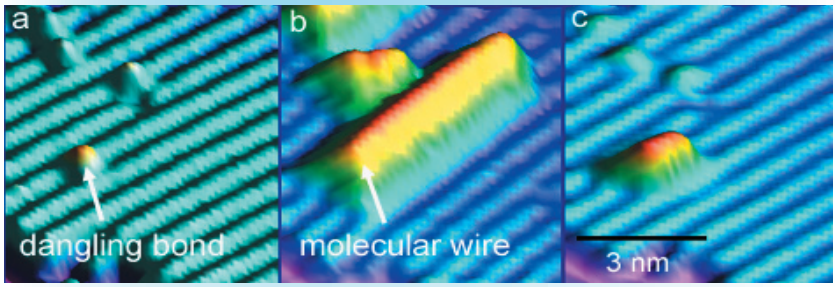


Figure 1: Scanning tunneling microscopy images showing (a) a dangling bond site, (b) the growth of a molecular wire from that site, and (c) the wire after shortening.

The dangling bond that remains at the end of the line can play an important role in the stability of the nanowire. Hossain observed the molecular lines' growth with various building-blocks at different temperatures. At lower temperatures the lines grew, whilst at higher temperatures the dangling bond reaction worked in reverse effectively erasing the wire.

Therefore growth of a wire depends on the stability of the terminal dangling bond and the rate of the reverse reaction. These results imply that the scope to grow nanowires using this technique could be wider than anticipated. The properties and growth of wires could be engineered using different building-blocks and temperatures, and capping the terminal dangling bond of a wire could also help make them more stable.

The next step is to look at other building-blocks. "Using various molecular systems, we intend to fabricate complex molecular lines leading to functional molecular circuits," says Hossain. ■

1. Hossain, M. Z., Kato, H.S. & Kawai, M. Competing forward and reversed chain reactions in one-dimensional molecular line growth on the Si(100)-(2 x 1)-H surface. *Journal of the American Chemical Society* **129**, 3328–3332 (2007).

The golden charm of high-energy particle collisions

Collisions of gold nuclei provide a unique look at the earliest stages of our universe following the big bang

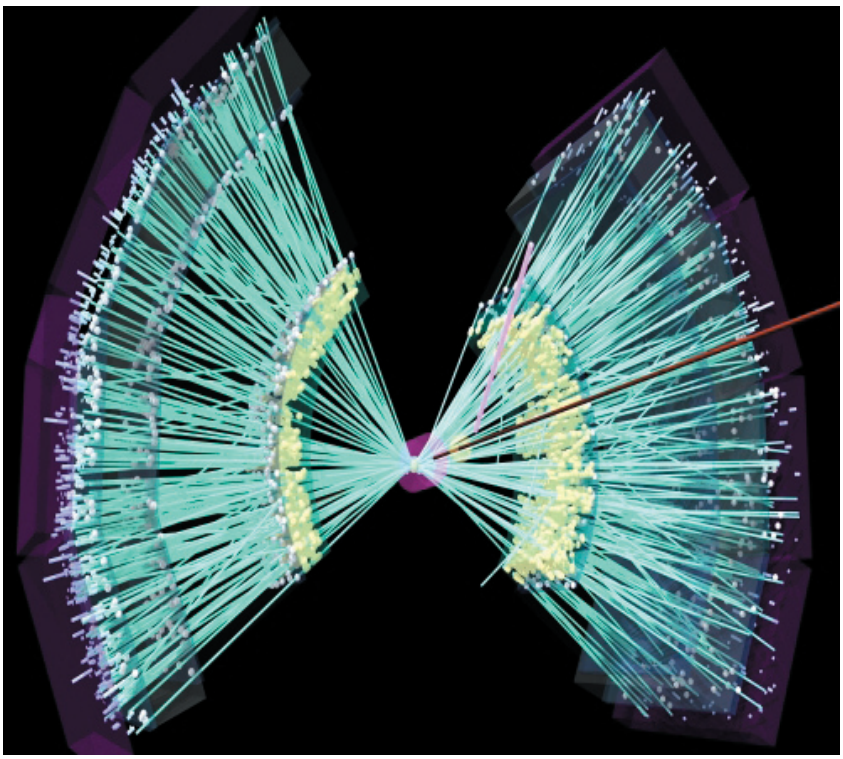


Figure 1: Collision of gold nuclei. The lines track the various particles generated by the collision that are tracked by the detector (blue, purple features).

Investigating the earliest stages of our universe is the mission of the international collaboration PHENIX, to which RIKEN contributes. Based at the US Brookhaven National Laboratory, the PHENIX researchers study the collision of gold nuclei at high energies with the aim of generating a type of plasma that they believe will replicate the universe at its earliest stages—before protons and neutrons were formed.

The plasma consists of the elementary particles known as quarks and gluons that normally exist inside protons or neutrons. Only the large energies at which the gold nuclei collide in the experiments can release the quarks and gluons and therefore create the right environment, albeit for a very short time, to study their interactions in the plasma (Fig. 1).

The researchers are particularly interested in the behavior of the heavier quarks; in this case, the 'charm' and 'bottom' quarks. These heavy quarks do not occur in normal matter, so their creation in such collisions provides experimental input for the verification of theoretical models describing the plasma properties.

However, before the quark–gluon plasmas can be studied in detail, the theoretical models need to be verified. The first step is to compare the results with those of proton collisions where no plasma is formed. These results, recently reported in the journal *Physical Review Letters*[1], provide the basis for correctly understanding the data obtained using the gold collisions, explains Yasuyuki Akiba from RIKEN's team at PHENIX.

The team's next step was to analyze the data obtained from the collisions of gold nuclei. They found that heavy quarks lose energy when propagating through the plasma[2]. Furthermore, the heavy quarks participate in the collective flow of the plasma, which proves that the charm and bottom quarks interact strongly with the plasma. This strong interaction between heavy quarks and the plasma suggests that the plasma behaves like a fluid with almost no viscosity.

These findings provide a significant insight into the properties of the plasma. However, as a next step it will be important to analyze each of the heavier quarks individually, because the heavier quarks are currently indistinguishable in the experiments. Therefore, "the PHENIX consortium is currently building a new detector for heavy quark measurements that can distinguish charm and bottom quarks in gold collisions," says Akiba. Already, the present results are of tremendous value to the theoretical understanding of the quark–gluon plasma. ■

1. Adare, A., Afanasiev, S., Aidala, C., Ajitanand, N.N., Akiba, Y., Al-Bataineh, H., Alexander, J., Aoki, K., Aphecetche, L., Armendariz, R., *et al.* Measurement of high-p_T single electrons from heavy-flavor decays in $p + p$ collisions at $\sqrt{s}$ = 200 GeV. *Physical Review Letters* **97**, 252002 (2006).
2. Adare, A., Afanasiev, S., Aidala, C., Ajitanand, N.N., Akiba, Y., Al-Bataineh, H., Alexander, J., Al-Jamel, A., Aoki, K., Aphecetche, L., *et al.* Energy loss and flow of heavy quarks in Au + Au collisions at $\sqrt{s_{NN}}$ = 200 GeV. *Physical Review Letters* **98**, 172301 (2007).

PHENIX spies unique light from collisions

Building blocks of matter studied in hot plasma of creation

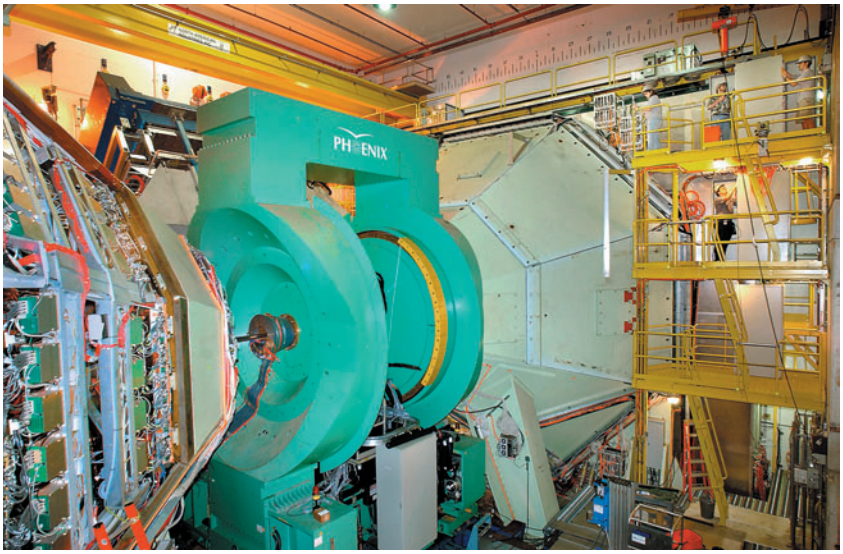
Scientists using intense collisions to study the fundamental constituents of matter say that our current theory about the make-up of the proton, the particle buried at the heart of every atom, is right on track.

Atoms are composed of a nucleus of protons and (usually) neutrons, surrounded by a cloud of electrons. Drill inside protons and you find quarks, held together by gluons. To find out more about how quarks and gluons behave, scientists working at the Relativistic Heavy Ion Collider (RHIC) at the Brookhaven National Laboratory (BNL) in Upton, US, smash gold atoms together to heat the matter to thousands of times the temperature of our sun, conditions mimicking those which existed just a fraction of a second after the birth of the universe.

In this state, quarks and gluons exist as a hot plasma. But they behave differently in other situations which are less hot and dense, such as collisions between protons themselves. Comparing the two cases can help to understand exactly how quark–gluon plasmas behave.

To provide a baseline for the gold collision experiments, the PHENIX (**P**ioneering **H**igh **E**nergy **N**uclear **I**nteraction e**X**periment) detector at RHIC (Fig. 1) studies collisions between protons traveling at 99.995% the speed of light, which produce explosions of other particles and particles of light called photons.

These photons, produced directly by the interaction of gluons and quarks in the impact, carry valuable information about the structure of the proton. And since photons are less likely to interact with matter than the other particle debris, they are unique probe of the quark–gluon plasma.



Brookhaven National Laboratory

Figure 1: The PHENIX detector at the Relativistic Heavy Ion Collider (RHIC).

"But the majority of the products of proton collision are pions—the lightest nuclear particles," says Kensuke Okada, who works at RIKEN's research centre at BNL and is part of the international PHENIX team. These pions decay into a pair of photons, confusing the photon signal from the experiment.

Okada and his colleagues have now developed a method to subtract the pion's photons from their results. The tactic allowed them to pick through the debris to study only those direct photons produced by quark–gluon interactions[1].

The team's results match the predictions of quantum chromodynamics, the theory that describes quark–gluon interactions and behavior, and support the idea that new states of matter are formed in the gold–gold collisions. Scientists can now use the photons produced in these collisions to hunt for more information about the way that gluons and quarks affect the properties of the proton, such as its spin, says Okada. ■

1. Adler, S.S., Afanasiev, S., Aidala, C., Ajitanand, N.N., Akiba, Y., Al-Jamel, A., Alexander, J., Aoki, K., Aphecetche, L., Armendariz, R., *et al.* Measurement of direct photon production in $p + p$ collisions at $\sqrt{s} = 200$ GeV. *Physical Review Letters* **98**, 012002 (2007).

Choreography of electrons in one dimension

A novel theory successfully describes the different interactions governing electrons in narrow quantum wires

A team of researchers from Argonne National Laboratory, US, RIKEN's Discovery Research Institute, Wako, and the University of Minnesota, US, has presented a unifying theory that is able to describe the electronic states in a nanostructure known as a quantum conducting wire for a broad range of parameters.

There have been previous approaches to describe the quantum states of electrons in these wires, most notably the so-called Tomonaga–Luttinger theory. However, these theoretical descriptions are commonly limited to a narrow range of system parameters, namely very low temperatures and small energies.

The fabrication of nanostructures, including quantum wires that are small enough to confine electrons to a narrow channel, heralds a new physical regime. As a consequence of this confinement, the electrons cannot travel through such quantum wires in an arbitrary manner. Instead, they have to choreograph themselves into finely tuned arrangements in search of the state with the lowest energy. However, the actual state the electrons eventually assume depends strongly on parameters such as temperature and the strength of interaction between the electrons (Fig. 1).

"We have derived a general formula for the electronic states of quantum wires that extends the Tomonaga–Luttinger theory and at the same time greatly simplifies the calculation of the various properties of the system," says RIKEN's Akira Furusaki, a member of the research team. The team's theory is published in *Physical Review Letters*[1].

The unified theory developed by Furusaki and colleagues initially separates the influence of the electric charge of the electrons from their spin—a fundamental quantum property of elementary particles that either can point 'up' or 'down'. Once the individual contributions are combined, however, the new model is able to describe a broad range of different quantum states. For example, it correctly reproduces the Tomonaga–Luttinger theory, where electrons as well as spins show signs of long-range order. In addition, the researchers are also able to predict the properties of the state where the spins are completely disordered.

Importantly, the researchers predict an asymmetry in the energy distribution of the electrons. "No one expected to see such an asymmetry, and it will now be important to verify these predictions experimentally," explains Furusaki. Although experiments on quantum wires have been performed before, they did not approach the extended parameter space made accessible by the new theory. Therefore, as experiments and additional theoretical studies venture into this new regime, further intriguing effects might be uncovered. ■

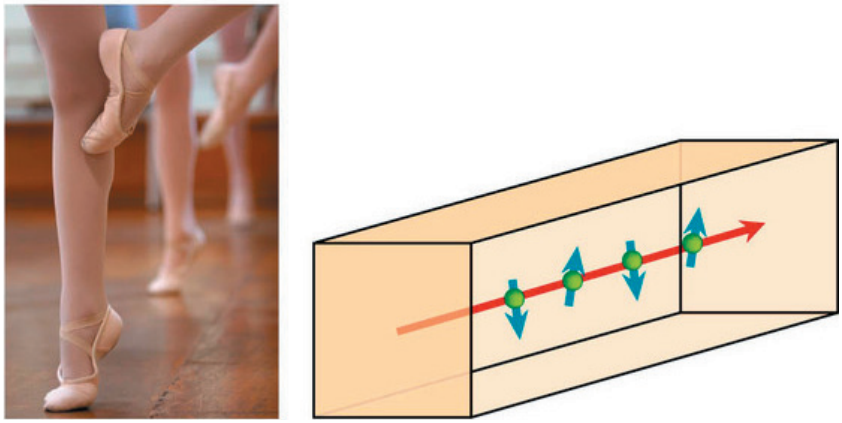


Figure 1: Choreography of electrons. Like dancers in a ballet (left), electrons (green) moving along a quantum wire (right) assume carefully ordered arrangements with each other depending on parameters such as temperature, or the strength of the interaction between the electron spins (blue).

1. Matveev, K. A., Furusaki, A. & Glazman, L. I. Asymmetric zero-bias anomaly for strongly interacting electrons in one dimension. *Physical Review Letters* **98**, 096403 (2007).

Calcium sensor illuminates learning and memory pathway

A new pathway involved in cerebellar learning and memory has been identified with the help of a transgenic calcium sensor

Researchers led by Thomas Knöpfel at the RIKEN Brain Science Institute, Wako, have identified a potential new mechanism for learning and memory in the cerebellum, using a transgenic mouse expressing a protein that acts as a calcium sensor by fluorescing when calcium ions are present[1].

In the cerebellum, a brain structure primarily involved with sensory-motor integration and control of movements, plasticity, or the ability to change strength, at synapses between parallel fibers (granule cell axons) and Purkinje neurons is thought to be the cellular basis of cerebellar learning. It involves a number of molecular mechanisms which permanently alter the ability of a neuron to convey signals, either positively (long-term potentiation, LTP) or negatively (long-term depression).

The molecular changes underlying cerebellar plasticity can occur in either the parallel fibers, as pre-synaptic expression of plasticity, or in the Purkinje neurons, as post-synaptic expression. But while post-synaptic mechanisms are well understood, mechanisms in the tiny pre-synaptic structures formed by the granule cell axons have been difficult to study. A transgenic mouse, which only expresses the calcium sensor in granule cells, allows the researchers to use optical imaging of pre-synaptic activity.

By using the transgenic mouse, as well as pharmacological agents, the researchers have demonstrated the requirement for a signaling cascade involving protein kinase A, NMDA receptors, which bind the neurotransmitter glutamate, and nitric oxide in generating pre-synaptic expression of LTP in parallel fibers (Fig. 1).

But they now have some new questions to answer, as they have determined that the NMDA receptors utilized in the proposed cascade are not found on either the parallel fibers or the Purkinje neurons, but instead are located on a third type of cerebellar neuron, the interneuron.

"Plasticity of the synapse between parallel fibers and Purkinje neurons is the foundation of cerebellar learning theory," says Knöpfel.

"That theory postulates that the cerebellum implements a learning algorithm and memory storage associated with the control of movements. Memory is stored as the strength of synaptic connections between parallel fibers and Purkinje neurons. The involvement of interneurons in pre-synaptic expression of parallel fiber LTP implies a reconsideration of the learning algorithm."

These findings, and complementary findings by other researchers looking at post-synaptic parallel fiber–Purkinje neuron plasticity, may ultimately lead to a new understanding of cerebellar learning mechanisms. ■

1. Qui, D-L., & Knöpfel, T. An NMDA receptor/nitric oxide cascade in pre-synaptic parallel fiber–Purkinje neuron long-term potentiation. *Journal of Neuroscience* **27**, 3408–3415 (2007).

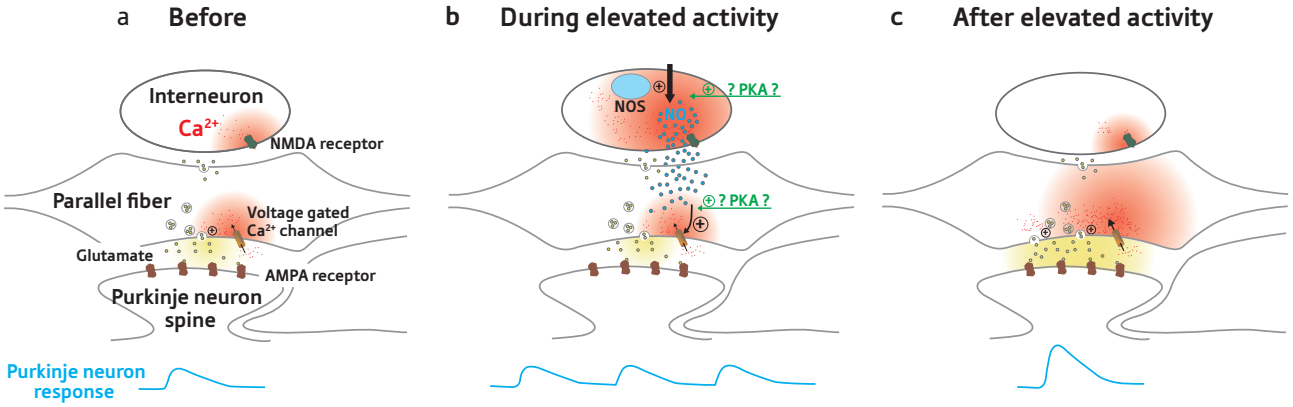


Figure 1: Illustration of the mechanism underlying pre-synaptic parallel fiber long-term potentiation (LTP).
(a) Under baseline conditions, parallel fiber activity triggers an influx of calcium (red dots) via voltage gated Ca^{2+} channels (orange) which in turn triggers the release of the neurotransmitter glutamate (yellow). Glutamate diffuses through the synaptic cleft to activate AMPA receptors (red) at the spine of Purkinje neurons. This leads to a modest excitatory response of Purkinje neurons (blue trace). Activation of NMDA receptors at interneurons causes a modest influx of calcium.
(b) During elevated parallel fiber activity (induction of LTP), larger amounts of calcium (red) flows into interneurons where it activates nitric oxide (NO) synthase (NOS). NO (blue circles) diffuses into parallel fibers and causes a persistent increase in pre-synaptic calcium influx. Protein kinase A (PKA) might support the action of NOS or NO.
(c) During expression of LTP parallel fibers release an increased amount of glutamate, leading to an enhanced Purkinje neuron response.

Asymmetric protein distribution mediates cell division

Japanese researchers begin to unravel a key signaling pathway in asymmetric cell division

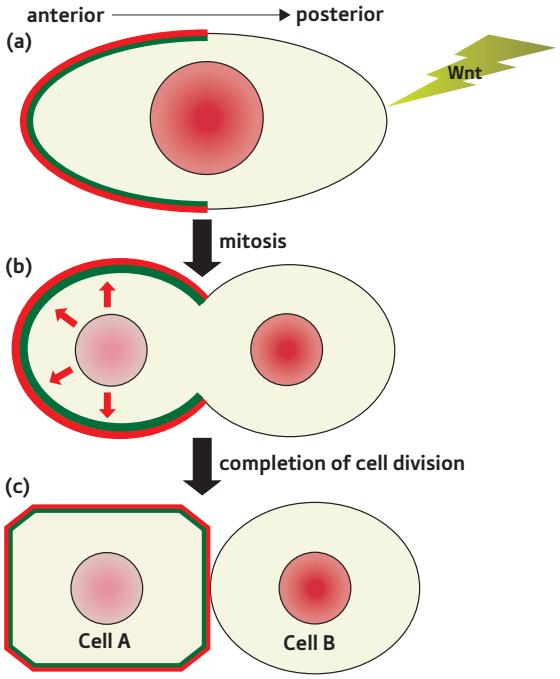


Figure 1: A schematic representation of asymmetric cell division in *C. elegans*.
(a) Wnt signal from one side of the cell polarizes the cell and induces asymmetric WRM-1(ß-catenin) (red) and APR-1(APC) (green) localization to the anterior cortex.
(b) Just before the completion of cell division, cortical WRM-1 (red) and APR-1 (green) stimulate nuclear export of WRM-1 (red arrows) from the anterior daughter cell's nucleus, resulting in the asymmetric WRM-1 localization between the daughter nuclei.
(c) Finally, asymmetric WRM-1 induces asymmetric cell fate determination between the daughter cells.

In many species, the protein ß-catenin plays two roles, in cadherin-mediated cell adhesion and in the Wnt signaling pathway that plays a crucial role in embryogenesis and morphogenesis. The nematode *Caenorhabditis elegans* has four ß-catenin proteins, one involved in cell adhesion and the other three associated with signaling.

In *C. elegans*, an atypical Wnt signaling pathway regulates asymmetric cell division, producing two daughter cells with different cell fates. In worms with mutations in this pathway, many cells divide symmetrically and fail to acquire proper cell fate. Mutations of the mammalian Wnt signaling pathway have been associated with tumor formation.

Kota Mizumoto and Hitoshi Sawa, from the RIKEN Center for Developmental Biology, Kobe, are unraveling the Wnt pathway, focusing on the *C. elegans* ß-catenin protein WRM-1, which localizes to the anterior cortex and the posterior nucleus during cell division, suggesting a role in asymmetrical cell division[1].

Initially, the researchers expressed WRM-1 as a fusion protein in a hypodermal cell of the nematode tail, engineered to localize uniformly throughout the cortex, rather than asymmetrically. Symmetric cell division then resulted in two hypodermal daughter cells—rather than a hypodermal and neural cell. This was also observed in mutant worms lacking the *wrm-1* gene, suggesting the uniform localization of the protein caused the defect in asymmetric cell division.

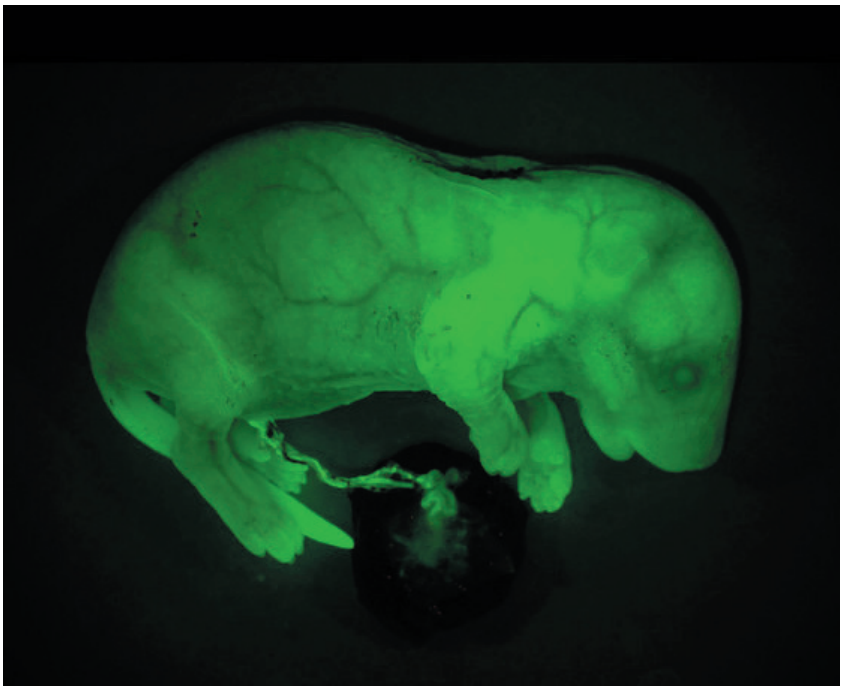
Next, they examined components of the 'destruction complex', which plays an important role in regulating ß-catenin activity by binding to ß-catenin when the signaling protein Wnt is not present. Inhibition or removal of one factor in the destruction complex, the APC homolog APR-1, caused both daughter cells to become neural cells, not hypodermal cells. The researchers also observed that APR-1, and another component of the destruction complex, PRY-1, localize to the anterior cortex, possibly together with WRM-1. Mizumoto and Sawa hypothesize that APR-1 is recruited by WRM-1 to the anterior cortex before and during cell division, while cortical APR-1 stimulates the export of nuclear WRM-1 to the anterior cortex at telophase, the final stage of cell division (Fig. 1).

"So far, it is not clear whether Wnt signaling regulates asymmetric cell division in mammalian cells," says Mizumoto. "However, the Wnt pathway is known to be required for stem cell maintenance. Because stem cells are thought to undergo asymmetric cell division, it is interesting to hypothesize that Wnt signaling maintains stem cell through regulating its asymmetric cell division as in *C. elegans*." ■

1. Mizumoto, K. & Sawa, H. Cortical ß-catenin and APC regulate asymmetric nuclear ß-catenin localization during asymmetric cell division in *C. elegans*. *Developmental Cell* **12**, 287–299 (2007).

When bad eggs are good

Eggs that fail to become fertilized can be used to obtain embryonic stem cells



Teruhiko Wakayama

Figure 1: A mouse developed with embryonic stem cells grown from aged, fertilization-failed oocytes. Expression of the green protein GFP shows that stem cells contributed to all body tissues.

Embryonic stem cells can transform into many different cell types, providing a potentially limitless source of cells for medical applications. However, the process of obtaining stem cells is controversial because it involves the collection of egg cells, or oocytes, from healthy women, and the destruction of embryos that have the potential to produce offspring. Now RIKEN researchers have demonstrated an alternative, by successfully obtaining stem cells from oocytes that failed to become fertilized during IVF treatment[1]. Such oocytes, called 'aged fertilization-failure' (AFF) oocytes, would normally be discarded in clinics.

The team at the RIKEN Center for Developmental Biology in Kobe has significantly improved the process of therapeutic cloning, otherwise known as somatic cell nuclear transfer (SCNT)[2]. The researchers collected AFF oocytes from mice, and used SCNT to replace each oocyte nucleus with the nucleus of a non-reproductive cell taken from laboratory mice engineered to express a protein called GFP that glows green under fluorescent light. The AFF oocytes were artificially activated to develop into early-stage embryos, or blastocysts, from which stem cells were derived. In general, the success rate for developing blastocysts from AFF oocytes was lower than for fresh oocytes, but those AFF oocytes that did reach the blastocyst stage had high success in producing stem cells.

The stem cells derived from AFF oocytes had the normal arrangement of chromosomes, and when they were injected into fresh mouse embryos they contributed to the development of healthy, fertile animals. Furthermore, each entire animal appeared green under fluorescent light (Fig. 1), proving that the stem cells are capable of differentiating into all cell types in the body.

However, none of the cloned blastocysts from AFF oocytes developed to full-term offspring. This suggests that AFF oocytes have deficiencies that hinder full-term development, but are sufficient for the generation of stem cells. The findings could address some ethical concerns about the method—if the blastocyst will never be able to develop, why not use it to acquire stem cells?

Project leader Teruhiko Wakayama believes that the techniques can be further improved to produce human stem cells, but some concerns will remain. "AFF oocytes are the best solution to end the ethical debate, but due to the low success rate, you must prepare a hundred AFF oocytes from hospitals for each patient," he says. "We must improve the success rate to reduce the number of used AFF oocytes.■

1. Wakayama, S., Suetsugu, R., Thuan, N.V., Ohta, H., Kishigami, S. & Wakayama, T. Establishment of mouse embryonic stem cell lines from somatic cell nuclei by nuclear transfer into aged, fertilization-failure mouse oocytes. *Current Biology* **17**, 120–121 (2007).
2. Kishigami, S., Mizutani, E., Ohta, H., Hikichi, T., Thuan, N.V., Wakayama, S., Bui, H.T. & Wakayama, T. Significant improvement of mouse cloning technique by treatment with trichostatin A after somatic nuclear transfer. *Biochemical and Biophysical Research Communications* **340**, 183–189 (2006).

Looking back into the brain's future

Molecular biologists determine a key compound that regulates brain development

RIKEN researchers are beginning to unravel details of the sophisticated regulation system which governs development of the forebrain in all non-marine animals with backbones, including mammals. The system is based around a protein known as YY1, which regulates the segments of DNA that organize the activity of the key gene directing development of the head region, *Otx2*.

The work is significant in that it demonstrates not only the subtlety of developmental regulation systems, but also the great impact they can have on evolution. The genes responsible for development of the head region in vertebrate animals have been highly conserved over time, suggesting that variation in regulation has been responsible for the vast evolutionary changes that have taken place.

Earlier this year, a research team from RIKEN's Center for Developmental Biology in Kobe showed that, with the exception of the bony fishes, development of the forebrain in vertebrate animals is initiated when the enhancer known as anterior neuroectoderm (AN) activates the *Otx2* gene[1]. Now, the same team has found that AN is itself is dependent on a promoter, and both are regulated by the protein YY1.

The region of the developing embryo where AN has its impact is very small and difficult to work with *in vivo*. But the team was able to proceed with its studies when it discovered that the AN enhancer was active in the laboratory cell line, F9.

The researchers found that, to activate *Otx2*, YY1 must bind to both AN and its promoter. Only YY1 with an acetyl group attached, however, will bind to AN, and this form of YY1 occurs only in the anterior head region, not elsewhere.

Figure 1: A present-day skate.

The group also found that the binding sites for YY1 are highly conserved in an evolutionary line extending from ancestral skates and coelacanth fishes through to mammals (Fig. 1), suggesting that the regulatory system has been in place a very long time.

While YY1 is necessary for *Otx2* to function it is not sufficient. Other compounds are involved in the regulatory process. Not only that, but YY1 itself is associated with and affected in different ways by a wide variety of other proteins.

The picture that emerges is one of a complicated regulatory system subject to highly subtle molecular influences, according to the team. There are still many things they would like to find out about it, such as how AN recognizes acetylated YY1 and what other compounds or co-factors are important. ■

1. Takasaki, N., Kurokawa, D., Nakayama, R., Nakayama, J. & Aizawa, S. Acetylated YY1 regulates *Otx2* expression in anterior neuroectoderm at two *cis*-sites 90kb apart. *The EMBO Journal* **26**, 1649–1659 (2007).

Fishing for evolution

Embryo studies of an elusive fish helps put vertebrate evolution in proper perspective

A research team from the Center for Developmental Biology in Kobe has successfully bred hagfish in captivity for the first time. Using the resulting embryos, the team shows that the cellular events leading to the development of the structure called the neural crest in this organism are almost identical to those seen in more advanced vertebrates.

The jawless hagfish is classified among the vertebrates, but considered a transitional class of animals lying somewhere between non-vertebrates and the jawed fish such as sharks. Lacking vertebrae, hagfish embryos were also thought to possess non-migratory neural crest cells—the sheet of embryonic cells in vertebrates that migrates and then differentiates directly into various structures, including the spinal ganglia. These features caused controversy over the proper evolutionary position of hagfish and whether they should be classified among vertebrates.

The research team, led by Shigeru Kuratani, thought that better understanding of the embryonic development of hagfish may help scientists better organize the evolutionary tree and reveal the factors leading to the emergence of true vertebrates. They collected male and female hagfish off the coast of western Japan (Fig. 1) and induced the females to spawn in a laboratory aquarium. This feat allowed them to identify seven early-stage developing embryos—the first study of such early hagfish embryos in over 100 years.

Histological and gene expression analyses revealed that the neural crest in hagfish embryos develops in a remarkably similar manner to those of higher-order marine vertebrates. The embryos also possess the migratory cells that populate the neural crest from afar[1]. Thus, the team concludes that the genetic and cellular mechanisms by which the neural crest develops existed in the latest common ancestor that eventually gave rise to all the vertebrate taxa existing today. They note that there is already a hint of this from a recent finding of 'putative crest cells' in embryos of tunicates, which are an early ancestor of hagfish and other vertebrates of the same phylum[2].

Given the time of divergence between hagfish and other vertebrates, Kuratani estimates that the cellular mechanisms giving rise to the vertebrate body plan could have existed in a common ancestor as long ago as 500 million years, during the Cambrian period. He believes that the hagfish "remains a key model for exploring vertebrate evolution and is now available for use in molecular embryological approaches to address fundamental questions in evolutionary developmental biology." ■

Figure 1: A hagfish collected from deep water off the coast of western Japan.

1. Ota, K. G., Kuraku, S. & Kuratani, S. Hagfish embryology with reference to the evolution of the neural crest. *Nature* **446**, 672–675 (2007).
2. Jeffrey, W. R., Strickler, A. G. & Yamamoto, Y. Migratory neural-crest like cells form body pigmentation in a urochordate embryo. *Nature* **431**, 696–699 (2004).

'Lonely guy' helps crops to flourish

New insights into the regulation of an important plant hormone could extend the agricultural potential of rice and other crops

The plant hormone cytokinin is known to play an essential role for growth in several tissues, including the shoot meristems—small clusters of stem cells responsible for the formation of above-ground organs like leaves, stems and flowers. The final stages of cytokinin synthesis are thought to represent a particularly important regulatory checkpoint for this hormone's activity, but the actual enzymes involved in cytokinin processing have proven elusive. "We have tried to identify the gene or genes for about the last three years," explains Hitoshi Sakakibara, from the RIKEN Plant Science Center in Yokohama.

His group hit a turning point when they began collaborating with Junko Kyozuka and her team at the University of Tokyo, who had identified a novel mutant rice strain with apparent shoot meristem defects. The strain was named *lonely guy* because, in addition to having reduced numbers of floral organs, flowers tended to contain only one stamen—the 'male' reproductive organ—and no pistil. Working together, the two groups proceeded to identify and clone the mutated gene responsible for these shoot meristem defects, which they termed *LOG*.

As described in the team's recent *Nature* article, initial efforts to characterize the protein encoded by *LOG* based on its structure proved difficult, although the researchers found genomic evidence that LOG might be involved in the processing of cytokinin[1]. Functional studies confirmed this, revealing LOG to be a member of a class of enzymes capable of directly activating cytokinin. This was a big surprise, according to Sakakibara: "The structure of LOG did not suggest this at all." Further molecular biology experiments provided additional indirect evidence that reduction of LOG activity leads to a reduction of cytokinin activity in the shoot meristem.

According to Sakakibara, these results are exciting for a number of reasons. "This work has revealed a novel metabolic pathway for cytokinin," he says. "This could make researchers reconsider how and where cyotkinin activity is being controlled." However, there may also be important implications for agriculture, and Sakakibara, Kyozuka and colleagues are now developing genetically modified strains that exploit the LOG pathway. "Since cytokinin is an important hormone regulating rice grain number, control of LOG expression in shoot apical meristem might have a positive effect on the yield of rice," explains Sakakibara. "We have transformed rice to increase the gene copy number, and are now propagating the seeds to compare the yields." ■

1. Kurakawa, T., Ueda, N., Maekawa, M., Kobayashi, K., Kojima, M., Nagato, Y., Sakakibara, H. & Kyozuka, J. Direct control of shoot meristem activity by a cytokinin-activating enzyme. *Nature* **445**, 652–655 (2007).

Genetic stress relief

New discovery sheds light on how plants respond to environmental stresses

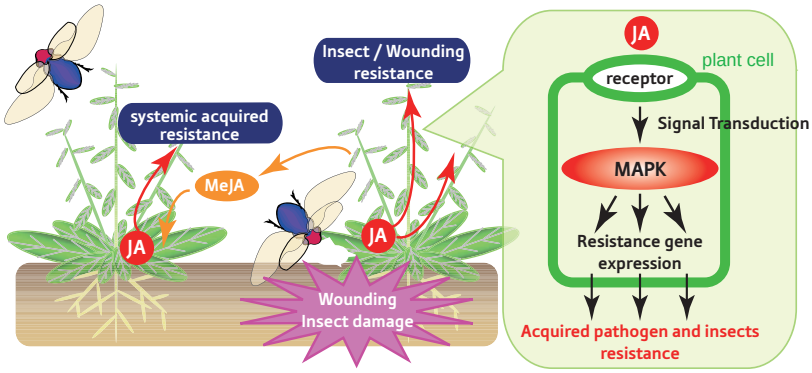
RIKEN researchers have identified a crucial process that regulates the genetic response of plants to environmental stresses such as physical wounding or pathogens[1]. The findings could improve the selective breeding of crops in order to achieve maximum productivity.

The Gene Discovery Research Team at the RIKEN Plant Science Center, Yokohama, is studying *Arabidopsis thaliana*—a small flowering plant often used as a model organism in genetics because of its small genome and short life cycle. Many genetic signaling functions in *Arabidopsis* are performed by reaction cascades among a family of proteins called the mitogen-activated protein kinases (MAPKs) and kinase-kinases (MAPKKs).

The researchers identified a new reaction cascade between one of the MAP kinase-kinases, MKK3, and a MAP kinase called MPK6. The cascade affects the activity of jasmonic acid, a plant hormone known to inhibit root growth and control responses to stress and ageing.

Several *Arabidopsis* plants were treated with the steroid hormone dexamethasone to mimic an environmental stress. Some of the plants were wild, while others were engineered to over-express MKK3. The MKK3-enhanced plants activated MPK6, which in turn caused increased jasmonic acid signaling. As a result, the engineered plants showed less evidence of stress-induced cell death than wild plants.

Furthermore, when jasmonic acid levels were artificially increased, plants with increased levels of MKK3 and MPK6 showed less root growth inhibition than wild plants. This implies that the MKK3–MPK6 cascade acts as a regulator of jasmonic acid activity.



Schematic diagram of the JA-mediated resistance mechanism

Figure 1: Schematic of the resistance mechanism mediated by jasmonic acid (JA) and regulated by mitogen-activated protein kinase (MAPK) cascades.

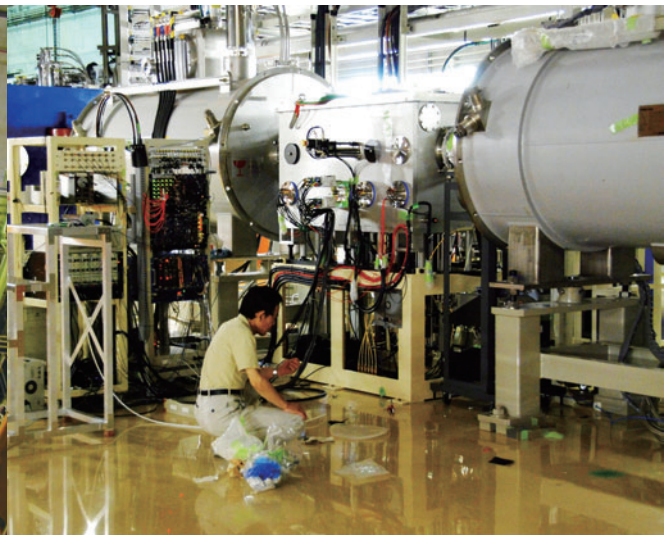
Fuminori Takahashi

An analysis of messenger RNAs from the plants showed that MKK3 increased the expression of 56 genes, including many genes that are known to act in response to external stresses. However, some other genes showed decreased expression.

Using the results of their work and previous studies, the researchers propose a model in which four distinct MAPKKs activate MPK6 and regulate different responses. The cascade MKK2–MPK6 occurs in response to cold and salt, while MKK4 and MKK5 activate the hormone ethylene in response to pathogens. The newly discovered cascade MKK3–MPK6 regulates jasmonic acid, controlling root growth and wounding response (Fig. 1).

"The research provides evidence of genetic modification of plants for resistance to pathogens and insects," says team leader Kazuo Shinozaki, "and MKK–MPK cascades play a similar role in other crops, so the results could be applied in crop breeding." However, he believes the model may still be incomplete. "In *Arabidopsis*, there are ten MAPKKs. Three MAPKKs (MKK8, MKK9 and MKK10) have not yet been analyzed." ■

1. Takahashi F., Yoshida, R., Ichimura, K., Mizoguchi, T., Seo, S., Yonezawa, M., Maruyama, K., Yamaguchi-Shinozaki, K. & Shinozaki, K. The mitogen-activated protein kinase cascade MKK3–MPK6 is an important part of the jasmonate signal transduction pathway in *Arabidopsis*. *The Plant Cell* **19**, 805–818 (2007).



Advancing the frontiers of nuclear physics

RIKEN Nishina Center for Accelerator-Based Science – Part 1
RIBF – Radioactive-Isotope Beam Factory

At RIKEN's Wako campus in the rolling hills of Saitama outside of Tokyo, researchers at the RIKEN Nishina Center for Accelerator-Based Science (the RIKEN Nishina Center) are busy extending the frontiers of nuclear physics. Using cutting-edge equipment, including the world's most powerful cyclotron, heavy ions are accelerated to close to the speed of light and smashed apart to create unstable isotopes. Scientists believe that these unstable isotopes play an essential role in the synthesis of the elements that form our universe.

The RIKEN Nishina Center, inaugurated April 1, 2006, includes the Radioactive Isotope Beam Factory (RIBF), consisting of five particle accelerators, including a linear accelerator, four ring cyclotrons, an RI beam separator and experimental apparatus.

The pride and joy of the facility is its superconducting ring cyclotron (SRC). Commissioned in 2006, it is located 20 meters underground, both for radiation shielding and to position its 8,300 metric tons solidly on a gravel bed. Its extremely powerful superconducting electromagnets make it possible to accelerate ions to create intense beams of unstable isotopes.

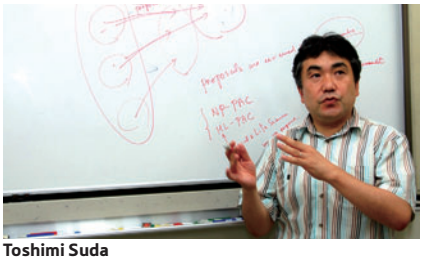
The ions start their journey through the facility in the linear accelerator, and are then sped up through three ring cyclotrons before entering the SRC and emerging as a beam of ions traveling at up to 70% of the speed of light. This beam then strikes a production target and the resulting fragments are converted into an RI beam containing thousands of different types of isotopes. This beam, to be useful for research, has to be purified to leave only the particles of interest. This is the job of BigRIPS, the first of a new generation of RI beam separators. Others are now being built in Germany and planned in the US.

"The role of BigRIPS is to collect the beam and separate the different types of isotopes spatially," says Toshiyuki Kubo, director of the BigRIPS facility. "It does this by analyzing the beam with 14 extremely powerful superconducting quadropole magnets. By carefully tuning the magnetic fields, we can separate the different kinds of ions, which are affected differently by the strong magnetic fields and focus the ions of interest on the secondary target, where their properties are studied."

According to Akira Goto, director of the Accelerator Development Group, RIBF provides "intensity that is the highest in the field, making this the best facility in the world for the production and study of RI beams."

Toshiyuki Kubo

Akira Goto



Toshimi Suda

In March 2007, in the first experiment ever performed at RIBF, a neutron-rich isotope of palladium, Pd125, was identified.

An important part of the center's research activities focuses on the study of so-called super heavy elements. In July 2004, the new heaviest element, with the atomic number 113, was created for the first time using the high-intensity ion beam from the heavy-ion linac, which is the first stage of the RIBF accelerator complex. We hope that the new element will be named 'rikenium' or 'japonium'.

RIBF's heavy ion beams are also being used in biological research, for example to induce genetic mutations in plants. RIKEN researchers recently developed a highly salt-resistant strain of rice, as well as new varieties of flowers.

Free and open collaboration—a crucial requirement for research

The field of nuclear physics thrives on free and open collaboration. But until now, external researchers had to hold positions within RIKEN, and that may have limited their free use of equipment in RIKEN.

Moves are now afoot to open the RIKEN Nishina Center's cyclotron facilities to external researchers. "This is an important and highly advanced facility, and should be considered a common instrument for all of humanity," says Toshimi Suda, the director of the RIKEN Nishina Center's User Liaison and Support Group.

The key to the new system is an independent body, the Program Advisory Committee (PAC), consisting of 17 top researchers from around the world, most representing large accelerator facilities. Its chairman is the director of GSI (Gesellschaft für Schwerionenforschung), one of major accelerator facilities in Germany.

The PAC is strictly independent of the RIKEN Nishina Center to ensure that proposals are evaluated on the basis of their scientific merit and feasibility only.

Interest has been intense. Since declaring the facility open six months ago, the PAC has received 32 proposals for experiments corresponding to 550 days of beam time.

Heiko Scheit—Exploring the edges of existence

Bizarre isotopes of ordinary, stable elements that contain large numbers of extra protons or neutrons, known as 'exotic nuclei', are a current focus of nuclear physicists. Created in the high-energy environment of the RIKEN Nishina Center's cyclotrons, these extremely unstable and short-lived isotopes—especially in their death throes as they decay and throw off gamma radiation—offer insights into the most fundamental properties of nature.

Heiko Scheit is a staff researcher at the RIKEN Nishina Center, performing experiments on exotic nuclei using the center's new SRC.

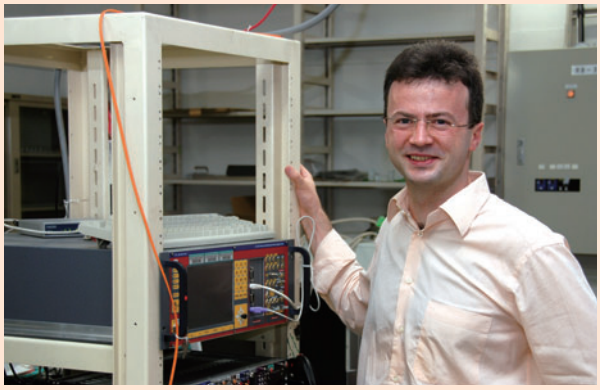
"These particles are very difficult to produce and difficult to study, since the more exotic they are, the more unstable and short-lived they become," he explains. So far about 3,000 have been observed and studied, and about 7,000 more are predicted by theory but have never been observed in the laboratory.

"Of particular interest in the study of the structure of exotic nuclei, are the so-called 'magic numbers'. Experiments investigating nuclides near stability have shown that some of them are especially stable and difficult to excite: these nuclides have a particular number of protons and neutrons, magic numbers, 2, 8, 20, 28, 50, 82 and 126."

These magic numbers form the cornerstone of the most successful nuclear model, the nuclear shell model. It is not clear, however, whether the common magic numbers (the ones listed above) also hold for exotic nuclei. In addition, various theories predict the properties of the most stable nuclei very well, but in exotic isotopes, once the number of protons and neutrons starts to diverge, yawning gaps appear between predicted and observed properties.

When Scheit and his team conduct experiments in October this year, they hope to find interesting new properties of these nuclei. "We also want to perform a systematic study of them, so that the results can be compared to theory over a wide range of proton and neutron numbers," Scheit adds. "That's where the challenge is in nuclear theory."

Specifically, he is interested in the so-called 'island of inversion', a region of the nuclear chart where exotic and rare isotopes have been identified but whose properties have not been established.



This kind of research advances nuclear theory, by testing the extent to which current models accurately describe the most exotic nuclei. It is also of interest, for example, to astrophysicists investigating the origin of the elements. "Exotic nuclei play a key role in this process," explains Scheit, "and reliable extrapolations have to be employed to predict properties of the most exotic nuclides that cannot yet be synthesized in the laboratory."

Very high energies and very intense beams are needed for this work. "The lab here is currently one of the best in the world, and will be for at least the next 5–10 years," Scheit notes. "With this facility I think we can extend the region of known nuclei much further."

You also need teamwork, which is available in abundance in the international environment of the RIKEN labs, according to Scheit. Despite the mix of cultures and languages, he has never had problems communicating or discussing issues with his Japanese or foreign colleagues. "Things are done a bit different here, but the differences are minor."

He came to RIKEN last November with his wife and one-year-old son from Germany, after a short stay in the US. They are enjoying life and work in Japan and plan to stay a long time.

RIKEN and A*STAR hold joint immunology and developmental biology meeting

The Biomedical Research Council of the Agency for Science, Technology and Research (A*STAR) of Singapore and RIKEN co-organized 'Joint Symposium 07', held on May 16 and 17 at Biopolis in Singapore. Biopolis is a biomedical research hub currently consisting of seven existing buildings, with two new ones, Immunos and Neuros, set to open soon as immunology and neurobiology research centers. Participating groups from A*STAR were the Singapore Immunology Network (SIgN), the Institute of Molecular and Cell Biology (IMCB), the Institute of Medical Biology (IMB), and the Genome Institute of Singapore (GIS), and from RIKEN the Research Center for Allergy and Immunology (RCAI) and the Center for Developmental Biology (CDB). Immunology was the focus of the first day of the meeting and the second day concentrated on developmental biology. These seemingly disparate scientific disciplines have much in common. Studies of blood-cell and lymphocyte development have been very informative models for our understanding of other developmental systems. Moreover, similar genetic programs, for example the involvement of hierarchies of transcription factors, regulate the development of immune cells as well as other cell types.

The symposium, organized by Kong Peng Lam and Mike Jones of A*STAR and Shin-Ichi Nishikawa and Takashi Saito of RIKEN, was broad in scope with 20 speakers from fields such as cell signaling, tumor immunology, lymphoid organogenesis, humanized mice, myogenesis, embryogenesis, and evolution. Nishikawa (CDB) described a new model of Peyer's patch (PP) organogenesis, which involves chemokine receptor-mediated sequential relocation of the cells that induce PP. The result is an influx and segregation of the various cellular constituents that make up the mature PP. Along a similar vein, Takeshi Watanabe (RCAI) described the generation of artificial lymph nodes (LN) that recapitulate conventional LN development but with intriguing differences, including a predominance of memory cells. He showed that these structures could be primed to suppress tumor-cell growth and ultimately hopes to extend these studies to humans. Jean Pierre Abastado (SIgN) has studied a spontaneous mouse-tumor model and found that tumor cells can attract and polarize immune cells, particularly macrophages, so that they support tumor-cell growth and inhibit immunity-mediated tumor rejection. Interference in this complex ecology may have therapeutic benefits. Phil Ingham (IMCB) is studying myogenesis in zebrafish and has made an EST (expressed sequence tag) library, the largest yet generated from this model organism. He has isolated several genes required for normal muscle development, and the library is publicly available to other zebrafish researchers, providing an important resource.

The goal of this Symposium was to allow senior and junior investigators from both countries to present data in areas of common interest in order to initiate and foster long-term collaborations. The participants all agreed that this was a very good beginning.

Thailand's minister visits SPring-8

On June 20, Yongyuth Yuthavong, Minister of Science and Technology of Thailand, with over 20 dignitaries including the director of Thailand's National Synchrotron Radiation Center, visited SPring-8 (the world's biggest synchrotron radiation facility) in Harima, Hyogo Prefecture.

Kenji Takeda, Executive Director of RIKEN, and Kanji Fujiki, Deputy Director-General of MEXT (the Ministry of Education, Culture, Sports, Science and Technology), gave a welcome address at the luncheon. Akira Kira, Director General of the Japan Synchrotron Radiation Research Institute, described SPring-8 and Hiroyoshi Suematsu, director of RIKEN Harima Institute, described the XFEL (X-ray Free Electron Laser). After a general account, they inspected SPring-8's central control room, beam lines and so on. Tetsuya Ishikawa, Project Leader of XFEL, showed the visitors the prototype accelerator of XFEL.

Masaki Yamamoto, Division Leader of Synchrotron Radiation Instrumentation, described X-ray protein crystallography at the RIKEN structural genomics beam line BL26.

Yongyuth asked many questions ranging from industrial applications to machine engineering. Finally, Yongyuth expressed his desire to strengthen scientific collaborations with SPring-8 to encourage the use of synchrotron radiation in industrial applications in Thailand.

JEM–EUSO—Investigating a perplexing mystery

Ultrahigh-energy cosmic particles are an intriguing puzzle in high-energy physics, and RIKEN is involved in a project to solve it. On May 16, the RIKEN proposal for the second utilization plan of the Japanese Experiment Module (JEM) on board the International Space Station was accepted, and its kickoff meeting was held on June 6 to 8 at RIKEN, Wako.

The particles have extremely high kinetic energies, as much as 10^{20} electronvolts, far greater than other cosmic-ray particles. They are also extremely rare—only 11 have been observed in 13 years of searching with the Akeno Giant Air Shower Array (AGASA), which has an effective area as large as the area enclosed by the Yamanote line in the Tokyo Metro. No one knows where they come from, or how they could have that much energy left over after the long journey through intergalactic and interstellar space.

To observe these rare phenomena, a telescope with an extremely wide field of view is needed, and it is being provided by the Extreme Universe Space Observatory (EUSO), to be installed on the JEM on the International Space Station, after JEM is launched in 2008.

Instead of looking out into space like a conventional telescope, EUSO will look down at the Earth from space, searching for streaks of ultraviolet fluorescence and Cerenkov radiation, which cosmic particles produce when they interact with the Earth's atmosphere.

EUSO incorporates a Fresnel lens, a very thin, wide-aperture lens made up of concentric rings, which provides a 60 ° field of view. The lens was made using ultraprecise grinding technology at the RIKEN Ohmori laboratory.

To detect the faint streaks of ultraviolet fluorescence and Cerenkov radiation that the particles produce as they enter the Earth's atmosphere, EUSO contains 6,000 photomultiplier tubes, which were developed and built in Japan.

EUSO is said to be launched by a Japanese H-IIB rocket and transferred by H-II transfer vehicle (HTV), and it is expected to detect about 1,000 ultrahigh-energy cosmic particles in the five years of its operation. From this data researchers hope to determine whether the particles originated from a single source or occur throughout the universe.

Fostering the spirit of entrepreneurship

RIKEN's long-standing culture of entrepreneurship is still delivering research with highly-valued commercial applications

Back in the 1920s and 1930s, RIKEN started the 'RIKEN *konzern*,' or the 'RIKEN conglomerate,' consisting of venture companies established to commercialize researchers' inventions. By 1940, RIKEN was flourishing with some 63 start-up companies—an unprecedented number for any research institute in the world at that time (see History of RIKEN, *RIKEN RESEARCH* **1(0)**, 18). The conglomerate was dissolved after World War II, but despite many years passing, RIKEN's findings still find application in high-profile commercial products (Fig. 1).

While Japan was still undergoing post-war rehabilitation in 1958, RIKEN made a fresh start and changed its status to a semi-public special corporation. RIKEN also sought to actively contribute to industry and society. Instead of aiming to create start-up companies, the organization actively licensed its intellectual property rights to other companies.

In 1966, a natural fungicide called 'polyoxin' was commercialized after its discovery by the laboratory of antibiotics study (see History of RIKEN, *RIKEN RESEARCH* **1(4)**, 18), and in 1967 another laboratory licensed to a private company its technology to manufacture single-crystal compound ferrite, a type of iron oxide used for magnetic heads. A decade later, four companies licensed RIKEN's anti-cancer agents called fluouracil derivatives. Later, a variable-area, electron-beam lithograph, invented by the information science laboratory, was commercialized by major companies such as Hitachi and Toshiba.

This series of technology transfers resulted in great success. Polyoxin generated as much as 540 million yen in revenue; part of the money was spent to develop uranium concentration technology for laser science research. An enzyme was also developed for commercial use from a study of microorganisms living in an extreme environment; it led to the development of the blockbuster detergent 'Attack' by Japan's consumer-goods giant Kao in 1986. In the 1990s, a study on hornets contributed to the creation of a popular sports drink 'VAAM'; made of 17 types of amino acids to help burn body fat and maintain physical stamina.

RIKEN's patent revenues were robust throughout the 1980s, with a peak of 165 million yen (US$1.37 million) in 1987. Then, the figures started to plunge, due to expiries of large patents and management's focus on strengthening basic research.

To increase this stream of revenue, RIKEN began to strengthen its management of intellectual properties based on the 'RIKEN Development Plan' stipulated under its fifth president Tatsuoki Miyajima. Then the seventh president, Akito Arima, further improved the system by reforming the organization's regulations on inventions. In 1997, RIKEN organized the first 'patent fair' to match its researchers with appropriate companies. Then, in 1998, RIKEN raised the motivation of researchers to file for patents by drastically increasing incentives for inventions.

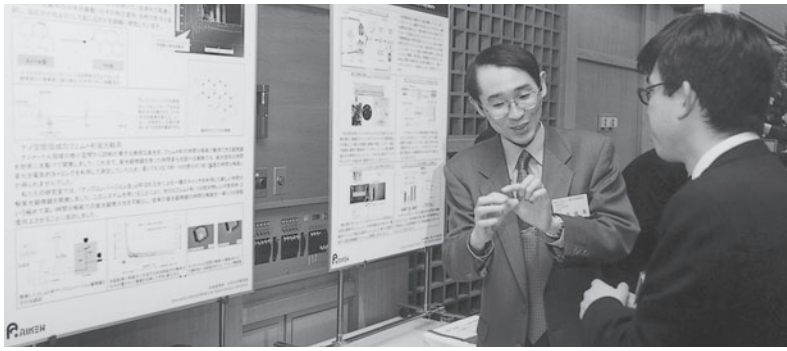


Figure 1: Every year RIKEN's interaction with industry becomes increasingly active.

These efforts saw the number of domestic patent applications surge to 264 in 2002 from around 100 in 1997. The patent income rebounded to 120 million yen in 2003 from just 32 million yen in 2000.

Arima also promoted his belief that RIKEN needed to revive the entrepreneurship spirit of Masatoshi Okochi, the third RIKEN president, to maintain its global competitiveness. In 1996, the first spin-off of a RIKEN laboratory in modern times was created to develop and sell advanced laser technology. To this end, RIKEN changed its traditional rules and allowed researchers to join a management board of a RIKEN start-up, which had been prohibited in the past. That innovative move influenced the government's deregulation policy regarding employment conditions of faculty members at national universities.

As increasing numbers researchers set about creating venture companies, RIKEN also started in 2001 to stipulate measures to support in-house entrepreneurs. One of such effort is the program 'RIKEN Venture Capital Corp.,' set up in 2005 to provide financial assistance. The current number of RIKEN venture companies totals 22; some are even aiming to list their shares on the stock market. Entrepreneurship is deep rooted in the 'RIKEN spirit' that actively encourages creative research. ■

For more information, please visit the URL:
http://r-bigin.riken.jp/bigin/engn/index.html

www.rikenresearch.riken.jp

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

RIKEN RESEARCH is a website (www.rikenresearch.riken.jp) and print publication intended to highlight the best research being published by RIKEN (www.riken.jp). It is written for a broad scientific audience and policy makers interested in science and aims to raise global awareness of RIKEN and its research.

For further information on the research presented in this publication or to arrange an interview with a researcher, please contact
RIKEN Public Relations Office
2-1, Hirosawa, Wako, Saitama, 351-0198, Japan
TEL: +81 48 467 4094
FAX: +81 48 462 4715
E-Mail: rikenresearch@riken.jp