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Protein's surprise role in shaping fruit flies

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Use salt for rich electronic states

Anion radical salts turn out to be a tasty treat for scientists

The world of condensed matter physics is full of intriguing effects that occur as a result of electrons interacting with each other. Well-known examples are magnetism and superconductivity. These effects were typically first observed in inorganic crystals and only later discovered in organic compounds. For example, superconductivity, the lack of any resistance in the electronic current flowing through a material, was first discovered in 1911, whereas it was only in 1980 that superconductivity has been shown for synthetic molecules.

The observation of such fundamental physics effects in organic molecules is not only exciting for scientists, but presents an intriguing possibility for future applications. Conventional electronic devices such as silicon transistors are fabricated on a nanoscale in a 'top-down' approach that relies on external technology. However organic molecules form naturally in a 'bottom-up' approach when presented with the right external conditions. Scientists are hoping that it will eventually be possible to use self-assembled organic molecules in electronic devices, or even as entire integrated circuits capable of performing complex mathematical operations.

Designer molecules

In recent years, the field has seen significant advances in the understanding of the physics of artificial designer molecules. This had led to an increasing complexity and enhanced functionality of newly designed salts. One such family of molecular compounds are the anion radical salts based on the $Pd(dmit)_2$ molecule (Fig. 1), studied by a team of materials scientists from the RIKEN Discovery Research Institute in Wako.

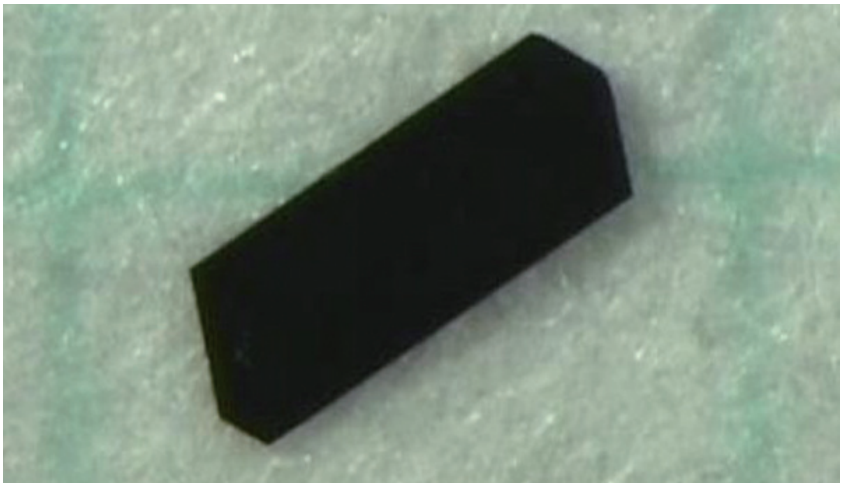

Figure 1: A photograph of one of the anion radical salts. The salts absorb visible light, which leads to their intense black appearance.

The salts exhibit a large variety of fundamentally different electronic states that is astounding. "The $Pd(dmit)_2$ system is a unique playground that contains rich physics," says Reizo Kato, whose team has been intensively investigating these salts. "It is an exciting challenge to understand the subtle behaviors of this molecular system."

Most of these effects are rooted in the crystal structure of the salts. The structure of crystals can be determined using x-ray diffraction imaging, where the intensity distribution of x-rays diffracted from a crystal is directly related to their structure. In previous experiments, Kato's team has shown that in $Pd(dmit)_2$ salts the anions arrange themselves into strongly coupled pairs, known as dimers, which in turn form a layered structure with a triangular lattice (Fig. 2). The combination of the $Pd(dmit)_2$ anion and various cations is crucial and determines the conducting properties of the salts in addition to external parameters such as temperature and pressure.

Small changes, big consequences

In their latest study, published in the *Journal of the American Chemical Society*[1], the team investigates the new system that contains $EtMe_3Z^+$ (Z=P, As) cations. Compared with previously studied cations, the $EtMe_3Z^+$ cations have a more drastic influence on the electronic properties of the system. "Surprisingly, in the molecule-based conductor, the insulating cation is not a spectator and its geometrical and physical properties strongly affect the conducting properties of the whole system. In that sense, all components are important and the molecular system is a stage where everyone must play a part," explains Kato.

As the RIKEN team discovered, the difference in the crystal structures of the salts and their electronic states is solely determined by a single atom Z in the cation, being either Phosphorus or Arsenic. "We did not expect that such a small change would lead to extraordinary differences in magnetic properties," confesses Kato. At low temperatures, the salt with $EtMe_3As^+$

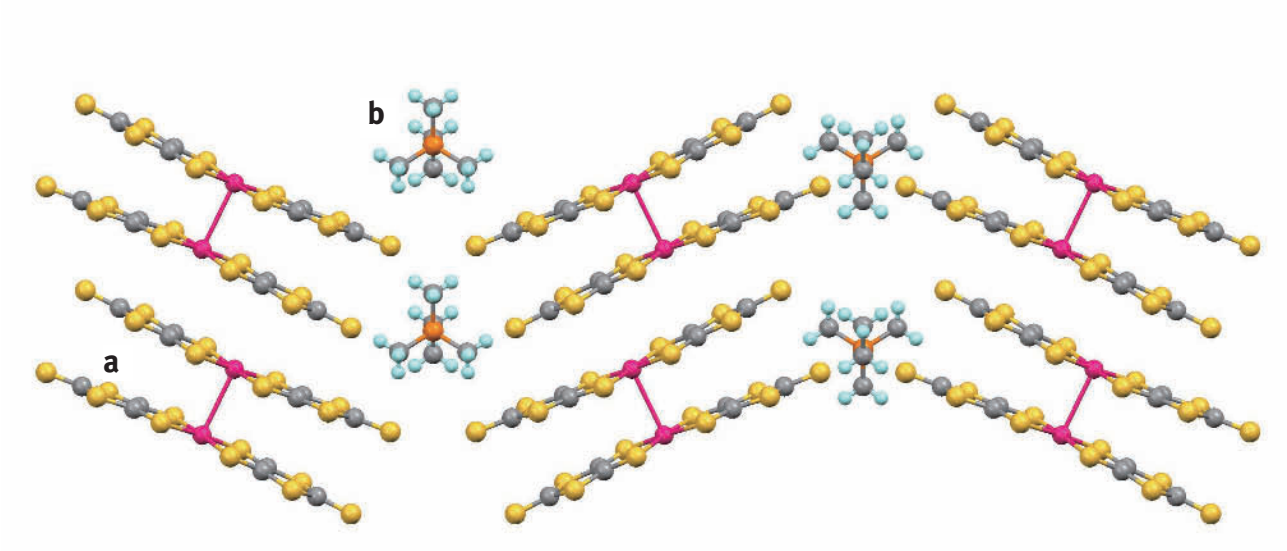

Figure 2: The crystal structure of the salt $EtMe_3P[Pd(dmit)_2]_2$ (dmit = 1,3-dithiole-2-thione-4,5-dithiolate). The anions (a) are paired into dimers. The dimers then arrange into layered structures with a triangular lattice, separated by the smaller cations (b).

cations is antiferromagnetic, while that with $EtMe_3P^+$ is non-magnetic. However, the two salts also share similarities. At room temperature, both salts conduct poorly and when hydrostatic pressure is applied they become metallic. Upon cooling under pressure, both turn superconductive.

The structural relationship between these salts and the similarities in some of their electronic properties suggest a unified framework. The RIKEN team shows that this is a direct consequence of the layered crystal structure and down to variations in the interaction between the dimers on the triangular lattice. For the two salts, the arrangement of how the dimers are packed into the layers (Fig. 2) varies slightly. This leads to changes in the electronic interactions between the dimers and as a consequence to the suppressed antiferromagnetism in the salt with the $EtMe_3P^+$ cation.

The dawn of a new molecular age?

The large variety in the electronic properties of these salts from introducing only subtle changes to their composition has convinced Kato that "these findings will open the way to a new solid-state molecular science". The flexibility with which the electronic states can be predetermined via the design of the molecules suggests new pathways to prepare well-defined electronic states. This could enhance our understanding of the fundamental physics governing these molecular systems.

In addition, it is possible to envisage the use of such molecules for novel applications and silicon technology provides yet another analogy to compare those two technologies. Replacing atoms in Silicon crystals with other elements such as Boron, Phosphorus or Arsenic—incidentally, the latter being the same elements as those used for the $EtMe_3Z^+$ cations—leads to materials with different polarity. The combination of these polar materials in a single device, the bipolar transistor, led to the microelectronics revolution of the 20th century. Thus, the utilization of anion radical salts with slightly different cations, and hence very different electronic states in a single device, could lead to similarly exciting developments. Whether there will be new physics, new technology or both, it will be thrilling to observe the progress in the field. ■

1. Kato, R., Tajima, A., Nakao, A. & Tamura, M. Two pressure-induced superconducting anion radical salts exhibiting different spin states at ambient pressure. *Journal of the American Chemical Society* **128,** 10016–10017 (2006).

About the researcher

Reizo Kato was born in 1955 in Yamaguchi, Japan. He received his BSc degree in 1979, MSc in 1981 and DSc in 1984 from the University of Tokyo. He was appointed as a research associate of the Department of Chemistry at Toho University in 1984, and promoted to lecturer in 1988. He joined the Institute for Solid State Physics, the University of Tokyo, as an associate professor in 1990. Since 1999 he has been the chief scientist and director of the Condensed Molecular Materials Laboratory at RIKEN. He received the Chemical Society of Japan Award for Young Chemists in 1990 and the IBM Japan Science Prize in 1995 for his work on molecular conductors. His research has focused on the development of new molecular materials, especially molecular metals and superconductors.

Fat lithium atoms hang on to their halo

Scientists measure stickiness of outlying neutrons

An outsized atom known as lithium-11 is yielding the secret of how it holds together. RIKEN scientists have proved that a pair of outlying neutrons, which form a halo around the central core of the atom's nucleus, play an important role in keeping it whole.

The nucleus of almost every atom contains protons and neutrons, bound together by the so-called strong force. Lithium atoms always have three protons, and usually like to team those with four neutrons. However, about 20 years ago physicists found that a lithium nucleus could carry as many as eight neutrons, making lithium-11. Two of those neutrons orbit the central nucleus like moons around a planet, forming a 'halo' which makes the overall nucleus as wide as the atomic giant, lead-208.

Lithium-11 is not easy to study, partly because half of all the atoms in any sample fall apart every eight milliseconds. The fat nucleus is also so fragile that any experiments to probe its structure must be done at relatively low energies.

Tohru Motobayashi and colleagues at RIKEN's Nishina Center for Accelerator-Based Science in Wako, along with collaborators from US and Japanese universities, have now carried out the most sensitive investigation of lithium-11 to date[1].

The team fired a stream of about 20,000 lithium-11 atoms per second at a lead target, which cracked the nuclei into lithium-9 and two separate neutrons. Measuring the momentum and direction of the three collision fragments allowed the scientists to calculate how tightly they were bound together in the first place.

Crucially, the team used two sets of neutron detectors so that they could detect the fragments of the collision with much greater accuracy than previous experiments.

Figure 1: An image of Borromean rings.

They found that the collapsing nucleus absorbed a characteristic amount of energy—about 0.6 MeV (mega electron volts), some 200 times less than the energy released when a radioactive uranium-235 atom breaks apart.

This matches theoretical predictions of how much energy should be required to rip the two halo neutrons apart, suggesting that the interaction between the two is very important in keeping the whole nucleus together.

Appropriately enough, the lithium-11 nucleus is known as a Borromean system, after the Italian Borromeo family crest. This carries three interleaved rings arranged so that if just one is removed, the whole system falls apart (Fig. 1). "Our new result … should provide fruitful information on the crucial properties of this intriguing Borromean system," the team says. ■

1. Nakamura, T., Vinodkumar, A., Sugimoto, T., Aoi, N., Baba, H., Bazin, D., Fukuda, N., Gomi, T., Hasegawa, H., Imai, N., *et al.* Observation of strong low-lying *E*1 strength in the two-neutron halo nucleus ^{11}Li. *Physical Review Letters* **96**, 252–502 (2006).

Using defects to improve quantum bits

Naturally occurring defects in quantum devices could be used to improve their performance

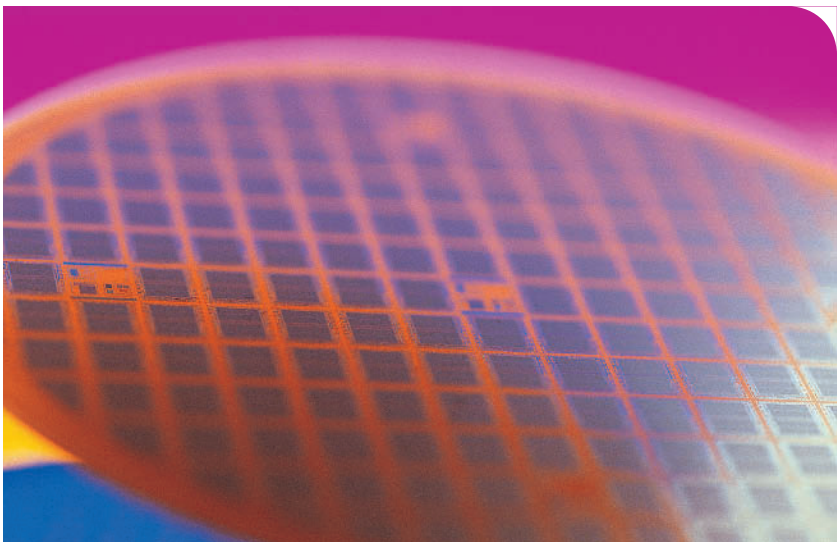

Figure 1: An image of a superconducting device.

Naturally occurring defects in superconducting devices (Fig.1) are usually thought of as an annoying source of noise, or imperfections that must be minimized.

Now, a team led by Franco Nori, a physicist at RIKEN's Frontier Research System, Wako, and the University of Michigan, USA, has shown that these defects can be used to perform quantum computing tasks[1].

To advance the speed of computers, researchers have been trying to fabricate superconducting devices to do quantum computations. These tiny devices are called quantum bits—or qubits for short. Just as 'bits' are the smallest unit of information stored in a classical computer, qubits are the basic units of quantum information. A qubit can be thought of as an oscillator where the information is stored in the exact form of the qubit oscillations. Any disturbance to these oscillations, such as electrical noise from a fabrication defect, degrades the information stored in a qubit in a process called decoherence.

Defects in artificial qubits have posed a major obstacle to performing calculations (Fig. 2). If a defect oscillates at the same frequency as the qubit, they can communicate at that frequency. Thus, the defect can quickly absorb the information from the qubit, perturbing the qubit oscillation, and causing very fast decoherence. Scientists therefore thought that the devices must be made as clean as possible to reduce the number of defects. Then Nori proposed the new idea that if these defects can keep oscillating without disturbance for a very long time, they could also be used as qubits.

The defects do, in fact, have some advantages over the artificially fabricated qubits. As the defects are usually microscopic, they are well-isolated from disturbances, unlike the entire device that is essentially immersed in a sea of electromagnetic noise. Furthermore, in a single artificial qubit, it is typical to find tens of 'good' defects that can oscillate without dissipation long enough to be usable.

Nori's team demonstrated theoretically that during fabrication full control of defects, which are uncontrollable, is not required. Good access to the qubits is all that is needed, and this can be achieved through the artificially fabricated device. "Think of atoms," says Sahel Ashhab, a member of the RIKEN team, "we do not need to spend any effort making them, and each species has different properties from the other; yet we can use them to do all kinds of quantum-mechanics experiments." ■

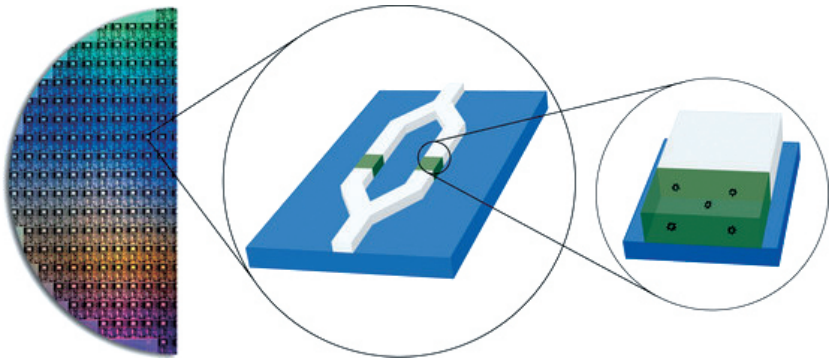

Figure 2: Microscopic defects in quantum circuits could themselves be used as quantum devices. The circles show progressively magnified views: an electronic chip (left), an artificial qubit (middle) and microscopic defects inside the qubit (right).

1. Zagoskin, A. M., Ashhab, S., Johansson, J. R. & Nori, F. Quantum two-level systems in Josephson junctions as naturally formed qubits. *Physical Review Letters* **97**, 077001 (2006).

Expanding the genetic code

Researchers add a third pair of 'letters' to DNA's natural alphabet

Molecular biologists at the RIKEN Genomic Sciences Center in Yokohama have constructed a synthetic pair of nucleotide bases. These bases can be incorporated into the genetic compounds of DNA and RNA, and replicated and transcribed by the standard enzymes found in cells.

The new pair, an imidazo-pyridine and a pyrrole-carbaldehyde known as Ds and Pa respectively, adds two more alternatives to the existing four 'letters' of the genetic code—guanine (G) and cytosine (C), and adenine (A) and thymine (T). The research team believes these 'unnatural' bases can be used to introduce molecular groups with novel and interesting properties into specific positions in DNA and RNA. Already the group has attached fluorescent tags to RNA by means of the new bases.

The unnatural base pair may even open the way towards building novel amino acids into 'unnatural' proteins, according to project leader, Ichiro Hirao.

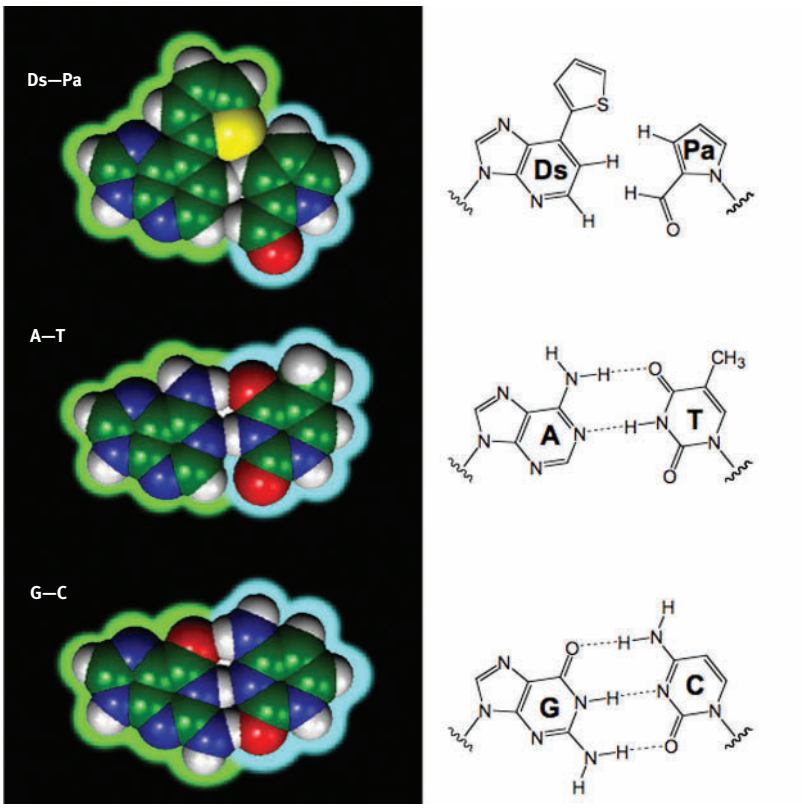

Figure 1: The unnatural base pair system, Ds-Pa, compared with the natural base pairs, A-T and G-C.

In a report of their work in *Nature Methods*[1], the researchers describe how Ds and Pa are driven by the affinity of the surrounding water molecules for each other to pack tightly together in a complementary fashion in what is known as a hydrophobic interaction. In contrast, the natural bases of A-T and G-C pair via an electrostatic interaction called hydrogen bonding (Fig. 1).

Although more than 50 such unnatural base pairings have been reported in the literature, this is the first that can be routinely manipulated by standard enzymes. The researchers showed in the laboratory that DNA incorporating Ds and Pa can be replicated and amplified with a high degree of fidelity using the polymerase chain reaction—although the process involves using modified energy-rich phosphate compounds. They also demonstrated that the unnatural base pair can be transcribed from DNA into RNA, which opens the possibility of using it to construct novel proteins or RNA-based compounds.

The researchers are now exploring the development of functional DNA and RNA with different active molecular groups attached via the new base pairs. "We are also trying to establish a translation system using unnatural base pairs for site-specific incorporation of various unnatural amino acids into proteins," says Hirao.

In addition, Hirao wants to try to incorporate the Ds-Pa base pair into a living organism, such as a bacterium. The organisms would be inherently safe, Hirao insists, because they cannot produce all of the compounds they require to function. For example, the researchers would have to supply some of the compounds required in replication and translation. ■

1. Hirao, I., Kimoto, M., Mitsui, T., Fujiwara, T., Kawai, R., Sato, A., Harada, Y. & Yokoyama, S. An unnatural hydrophobic base pair system: site-specific incorporation of nucleotide analogs into DNA and RNA. *Nature Methods* **3**, 729–735 (2006).

Naturally occurring peptides suppress plant development

Interdisciplinary research demonstrates how peptides control plant stem cell development

A team of Japanese plant biologists and biochemists has identified a naturally occurring peptide that suppresses development of critical vascular structures in plants.

As in animals, development of plants includes early 'body patterning' and differentiation of primitive stem cells into specialized cells, such as the vascular structures for transporting water and nutrients.

Reporting in *Science*[1], the team led by the University of Tokyo's Hiroo Fukuda, studied the development of the main conductive structures in plants required for water transport—the so-called 'tracheary elements'.

Studying cells from the plant *Zinnia elegans* (Fig. 1) and a process called trans-differentiation, in which specialized photosynthetic cells become specialized tracheary elements, the team found an inhibitor of tracheary element formation. They called the inhibitor the 'tracheary element differentiation inhibitory factor' (TDIF). Preliminary studies indicated TDIF was a protein, but its molecular nature was not immediately clear.

Biochemical analyses by Naoshi Dohmae from the RIKEN Discovery Research Institute in Wako identified TDIF as a 12 amino acid peptide. "We fractionated TDIF from plant materials in a filtered culture medium using a combination of high performance liquid chromatography techniques (ion exchange, gel filtration, reversed-phase)," explains Dohmae. "Finally we isolated TDIF using reversed-phase high performance liquid chromatography. We then determined the TDIF sequence by tandem mass spectrometry and standard peptide sequencing."

Figure 1: The plant *Zinnia elegans*.

Dohmae's work enabled TDIF to be identified as a member of the CLE family of peptides. All plants encode approximately 26 small peptides referred to as 'CLV3/ESR-related' or 'CLE' peptides that have conserved amino acid sequences. Previous studies have shown that CLE peptides likely provide essential cues for normal plant development.

Using the sequence data provided by Dohmae's team, Fukuda's team searched protein databases for homologies to TDIF and found matches with the CLE peptides from the plant *Arabidopsis thalania*.

The CLE peptides of *Arabidoposis* are grouped into several clades, or groups, based on sequence homology. Tests on all 26 *Arabidoposis* CLE peptides of 12 amino acids (dodecapeptides) for TDIF activity by Fukuda and colleagues demonstrated two counteracting activities, one that promotes stem cell differentiation, such as the CLE3 peptide of *Arabidoposis*, and one that inhibits stem cell differentiation, such as TDIF, during vascular development.

This work demonstrates that naturally expressed peptides provide two key opposing functions during plant stem cell differentiation.

Dohmae's biomedical analyses, crucial for identifying the biochemical nature of TDIF, demonstrate the importance of basic biomolecular characterization techniques in scientific advances. ■

1. Ito, Y., Nakanomyo, I., Motose, H., Iwamoto, K., Sawa, S., Dohmae, N. & Fukuda, H. Dodeca-CLE peptides as suppressors of plant stem cell differentiation. *Science* **313,** 842–845 (2006).

Reviving sperm from the dead

Research shows viable DNA can be retrieved from the sperm of frozen mice to fertilize eggs

Researchers from RIKEN, along with colleagues in Japan, England and Hawaii, have developed a simple and cost-effective way to safely freeze and thaw mouse sperm and produce normal offspring (Fig. 1).

Mice are an important tool in biomedical research because their genes can be manipulated to develop models of human disease. But sharing of specially bred or genetically engineered mice between laboratories has proven difficult, expensive, and time-consuming: in-coming mice need to be quarantined to safeguard against introducing disease to existing colonies. Sending frozen sperm from these mice is the ideal alternative, but mouse sperm has proven to be quite sensitive to freezing and thawing, and has yielded poor results with *in vitro* fertilization (IVF).

Now a team led by Atsuo Ogura from the RIKEN Bioresource Center, in Tsukuba, has shown that whole male mouse testes can be frozen and then used as a source of sperm up to a year later. While the sperm after thawing are technically 'dead' (that is, they are non-motile), their DNA-containing nucleus can be used to generate viable pups by a standard technique used in assisted fertilization known as the intracytoplasmic sperm injection (ICSI) technique.

The team showed that no cryoprotectant was needed to safely freeze the sperm and that the proper temperature to achieve this result was only -80 °C, a temperature easy to maintain in most laboratory freezers. Ogura and colleagues also proved that the samples can be shipped world-wide on dry ice. Their results are reported in the *Proceedings of the National Academy of Sciences*[1].

Figure 1: A healthy pair of mouse offspring generated from tissue of frozen testes.

"We expect that by using our simple freezing technique researchers will be able to freeze sperm from their genetically engineered mice, which are precious resources, without the help of cryopreservation experts," says Ogura. He also hopes that this technique will easily allow mouse deposition from researchers to mouse banking centers, such as the RIKEN Bioresource Center, since it avoids microbial contamination.

The researchers also showed that sperm extracted from the testes of frozen carcasses of mice, which were stored at -20 °C for 15 years, yielded viable offspring at rates similar to their other experiments using freshly frozen testes. This result suggests that frozen carcasses of other animals, such as those long extinct, could be used as a source of sperm to revive the species. The possibilities explored in science-fiction novels such as *Jurassic Park* may now be a step closer to reality. ■

1. Ogonuki, N, Mochida, K., Miki, H, Inoue, K. Fray, M., Iwaki, T., Moriwaki, K., Obata, Y., Morozumi, K., Yanagimachi, R. & Ogura, A. Spermatozoa and spermatids retrieved from frozen reproductive organs or frozen whole bodies of male mice can produce normal offspring. *Proceedings of the National Academy of Sciences USA* **103**, 13098–13103 (2006).

Understanding optimized immune therapy

A single agent efficiently activates multiple arms of the immune response

In clinical trials worldwide, therapies capable of harnessing the power of the immune system show promise in treating infections and malignancies.

At the RIKEN Research Center for Allergy and Immunology in Yokohama, Shin-ichiro Fujii and colleagues have identified molecular mechanisms underlying the potent immunostimulatory capability of one such therapy. Their focus was on the glycolipid α-galactosylceramide (α-GalCer), which triggers strong protective immune responses.

Within the body, 'foreign' substances such as microbes or abnormal tumor cells are captured by and displayed on the surface of dendritic cells (DCs). Passing immune cells called natural killer T (NKT) cells are equipped with sensors that allow them to recognize bits of foreign substances decorating the surfaces of DCs. NKT cells that detect foreign substances relay warning signals, in the form of cytokines and co-stimulatory molecules. These signals alert other immune cells to the presence of 'non-self'.

It has been established that when α-GalCer is presented by DCs, it activates NKT cells, and exhibits therapeutic activity in mouse models of cancer and infectious disease. However, a slightly modified version of α-GalCer, called α-C-GalCer, exhibits 100 and 1,000-fold greater potency in fighting tumor metastases and malaria infections, respectively.

Fujii and colleagues examined the consequences of intravenous injections of graded amounts of α-GalCer or α-C-GalCer into mice (Fig. 1). As reported in the *Proceedings of the National Academy of Sciences*[1], they found that substantially lower doses of α-C-GalCer were needed to effect release of cytokines from DCs and NKT cells. Further, α-C-GalCer selectively amplified production of cytokines that are particularly adept at killing transformed and infected cells.

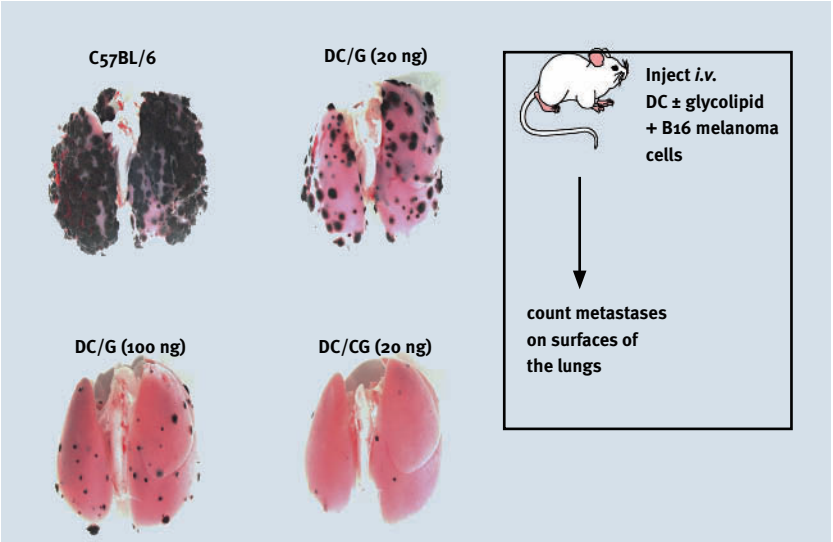

Figure 1: α-C-GalCer inhibits tumor metastasis in mice. When co-injected with tumor cells, DCs pulsed with low doses of α-C-GalCer (CG/DC) effectively suppress the spread of tumor cells (black spots) to the lungs. Higher doses of α-GalCer (G/DC) are required to achieve the same effect.
PNAS/National Academy of Sciences/103/11255 (2006)

Although both glycolipids were equally capable of promoting the appearance of co-stimulatory molecules on the surface of DCs, less α-C-GalCer was needed to trigger expression of co-stimulatory molecules on NKT cells.

When co-injected with tumor cells, low amounts of α-C-GalCer were sufficient to elicit immune protection against subsequent challenges with tumor cells.

Less time was required to 'load' α-C-GalCer onto DCs, and DCs loaded with α-C-GalCer retained their ability to stimulate NKT cells for a prolonged period of time. "These observations suggest that more efficient and stable presentation underlie the enhanced potency of α-C-GalCer, although factors other than stability might also contribute," says Fujii.

The team's work highlights facets of the immune response that correlate with clinical efficacy, which may be important parameters to consider during future experiments aimed at designing optimal immune therapies. ■

1. Fujii, S., Shimizu, K., Hemmi, H., Fukui, M., Bonito, A.J., Chen, G., Franck, R.W., Tsuji, M. & Steinman, R. Glycolipid -C-galactosylceramide is a distinct inducer of dendritic cell function during innate and adaptive immune responses of mice. *Proceedings of the National Academy of Sciences USA* **103,** 11252–11257 (2006).

Figuring out the shape of things

Two previously identified proteins play an unexpected role in regulating the formation and growth of a variety of important structures in the fruit fly

Respiration in the fruit fly, *Drosophila*, is mediated by the tracheal system, a network of branched tubules composed of polarized epithelial cells. To better understand the process by which these epithelia form, Shigeo Hayashi's team at the RIKEN Center for Developmental Biology in Kobe, along with colleagues at the University of Tokyo, National Institute of Genetics, and Tokyo Metropolitan University, screened a large set of *Drosophila* mutants to identify genes whose misexpression disrupted the development of the tracheal epithelium[1].

This screen led to the identification of a surprising candidate gene, encoding the protein kinase IKKε. Tracheal epithelia in fruit fly embryos overexpressing IKKε exhibited loss of polarity, as well as defects in the localization of F-actin, a cytoskeletal protein important for cell morphology and migration. In cultured *Drosophila* cells, reduction of IKKε activity dramatically affected cell shape, with mutant cells extending numerous branches to assume 'serrate' or 'stellate' morphologies, as opposed to the 'smooth' morphology of wild-type cells. Similar effects were seen in live embryos, where reduced IKKε activity led to defects in the late-stage development of tracheal branches. This manifested by abnormal splitting, turning and duplication of tracheal termini. Further support for IKKε regulation of F-actin came from the observation of defects in other structures, including the ends of the antennae (Fig. 1).

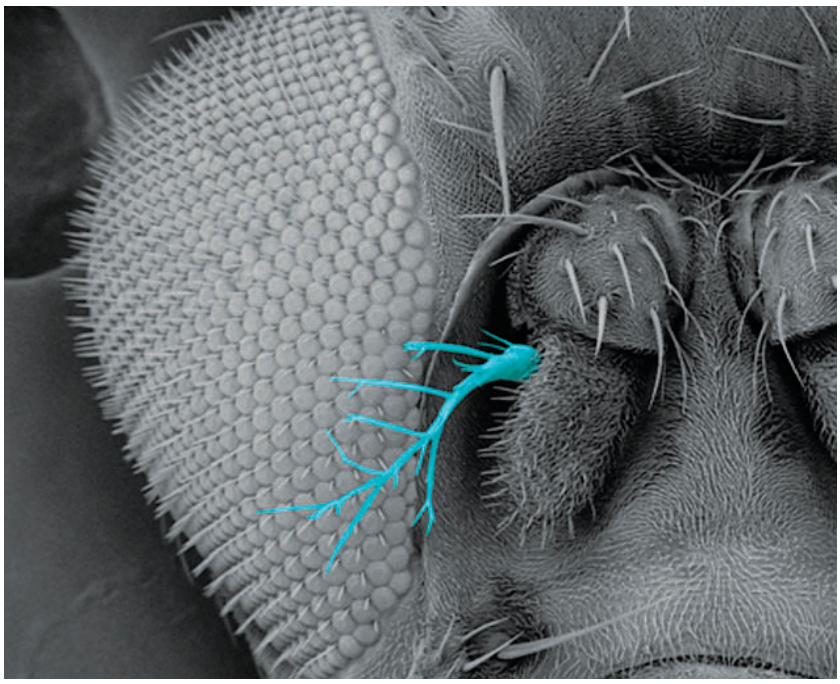

Figure 1: Expression of a 'dominant negative' form of IKKε, which interferes with function of the endogenous protein, results in excess lateral branching of the antenna (blue), and demonstrates a critical role of IKKε in proper formation of this structure.

Antenna development is also controlled by the protein DIAP1. Overproduction of DIAP1 exacerbated the developmental defects resulting from IKKε deficiency, but mitigated the effects of IKKε overexpression. On the other hand, IKKε appears to negatively regulate DIAP1 expression, suggesting that IKKε and DIAP1 play opposing roles in regulating F-actin-dependent morphogenetic events. DIAP1 is known to function as an inhibitor of caspases—the proteases that trigger apoptosis, the process of programmed cell death. Overexpression of IKKε led to increased apoptosis in cultured cells. However, it appears that under normal conditions, the roles of IKKε and DIAP1 in cellular morphogenesis are independent of their roles in regulating apoptosis.

Many of these findings were unexpected, according to Hayashi. "The IKKε family of protein kinases is known to include regulators of immune response, so we were very surprised to find that IKKε has a completely different role in F-actin-dependent cellular morphogenesis," he says. "We were even more surprised to find that IKKε functions as a negative regulator of the non-apoptotic functions of DIAP1." As these pathways are retained in vertebrates, these data may also provide insights into the role of caspases in the development of higher organisms. ■

1. Oshima, K., Takeda, M., Kuranaga, E., Ueda, R., Aigaki, T., Miura, M. & Hayashi, S. IKK regulates F actin assembly and interacts with *Drosophila* IAP1 in cellular morphogenesis. *Current Biology* **16,** 1531–1537 (2006).

Poles apart: new mechanisms in cell polarity

Real-time visualization of cytoskeleton-associated proteins reveals their roles in the development and maintenance of cell polarity

Researchers at the RIKEN Center for Developmental Biology in Kobe have identified important molecules that are essential for the establishment and maintenance of the anterior-posterior axis in the developing roundworm, *Caenorhabditis elegans*.

To develop a proper body plan, organisms must first establish an initial asymmetry. Asako Sugimoto, senior author of this new study[1], explains that "to make various cell types, some cells have to produce two daughters that will have distinct cell fates. Breaking of symmetry in the mother cell is the first step in this process."

Asymmetry first occurs when the sperm penetrates the egg, breaking its symmetry, and thus establishing a 'pole' within the one-celled embryo. Eventually a critical anterior-posterior (A-P) axis develops that allows future cell division to occur so that the worm can develop and mature normally. But, until now, it was unclear which molecules were important in establishing the A-P axis.

Sugimoto and her co-worker, Fumio Motegi, targeted three proteins (CDC-42, RHO-1, and ECT-2) associated with the cytoskeleton, since the cytoskeleton is important in the polarization process. Using a well-established genetic technique known as RNA-mediated interference (RNAi), they were able to eliminate the individual expression of each of these three proteins in newly fertilized *C. elegans* embryos.

The researchers then followed the motility of fluorescently-labeled proteins that were known to flow along the A-P axis to see how they behaved in the mutated embryos.

From these experiments, Sugimoto and Motegi were able to show that the A-P axis develops in two phases: in Phase I, RHO-1 and ECT-2 are required to establish the axis; while in Phase II, CDC-42 is required for its consolidation and maintenance. They then confirmed this finding by fluorescently labeling these three proteins and directly visualizing the dynamics and timing of their accumulation in the anterior pole (Fig. 1). Recent findings on the establishment of the A-P axis in *C. elegans* by two other groups[2,3] are consistent with this study.

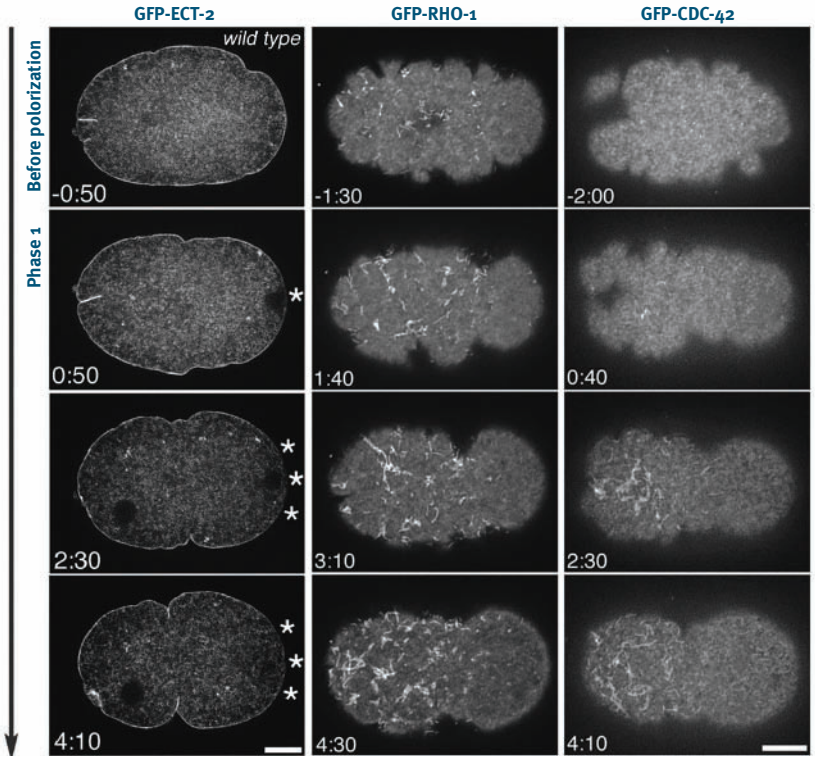

Figure 1: Anterior migration of fluorescently labeled ECT-2, RHO-1, and CDC-42 in a *C. elegans* embryo.

"Advancement of live-imaging techniques now enables us to examine highly dynamic cellular processes in live embryos and animals," says Sugimoto. "And, the combination of live-imaging and RNAi techniques is a powerful tool to dissect dynamic processes at a genetic level."

Sugimoto also thinks that it is highly likely that the mechanisms they have identified are also used in higher animals. ■

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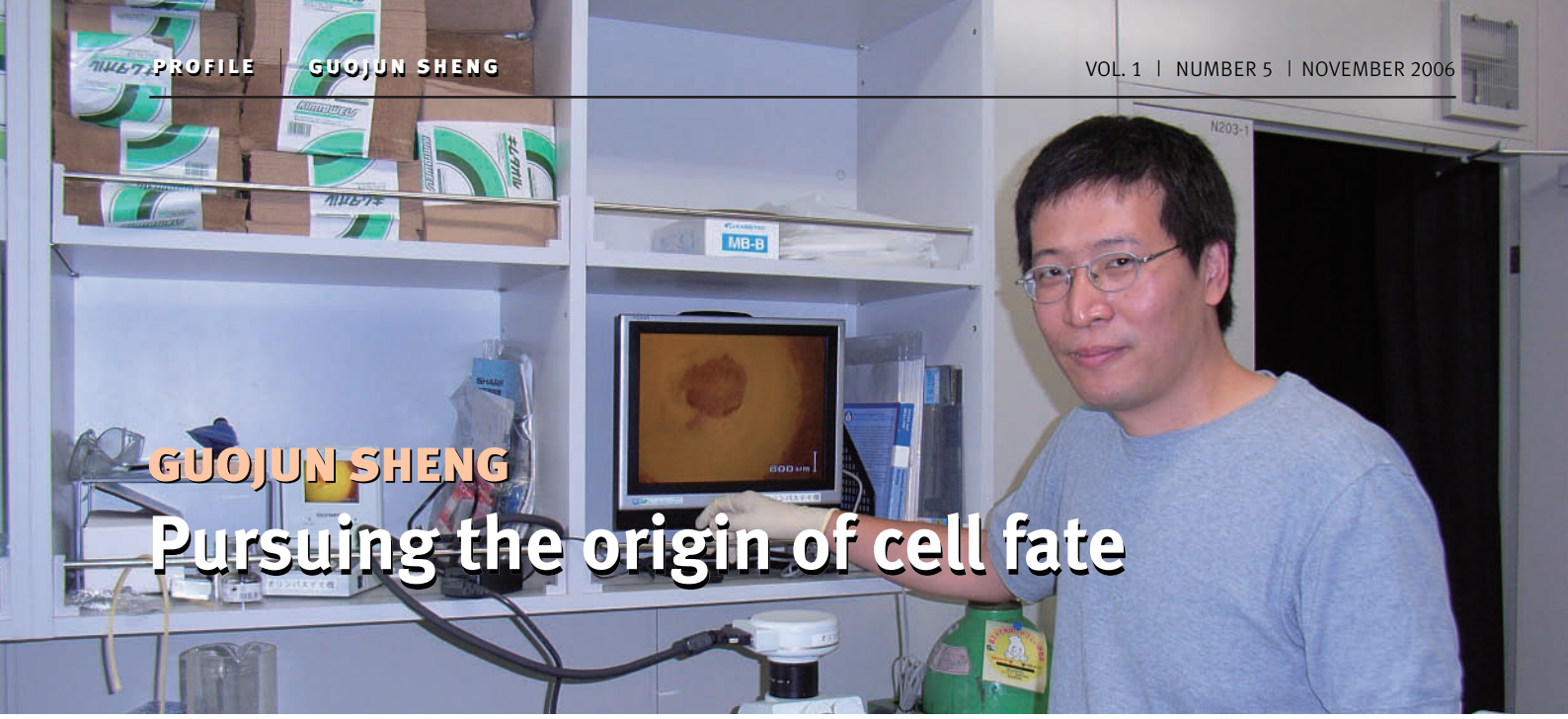


GUOJUN SHENG
Pursuing the origin of cell fate

A Chinese embryologist at RIKEN is looking into a classic question of what determines cells to become specific parts of a body

In any animal, a single cell, or a fertilized egg, repeats cell divisions to become a multicellular organism. From a very early stage of development, each cell knows exactly what part of a body it will become and follows a complicated blueprint every step of the way. "The fascination is to see simple things step by step become complicated ones," says Guojun Sheng, a Chinese embryologist at the RIKEN Center for Development Biology (CDB) in Kobe.

As a student Sheng became interested in how embryonic cells form patterns, differentiate into new roles, proliferate and coordinate each other or move around to establish a complex structure. Now he is trying to answer some of these questions by studying chickens. The leader of the Laboratory for Early Embryogenesis, Sheng also orchestrates his lively, ten-member laboratory to produce impressive insights, such as the unexpected role of a signaling molecule in regulating the differentiation of blood and vessel cells.

Shifting from flies to chicks

Born on a small island near Shanghai in 1968, Sheng initially became interested in chemistry influenced by his older sister, who was a high-school chemistry teacher. But he chose biology because he liked observing living things with a simple microscope and, he recalls, "biology looked trendier than chemistry."

Sheng majored in microbiology at Fudan University in Shanghai, studying all the main aspects of basic biology. After graduating in 1990, he flew to New York and enrolled in Rockefeller University to investigate the early development of the fruit fly, *Drosophila*, using molecular genetics. He joined the Laboratory of Claude Desplan, a renowned developmental geneticist from who Sheng says he learned tremendously about genetics and molecular biology, and about how to conduct science.

The turning point came during his six-year stay at Rockefeller, when Sheng spent six weeks at the famous Marine Biological Laboratory in Woods Hole, Massachusetts. Developmental biology started from embryology, but at that time the trend was to use molecular genetics as a method and *Drosophila* as a model. Sheng was no exception. But after working directly with embryos at the independent scientific institution, he was drawn into hands-on embryology. "The genetics of *Drosophila* is a key field. But I felt I was more interested in looking at embryos and manipulating them," Sheng says.

In 1998, Sheng went to Columbia University, New York, to join the laboratory run by Claudio Stern—one of the world's greatest embryologists, he says—as a postdoctoral fellow. He started to observe a chicken egg, which is not a useful model in studying genetics systems but is highly visual and extremely good for observing and manipulating embryos. It also hatches in just 21 days, making it easy to observe the detailed processes of development. In 2001, when Stern moved to the University College London, in the United Kingdom, Sheng went with him.

Tending an embryo

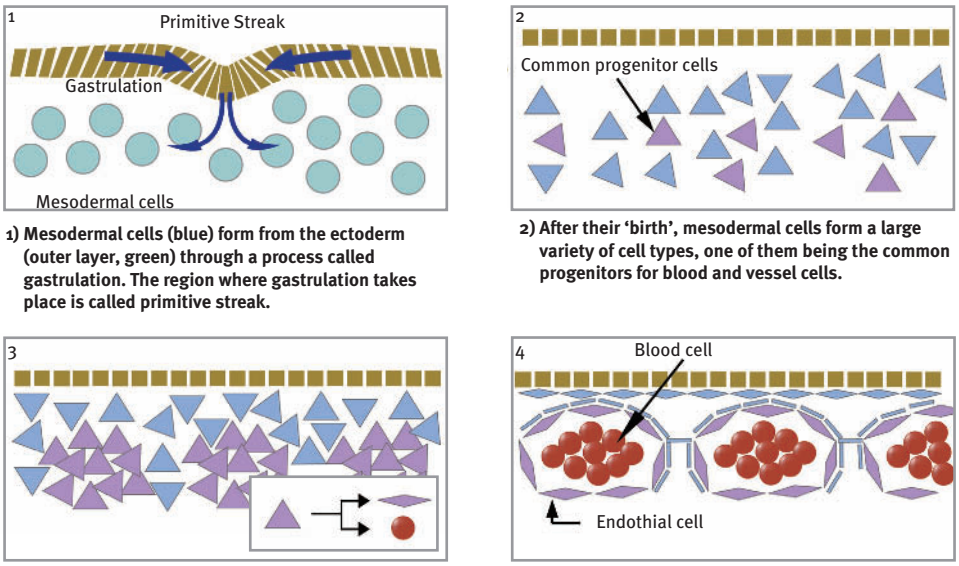
It took Sheng a few years to feel comfortable working with an embryo. That's because it requires a lot of skill to manipulate it, such as an ability to scratch it slightly to move out a layer, or to inject genes into the right places in cells. But he says he can now tell how an embryo is 'feeling' in a small culture dish.

In a fertilized chicken egg, an embryo sits like a white dot on the surface skin that covers a yolk. Using an embryo between half a day and two days old, Sheng's team looks at gastrulation, the earliest development phase. This is when the three germ layers—ectoderm, mesoderm and endoderm—are formed and their fate as specific organs and tissues is determined. Ectoderm mainly becomes skin and nerve cells. Some ectoderm cells move inwards at a region called the primitive streak, and become mesoderm, which gives rise to bone, blood, vessels and muscle. The endoderm becomes the digestive tract, liver, pancreas and lungs.

As a postdoc, Sheng concentrated on which cells stay in the ectoderm to become neural ectoderm and which move in to become mesoderm—in Sheng's words, "how to separate your brain from your skin".

Researchers knew that signaling molecules called FGFs (fibroblast growth factors) were crucial in the early formation of neural ectoderm and mesoderm. But how do cells receiving FGF

Figure 1:



1) Mesodermal cells (blue) form from the ectoderm (outer layer, green) through a process called gastrulation. The region where gastrulation takes place is called primitive streak.

2) After their 'birth', mesodermal cells form a large variety of cell types, one of them being the common progenitors for blood and vessel cells.

3) These cells coalesce to form numerous little aggregates called blood islands.

4) Blood and endothelial (vessel) cells differentiate into morphologically distinct cell types within each blood island. All these steps take place before the establishment of heart beating and circulation, and intricate molecular signaling at every step ensures proper execution of cell fate decisions.

Figure courtesy of Masahiro Shin, a research scientist at Sheng's lab

signals decide their response? Sheng found that a transcription factor known as Churchill plays a key role[1]. Churchill is induced slowly by FGFs – but then surprisingly at some point starts to inhibit FGFs' induction of mesoderm markers and of epithelial–mesenchymal transition (EMT), a process required to determine a cell's fate. Defects in EMT could be linked with cancer and other diseases.

CDB's third foreign team leader

After having some fruitful time in London, Sheng started to look for a new job, and a Japanese colleague recommended the CDB. He had other offers, but "nowhere else could match the environment and expertise", he says. He moved in 2004 to become the third non-Japanese team leader at the CDB in Kobe. In this cozy port city, Sheng spends his weekend driving up to a near mountain with his family and enjoys the changing faces of nature.

Recently, Sheng's focus has shifted to the mesoderm. His team is looking into how a subset of mesoderm differentiates into blood and endothelial cells, some of which become blood vessels (Fig.1). Many genes are initially expressed in both cell types but later are restricted to one or the other. "These things are not well understood," he says.

In a recent finding that has drawn much attention from blood scientists, Sheng's team suggested a new pathway for FGFs: inhibiting blood-cell formation and somehow promoting endothelial formation[2]. Sheng is also looking into stem cells, and working with other researchers to develop a higher-resolution technology to observe cell movements.

Fumie Nakazawa, a research scientist who joined Sheng's lab from the Tokyo Medical & Dental University two years ago, says she likes the he way keeps the lab open to new ideas and lets members work at their discretion. Another important factor that attracted her was "an English-speaking environment," she says.

Feeling dynamism

The CDB uses English as a common language, even though 90 per cent of its researchers and staff are still Japanese. Sheng says the number of foreigners doesn't matter to an international mindset. He appreciates the CDB's open culture which lets him chat with other researchers freely regardless of rank. "Talking about completely unrelated work could suggest fresh solutions in my areas of study," Sheng says.

Masatoshi Takeichi, the CDB Director, praises Sheng's outgoing and friendly personality: "He shows great interest in fields outside his own ones, and spurs active discussions. That is really helping to push forward the CDB's research overall."

"It's important to feel the dynamics, something really cooking. Ideas and people get mixed and go," says Sheng, adding he's satisfied with his current situation. "That's really important." ■

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About the researcher

Guojun Sheng was born on Zhoushan island, China in 1968 and graduated from Fudan University in 1990. In 1997, he earned his PhD in molecular genetics at Rockefeller University, New York. He took a six-week embryology course at the Woods Hole Oceanographic Institution's Marine Biological Laboratory, US, in 1996. From 1998 to 2001, he worked as a postdoctoral fellow at the Department of Genetics and Development, Columbia University, New York, then moved to the Department of Anatomy and Developmental Biology, University College London, from 2001 to 2004. Since 2004, he has been leading the Laboratory for Early Embryogenesis at the RIKEN Center for Developmental Biology.

Further information is available at http://www.cdb.riken.jp/en/index.html

Clarifying protein atomic structures for drug discovery

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To aid drug discovery, researchers at RIKEN's Structural Biophysics Laboratory are clarifying protein structures at the atomic level. Using bovine rhodopsin as a mammalian model, the team was the first to elucidate the atomic structure of a very important target in drug discovery—the so-called G-protein coupled receptor (GPCR). Recently, the team also succeeded in elucidating the catalytic mechanism by which an enzyme, named sphingomyelinase, snips off a lipid. These findings are expected to pave the way to new drug discoveries.

Crystal structure analysis of bovine rhodopsin

The finding on GPCR was published in August 2000 in the US magazine *Science*[1] and has since been cited in some 2,000 international scientific papers. The paper was published by a collaborative team at the Structural Biophysics Laboratory of the SPring-8 Center and Washington University in the US.

Team leader, Masashi Miyano, Chief Scientist of the Structural Biophysics Laboratory, says that the attractiveness of the paper lies in the importance of GPCRs as targets for drugs to cure various diseases. "More than 50% of the drugs used currently exert therapeutic actions via stimulation or reduction of the function of GPCRs induced by the drug binding to this receptor," he explains. "Thus, pharmaceutical companies are eager to know GPCR atomic structures ahead of their competitors."

As GPCRs are embedded in the cell membrane, they can interact with the environment inside and outside the cell. As such, they play a crucial role in receiving communication signals from other cells. This occurs when signalling molecules, called ligands, bind to the active site of the receptor outside the cell, which in response, activates certain cellular responses. Much drug development today is focused on finding chemicals that modulate the ability of ligands to bind with GPCRs, thereby either inhibiting or accelerating cellular processes.

To date, about 1,000 kinds of GPCRs have been identified in the whole human genome. Their corresponding ligands vary with about half of them being odorant receptors. Hormones, metabolites, proteins, lipids, sugars, amino acids, and viruses are also recognized by specific GPCRs. Due to the biological and medical importance of these receptors, various GPCRs have been studied since the 1980s to clarify how they transmit information.

In 2000, Miyano and colleagues elucidated the world's first atomic structure of GPCR (Fig. 1) using rhodopsin, which exists in photoreceptor cells, or rod cells in retina. Its ligand is a photon. "The mechanism to transduce information is considered to be common among all GPCRs, which means they should have a common structure for the shared common activation mechanism of G-proteins," Miyano explains. "Clarifying the atomic structure of rhodopsin must have had a significant influence on the development of novel GPCR-targeted drugs. In fact, pharmaceutical company researchers have often told us directly that 'it was really beneficial to our R&D'."

A mature and functional protein has its own defined spatial shape—a three-dimensional structure—in which the polypeptide chain coded by the DNA of the gene, as an amino acid sequence in the genome, is folded properly. In other words, a protein with an incorrectly folded polypeptide chain does not function and is even toxic in spite of having the same amino acid sequence. Miyano's teams' dogma is: 'Function and structure are two faces of the same thing in a biological system'. Thus, they aim to understand living organisms from the standpoint of the three-dimensional structures of proteins.

Miyano has two guiding principles in doing research. "One is to observe the three dimensional structures of proteins at the atomic level," he says. "We will be able to interpret and regulate the function of proteins only by using the three dimensional structure of the protein at the atomic level."

Miyano utilizes x-ray crystallographic analysis, not nuclear magnetic resonance analysis or electron microscopic analysis, to determine the protein structure at the atomic resolution since it is most economic and feasible. "Crystallization of a target protein was one of the biggest obstacles in analyzing the atomic structure using x-ray crystallography," notes Miyano. "Some proteins took several years to crystallize. However, continuous and extended efforts on protein crystallization have been making it easier with the exception of some specific proteins."

The teams' second guiding principle is 'focus on drug discovery'. Proteins are functional when their active sites bind molecules such as other proteins and lipids. This means it is possible to modulate protein function if researchers understand the structure of a target protein at an atomic level. Researchers can also artificially synthesize chemicals that are designed to bind the active site of the target protein. "Designing those molecules will lead to the development of new drugs," says Miyano.

Membrane proteins are a difficult research target

Since clarifying the structure of bovine rhodopsin in 2000, using the world-leading Synchrotron Radiation Facility, at SPring-8, researchers around the world have failed to clarify the crystal structure of other mammalian GPCRs. "Some groups have succeeded in analyzing the crystal structure of bovine rhodopsin," comments Miyano. "However, they have clarified bovine rhodopsin at the same inactive state as we did. Nothing is new except for achieving a higher resolution. We are also trying to analyze the crystal structure of other GPCRs, but it really is a big challenge."

The reasons why the crystal structure of bovine rhodopsin alone has been analyzed are readily identifiable. In general, a large amount of protein is needed to grow crystals. Researchers often use the bacterium *Escherichia coli* for over-expression of protein. However, this method cannot be applied easily to proteins like GPCRs that are embedded in the cell membrane. As bovine rhodopsin is concentrated in cow retinas, it can be obtained in bulk from meat-processing plants. Unfortunately, this is the only natural source of GPCR so readily available.

The crystallization of membrane proteins is also extremely difficult

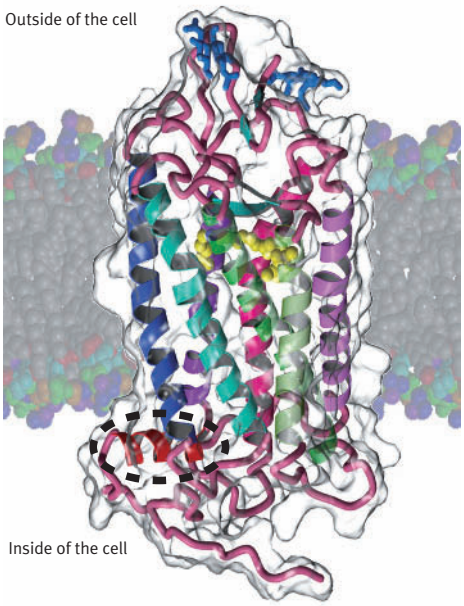


Figure 1: Three dimensional structure of bovine rhodopsin
Bovine rhodopsin is one of the g-protein coupled receptors (GPCRs). Rhodopsin is embedded in the cell membrane of a visual cell, and has the seven transmembrane α-helix structure. A newly-discovered, short helix 8 structure (indicated by the dotted circle) follows the seven transmembrane helix structure in the membrane surface inside the cell.

because they are embedded in the cell membrane. In the case of rhodopsin, Tetsuji Okada at Washington University spent five years growing crystals of bovine rhodopsin. He started the research when he was a special post-doctoral researcher at RIKEN. Okada is now the Chief Scientist at the Japan Biological Information Research Center, National Institute of Advanced Industrial Science and Technology.

"If we know the atomic structures of GPCRs from various signal transduction systems or from different living organisms, we can compare their structural differences and similarities. And this would be a significant step toward the elucidation of the structural basis of the signal transduction mechanism through GPCRs," says Miyano. To this end, his team is also working on the over-expression of membrane proteins using yeast and insect cells to crystallize membrane proteins.

Further explaining why it is so difficult to crystallize membrane proteins Miyano says: "To grow a protein crystal, we have to determine the conditions for the precipitating agents and pH (proton concentration in water) for each protein. We also have to take surfactants into account which makes the task more difficult. Cell membranes are made of lipid molecules. To extract membrane proteins from cell membranes, we need surfactants that can make oily lipids dissolve in water. It still usually takes a few years to crystallize a membrane protein. Yet we often fail to grow high-quality crystals that can be used for structure analysis."

Worldwide, scientists are competing fiercely to overcome the challenges in analyzing the structure of GPCRs. In Europe, a project called MePNet (Membrane Proteins Network) is proceeding. Under such circumstances, how can Miyano come out a winner?

"The next two or three years will be critical. What is necessary is not a major breakthrough, but a succession of small things integrated together step by step," Miyano says. "I am sure that we will succeed in clarifying the structure of new GPCRs again—by making the most of the dexterity and good teamwork for which Japanese people are characterized. Also the SPring-8 facility houses one of the world's best instruments to look at such things."

Lipids, another research target

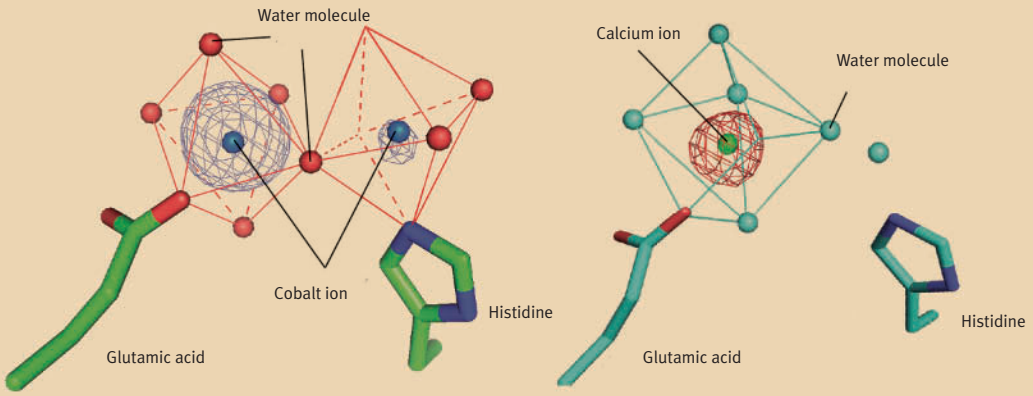
Lipids are another axis of research target in Miyano's laboratory. Lipids, along with proteins, are a major component of living bodies, and are also the main component of the cell membrane. "We pay attention to lipids because lipids themselves are 'mediators'," comments Miyano.

Many types of mediators are produced in living organisms. They are substances that bind to proteins to transmit signals and control the functions of living organisms. "What is interesting about lipid mediators is the fact that a single lipid changes into various derivatives, each of which exerts different functions," Miyano says. For example, a lipid is broken down into segments by enzymes in a variety of ways, or it can be modified by various sugars and phosphoric acid. Some lipid mediators function through the specific GPCRs as selective modulators. "We advance our research activities based on the assumption that assuming that we may be able to control various functions acting in living organisms by clarifying the interaction between lipids and proteins."

The Structural Biophysics Laboratory, in cooperation with Tokushima Bunri University, have clarified the crystal structure of a protein called sphingomyelinase, and elucidated its catalytic mechanism. This protein is an enzyme that serves as scissors to snip the lipid called sphingomyelin.

Hideo Ago, a senior research scientist at the Laboratory explains the background to this research. "The functions of some proteins are controlled by the number and kind of metal ions that bind to the proteins. It is also known from biochemical experiments in the 1970s that there are two types of metal ions; one enhances enzymatic activity significantly, and

Figure 2: **Two types of metal ion binding structures at the active site of sphingomyelinase.**



Water-bridged divalent metal ions binding structure
Enzymatic activity is significantly increased when both glutamic acid and histidine bind a divalent metal ion and the two metal ions are bridged by a water molecule. Cobalt ions are used for this structural analysis. Within an organism, magnesium ions work the same way.

Calcium ion binding structure
Enzymatic activity of this model is not much enhanced because the calcium ion is larger than the magnesium ion or cobalt ion. Thus the bound calcium ion at the glutamic acid prevents binding of another metal ion at the histidine.

the other enhances enzymatic activity slightly. However, the mechanism had remained a mystery," Ago says.

When sphingomyelinase snips sphingomyelin, it produces ceramide and phosphocholine that serve as mediators. Sphingomyelin is also considered to form a structure on the cell membrane, called a 'raft', upon which the GCPRs are floating. "We began this research assuming that if the crystal structure of sphingomyelinase is clarified and its catalytic mechanism is elucidated at the atomic level, we can expect a new interesting approach that links lipids, cell membranes, and even GPCRs," says Ago.

Figure 2 shows the metal ion binding structures at the active site of sphingomyelinase that the team has clarified to date. Enzymatic activity is greatly enhanced when both glutamic acid and histidine bind a metal ion each, and a water molecule is placed in between the two metal ions (Fig. 2, left). In contrast, enzymatic activity is not much enhanced when a calcium ion binds to the glutamic acid (Fig. 2, right). The water-bridged double divalent cations are considered to be essential in enhancing enzymatic activity significantly. When a calcium ion has bound to the glutamic acid, there is no room left for another ion to bind to the corresponding histidine because the size of a calcium ion is larger than a metal ion which enhances the enzymatic activity. "The mechanism is simple and convincing when you look at the structures," notes Ago. "We succeeded in clarifying the structure because we managed to observe the structure at the atomic level."

In this study, the team used sphingomyelinase from *Bacillus cereus*. However, humans have similar enzymes. "It has been found that the function of human sphingomyelinase is related to apoptosis in nerve cells and lung emphysema caused by cigarette smoking," says Ago. "Furthermore, ceramide, which is produced when sphingomyelin is broken down, are related to skin moisture retention. Thus we think the findings of this research will lead to the discovery of various drugs in the future."

Future research

In considering the future of his teams' research Miyano says, "We will do what we can do now. As a first step, we are trying to clarify the crystal structure of new GPCRs as soon as possible. We will also advance the use of SPring-8, and address the challenges to be faced, no matter how difficult." Miyano also considers that now is a very interesting time to be involved in research. "Things we have never imagined are being clarified one after another—protein research is truly very interesting," he notes. "However, although it is really interesting, we cannot afford to take enough time to appreciate and enjoy it. This, I think, is the tragedy of today's life science." ■

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About the researchers

Masashi Miyano

Masashi Miyano was born in 1953 near Lake Hamana in central Japan. After graduating from Tohoku University Graduate School in 1978, Miyano joined the Japan Tobacco & Salt Monopoly Company (currently Japan Tobacco) as a bio-organic chemist where he worked on tobacco alkaloids and the biophysical chemistry of a major plant protein. As a post-doctoral student, Miyano studied protein crystallography at the University of California, San Diego. He then went on to become the team leader of JT Life Science Laboratories. In 1989, Miyano returned to San Diego to work in a joint R&D program with Agouron Pharmaceuticals (now Pfizer San Diego). Before moving to RIKEN in 2000, Miyano worked on drug discovery using structure-based drug design at the JT Central Pharmaceutical Research Institute.

Hideo Ago

Hideo Ago graduated from Osaka University in 1991 where he started studying the structure of proteins using x-ray crystallographic analysis. From 1991 to 1994 at the Life Science Research Laboratory of Japan Tobacco Inc., he studied the structural biology of plant proteins that have anti-viral activity. In 1995, Ago received his doctorate from Tokyo Institute of Technology. From 1995 to 2001 at the Central Research Institute of JT, before moving to RIKEN, he studied the structure of proteins involved in the production of lipid mediators and viral replication for drug discovery.

Visit by the Emperor and Empress

On October 3, Their Majesties Emperor Akihito and Empress Michiko visited the Wako institute. The Emperor had been to the institute fourteen years ago, while it was the first visit for the Empress. Over a hundred employees gathered to welcome the Emperor and Empress when they arrived. The Imperial couple firstly received an introduction to RIKEN's history and organizational structure from the President, Ryoji Noyori, and some of the Executive Directors. Next, they visited several laboratories to hear about the discovery of the new element 113, the structural analysis of the cerebral cortex, and other recent achievements, from the researchers themselves. The researchers reported that both the Emperor and Empress asked knowledgeable questions. ■

Conference on Lifescience Grid

The third international LSGrid conference on the use of grid computing in the life sciences was held on October 13 and 14 at RIKEN's Genomic Sciences Center in Yokohama. The idea of grid computing is inspired by the electric power grid. Just as people can use mains electricity anywhere and at any time simply by plugging things in, so with grid computing they can freely use all the computers on a network, from desktop computers to supercomputers. Experiments aimed at achieving grid computing are now underway in several fields.

Grid computing has been attracting attention from researchers in the life sciences who want to analyze enormous amounts of data about DNA and proteins and then use this data to develop new drugs and investigate the causes of diseases. This is what motivated Akihiko Konagaya, director of the GSC Advanced Genome Information Technology Research Group, to organize the first LSGrid conference, in Kanazawa in 2004.

This third LSGrid conference was held in Yokohama to promote cooperation between Asian countries. More than More than eighty researchers attended from twelve countries: India, Indonesia, Pakistan, Korea, Taiwan and Vietnam, as well as European countries and the USA. The keynote speech was by Paul Gilna of the Community Cyberinfrastructure for Advanced Marine Microbial Ecology Research and Analysis project, known as CAMERA. He explained the basic infrastructure for meta-genome information processing. Other speakers presented examples of applied research, including virtual screening for the development of a vaccine for malaria.

As an experiment, this LSGrid conference included a panel discussion for ASEAN countries on the construction of a worldwide grid. Representatives described what their countries are doing for this effort, and there were lively debates about the construction of the international grid environment that will be necessary in the future. Next year's LSGrid conference will be held in Glasgow, Scotland. ■

Volume-CAD (VCAD) systems symposium

On October 18 and 19, a symposium on Volume-CAD (VCAD) systems was held in Wako. Whereas CAD (computer-aided design) software also describes the shapes of objects, VCAD software describes their internal structures and physical properties as well, and is expected to lead to major improvements in manufacturing processes. The development of this technology was begun in 2001 by Akitake Makinouchi, who is now director of the VCAD System Research Program at RIKEN.

The theme for the first day of the symposium was "Applications of VCAD to manufacturing industry". Speakers explained how VCAD systems can manipulate both design data and measurement data about the insides of objects, and how this will make it possible to manage production consistently from simulation through to manufacture. In July the VCAD System Research Program released nine pieces of software that are essential for making VCAD models. This software is available to the public for free, and is initially aimed at the Japanese market.

The second day's theme was "Applications of VCAD to the life sciences". VCAD is useful not only for industrial manufacturing but also for modeling natural objects such as human bodies and cells, and several research projects were presented that demonstrate this. In a lecture referring to the Next-Generation Supercomputer, a national project that is being carried out by RIKEN, it was reported that running VCAD software will be one of the major uses for this supercomputer. The wide range of topics discussed at the symposium gave an indication of the breadth of VCAD research.

In his closing remarks, Akitake Makinouchi said, "I am very pleased and impressed to see that the use of VCAD in product development is continuing to gather pace, and that it is finding applications in the life sciences. This encourages me to work on further developments". ■

Lighting the way for lasers

Since RIKEN initiated Japan's laser research in the 1960s, its researchers have been improving the technology to design this artificial light more easily

The laser is one of the greatest inventions of the 20th century. It was first demonstrated in the US in 1960. In Japan, a laser research team at RIKEN initiated the research into this new form of light. This team also played an important role in pioneering open-research systems and flexible employment at RIKEN.

Lasers are produced by amplifying photons in a coherent state and have a range of applications. They are used in medical equipment, as well as a tool to develop electronics and study various scientific phenomena.

At RIKEN in 1960, researchers started by studying the predecessor to the laser—the maser, which is the acronym for Microwave Amplification by Stimulated Emission of Radiation. Two years later, other researchers started to develop a fine-processing technology using lasers. Then in 1968, RIKEN launched a three-year laser project with the aim of deepening its basic fundamental research knowledge and developing new technical applications. The project was a watershed because it spurred many other laboratories to become interested in lasers that ultimately strengthened RIKEN's laser research potential.

As laser research became increasingly important, in 1976 RIKEN launched a large-scale project on laser science research, which eventually ended in 1997. During these 21 years, the laser team became the first at RIKEN to employ contracted or post-doctoral researchers, and to promote domestic and international collaborations with researchers in other disciplines, such as semiconductor research and chemistry.

From 1976 to 1985, the researchers focused on strengthening the basis of laser science overall. One of the most notable events was three key laboratories collaborating to develop a laser for use to separate isotopes from various elements, with the focus on uranium.

In natural uranium, 99.3% is an isotope called uranium-238 and 0.7% is uranium-235. Only the latter triggers a fission chain reaction. To use uranium as a nuclear fuel, the uranium-235 has to be enriched to 3% of the total. RIKEN's researchers thought the use of a laser would drastically reduce the time and cost of enriching uranium than using a conventional gaseous diffusion device.

Other researchers worldwide were also developing similar lasers, but often failed to separate uranium-235 from gaseous uranium hexafluoride due to the difficulty in creating the optimal wavelength of 16 micrometers, which was called the 'magic number.'

But RIKEN successfully developed a tunable infrared laser that could emit the world's strongest 16 micrometer light that reacted only to the uranium-235 to dissociate it.

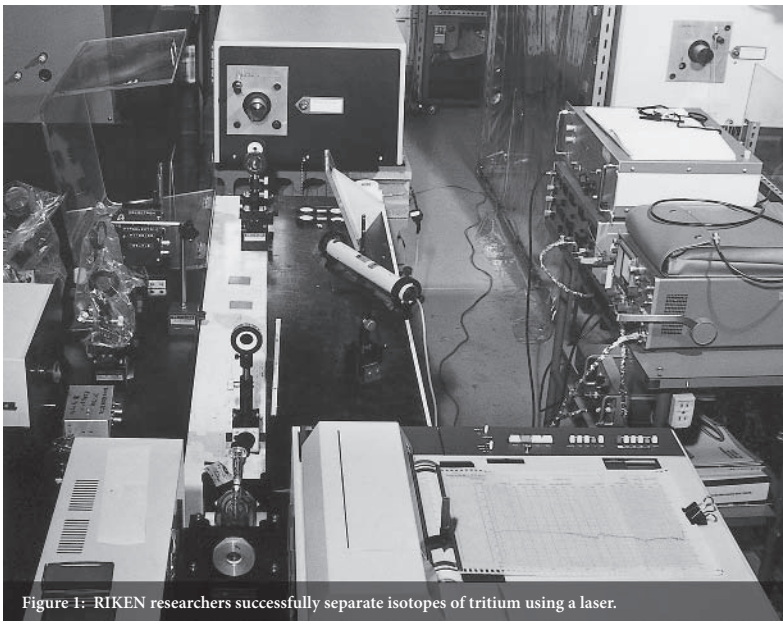

Figure 1: RIKEN researchers successfully separate isotopes of tritium using a laser.

RIKEN researchers first succeeded in this project by cooling a 16 micrometer laser cell to an extremely low temperature. But this method was ineffective, so they later developed new technology capable of working at room temperature, which greatly increased the productivity of uranium enrichment.

Other achievements of the researchers include the separation of tritium isotopes using a carbon-dioxide laser, and the development of an excimer laser for use in semiconductor lithography (Fig.1).

In the 1980s, researchers began investigating untapped wavelengths—x-rays—in a bid to seek further advanced applications of lasers. New research projects that started at that time, including the generation of intense, soft x-ray laser pulses, have recently begun to yield results.

By the beginning of the 1990s, high-quality visible and infrared lasers were developed. In 1997, the 'laser science research' project ended, but the newly established Laser Technology Laboratory, headed by laser scientist Katsumi Midorikawa, picked up the reins. Midorikawa created a new research concept called 'coherent science,' which takes advantage of a laser's coherent waves to control materials. For example, microscopes equipped with a laser often killed cells inadvertently during observations, so researchers at RIKEN designed a novel laser with complex, ultra short-pulse waves that didn't kill cells.

The current focus of research is creating lasers with shorter and shorter pulses at the interval of attoseconds, or quintillionths of a second, while looking for new chemical reactions that interact with a laser. ■

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