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Mobilizing calcium ions

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How plants work with sulfur

Researchers unravel a process essential to human wellbeing

Molecular biologists from the RIKEN Plant Science Center in Yokohama have identified a key genetic transcription factor which increases the efficiency of uptake and use of sulfur by plants in sulfur-deficient environments.

The work is significant as all plants need sulfur for growth. Many agricultural species—particularly the Brassicas, such as canola, broccoli and cabbages—require sulfur fertilization for maximum crop yields.

Sulfur also is an essential nutrient for animals, including humans, as it is an active part of two amino acids of critical importance to the structure of proteins. While plants can take up sulfur from the soil in the form of sulfates, animals can only acquire sulfur by eating plants or other animals.

The research could lead to more efficient use of fertilizers, according to team leader, Hideki Takahashi. This would reduce over-application and consequent pollution of waterways. Plants also use sulfur to make defensive compounds. One major group of these produced in Brassicas, the glucosinolates, is well known for protecting humans against cancer. "Our work may help us engineer increased glucosinolate production in Brassica crops," Takahashi says.

Boosting sulfur uptake

In response to sulfur deficiency, plants are able to boost their ability to take up sulfates from the soil, to release and transport sulfur within themselves, and to break down their sulfur-rich defense chemicals, such as glucosinolates, and recycle the sulfur they contain. This response is highly coordinated across the whole plant. So the research team reasoned that it might be initiated by a single critical regulator, perhaps a transcription factor, the essential link in the process of transcribing genes into messenger RNAs that generate the proteins and enzymes necessary to maintain the cellular function. Such a regulatory factor could simultaneously switch on the production of several different proteins necessary to the adjustment of the plant's metabolism in response to the sulfur supply shortage.

Finding and verifying the critical factor

In a recent paper in *The Plant Cell*[1], Akiko Maruyama-Nakashita and researchers from Takahashi's team describe how they were able to track down this critical factor—which they called SLIM1—in the thale cress, *Arabidopsis thaliana*. They did so by carefully fashioning a gene which produces a fluorescent protein under the control of the most important sulfate transporter—the ion transporting protein responsible for sulfate uptake—generated in response to low sulfur conditions (Fig. 1). This genetically modified plant fluoresces when this sulfate transporter is present and active under low sulfur conditions.

Using this 'sulfur indicator' plant, the team searched for those individuals which did not fluoresce under low sulfur conditions, suggesting they possessed a mutant form of SLIM1, unable to stimulate the production of the sulfate transporter. The mutants they discovered led them back to the SLIM1 gene itself. They then set out to demonstrate SLIM1's involvement in the other parts of the coordinated response.

First, they were able to verify SLIM1's overall involvement in the process. They did so by adding and activating the normal SLIM1 gene to plants which carried the mutant form of the gene. This restored the typical response to sulfur-deficient environments. Further, the team demonstrated how widespread and important the mechanism is in plants, by using the rice plant version of the SLIM1 gene to compensate for its inactivity in *Arabidopsis* mutants.

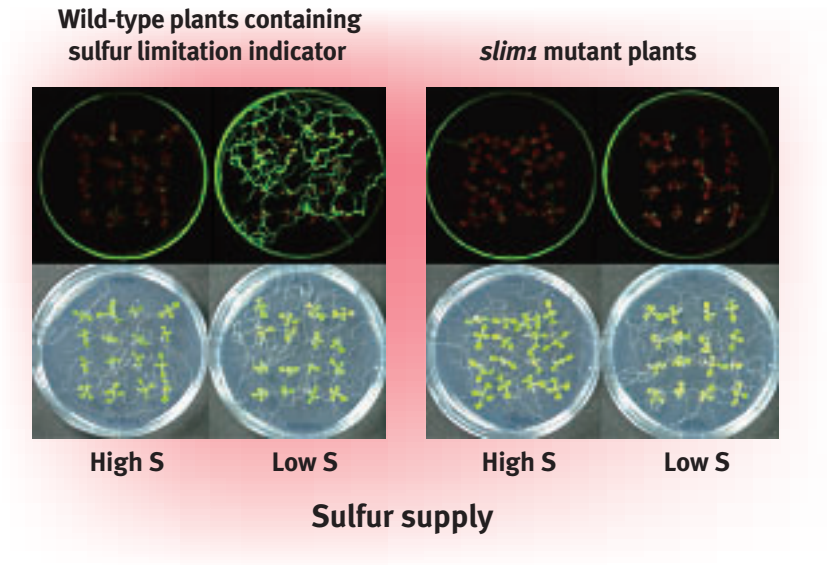

Figure 1: *Arabidopsis* plants carrying the normal SLIM1 gene fluoresce under low sulfur conditions (left). Plants with a mutant version of SLIM1 do not fluoresce (right).

Then, the group used gene chip technology to determine which genes SLIM1 switches on or activates, and hence which compounds and biochemical pathways it affects. Gene chips are able to show which genes are active in cell extracts, and which are not. The researchers compared gene activity under sulfur-deficient conditions in the roots of plants with normal and with mutant SLIM1 genes.

The results indicate that under sulfur-deficient conditions SLIM1 is responsible for switching on genes for sulfate transporters and enzymes that break down glucosinolates, and for switching off genes for the enzymes that produce the glucosinolates in the first place. This work could be cross-referenced and verified by studying the compounds that are produced and accumulated in the mutant plants when they are grown in sulfur-deficient conditions.

The researchers were also able to link SLIM1 back to a gene already named in the *Arabidopsis* genome. They showed that the mutants of the SLIM1 gene fall into the region of the *Arabidopsis* genome which encodes one of a family of six transcription factors known collectively as ethylene-insensitive-like (EIL) genes. The SLIM1 gene is, in fact, EIL3. They verified that this is the only member of the family specialized for the sulfur response by using each of the six EIL genes in turn to try to compensate for SLIM1 in the mutant plants. Only EIL3 was able to do so.

Clearly, not all the impacts of SLIM1 are caused directly by its protein product. The transcription factor sets off a cascade of downstream biochemical reactions. So the research team is now looking more closely at the SLIM1-mediated response, says Takahashi, to track down the additional protein factors necessary for the metabolic response in plants.

One goal is to engineer plants adapted to low-sulfur environments. Another, says Maruyama-Nakashita, is to explore the potential for producing a wider range of edible crop plants that incorporate cancer-protective glucosinolate compounds. ■

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About the researchers

Hideki Takahashi was born in 1967, and graduated from Kyoto University in 1990 with a major in biochemical engineering. From 1990 to 1993, he worked as a research associate at Idemitsu Petrochemical, and then earned his doctorate in pharmaceutical sciences from Chiba University in 1998. From 1998 to 2000, Takahashi served as a postdoctoral fellow at Chiba University and the Carnegie Institution of Washington, US. In 2000, he was appointed as the leader of the Plant Nutrition and Basal Metabolism Research Team at the RIKEN Plant Science Center.

Akiko Maruyama-Nakashita was born in 1971, and graduated from Tohoku University in 1995 with a major in biology. In 1997, she earned her masters degree in biology, and obtained her doctorate in biology in 2000, both from Tohoku University. After working as a postdoctoral fellow at Chiba University for a year, she joined Takahashi's laboratory at RIKEN in 2001 as a research scientist.

Driving for smaller engines

Quantum demon could boost the efficiency of tiny nano-engines

A microscopic engine that relies on the subatomic world for its power has been proposed by RIKEN researchers. If realized, it could be one of the smallest engines ever built.

Conventional heat engines, such as those that power your car, transform heat into useful work. In the case of the car engine, this is done by the expansion and contraction of gas produced in fuel combustion.

Franco Nori and Yu-xi Liu of RIKEN's Frontier Research System, Wako, and the University of Michigan, US, and colleagues from the Chinese Academy of Sciences in Beijing, are investigating the quantum equivalent of these engines.

These proposed quantum heat engines produce work using quantum matter—subatomic particles, for example—as their working matter, instead of heated gas.

The quantum nature of the working substance means that quantum heat engines should have unusual and exotic properties. Under some conditions, quantum heat engines are predicted to surpass the maximum limit on the amount of work done by a conventional engine.

The scientists now believe that they have a practical design for a quantum heat engine based on superconducting circuits.

The design relies on a concept called Maxwell's demon, originally coined by eminent physicist James Clerk Maxwell in the nineteenth century as an imaginary way to get round the second law of thermodynamics. This law insists that heat cannot flow spontaneously from a cold material to a hotter material, and that any system will naturally tend towards greater disorder.

Maxwell suggested that an imaginary demon might be able to make heat flow the 'wrong' way by strategically opening and closing a portal between two chambers, allowing only the faster, hotter molecules through into one of the chambers, which would gradually heat up.

This apparent contradiction to the second law can be resolved by considering the amount of information held in the system, along with its heat. Information—in the form of encoded quantum states—can also contribute to the amount of order in the system. So Nori's team propose a 'demon' that processes quantum information as it helps to shuttle heat from one place to another. The team's calculations show that this combination allows their demon to balance the system's thermodynamic books, and potentially create an efficient engine[1,2].

The work is also helping to reveal new aspects of quantum theory: "Quantum heat engines can highlight the difference between classical and quantum thermodynamic systems, and help us understand the transition and boundary between them," says Nori. ■

1. Quan, H. T., Wang, Y. D., Liu, Y.-X., Sun, C. P. & Nori, F. Maxwell's demon assisted thermodynamic cycle in superconducting quantum circuits. *Physical Review Letters* **97,** 180402 (2006).

2. Quan, H. T., Liu, Y.-X., Sun, C. P. & Nori, F. Quantum thermodynamic cycles and quantum heat engines. Published online at:http://arxiv.org/PS_cache/quant-ph/pdf/0611/0611275.pdf

The staircase to magnetic order

Researchers record the first direct observation of the correlation between magnetic order and crystal structure in strong magnetic fields

Condensed-matter physicists have a strong interest in transition metal oxides, ceramics based on elements from groups 3 to 11 of the periodic table such as manganese or copper. Many unusual magnetic phenomena are observed in transition metal oxides, as their crystal structure often leads to a complicated arrangement of the atomic magnets. One example is the compound $CuFeO_2$ in which the magnetic iron atoms arrange themselves in a hexagonal lattice that is slightly distorted due to an effect known as magnetic frustration (Fig. 1a). At low temperatures, $CuFeO_2$ shows an antiferromagnetic order, where some of the iron magnets point upwards, others downwards.

A team of researchers from the RIKEN SPring-8 Center in Harima and from the universities of Tokyo and Osaka have now unraveled the complex interplay between this magnetic arrangement and the hexagonal structure of $CuFeO_2$. In experiments published in the journal *Physical Review B*[1], the team used a newly designed magnet capable of reaching very high magnetic fields to study the influence of an increasing magnetic field on the material's magnetic order and its crystal structure.

Measurement of the crystal structure of transition metal oxides in high magnetic fields has been a particularly challenging problem for physicists, mainly owing to design restrictions of the experimental setup. "Previously, such high magnetic fields had been unavailable for the necessary diffraction measurements, and a high-field magnet had first to be developed for the x-ray diffraction experiments at SPring-8," explains Koichi Katsumata from the RIKEN team.

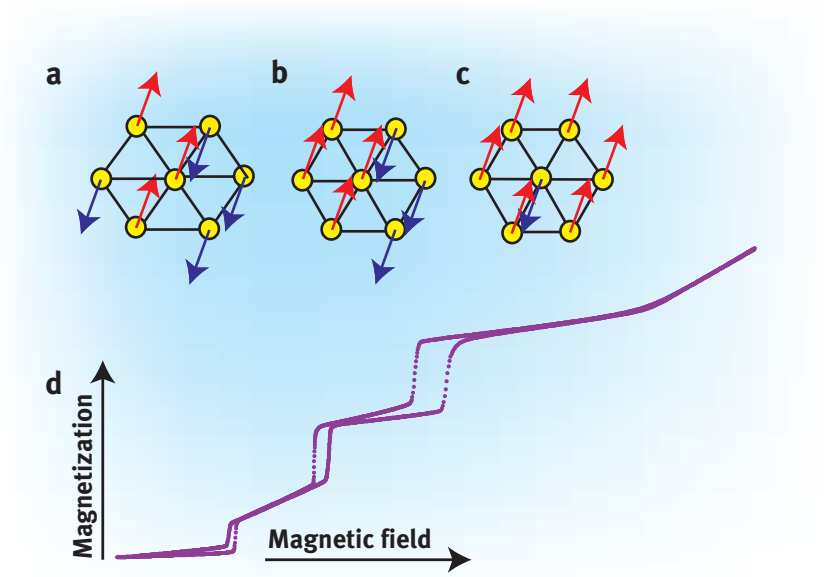

Figure 1: The relationship between magnetic order and the crystal structure of $CuFeO_2$. With an increasing magnetic field, the magnetic moments (blue and red arrows) align (a-c), leading to steps in the magnetization (d). Simultaneously, the hexagonal lattice becomes more symmetric.

The team found that as the magnetic field is increased, the magnetic moments of the structure (Fig. 1a) rearrange themselves more and more in direction of the field (Fig. 1 b, c) until the upward-pointing moments dominate. In parallel to this successive alignment, the magnetization of $CuFeO_2$ shows a number of steps (Fig. 1d). Importantly, the team observed that as each step occurs, the crystal structure also changes and the iron hexagons become more symmetric. This represents the first direct evidence that at low magnetic fields the antiferromagnetic order leads to a distortion of the regular hexagonal array, and therefore minimizes magnetic frustration.

Armed with such unique experimental capabilities, the team will now study materials with similar behavior. "Many interesting phenomena are expected to occur in magnetic materials as well as superconductors at high magnetic fields," explains Katsumata. Clearly, transition metal oxides offer abundant opportunities to learn more about the fundamental physics governing such intriguing effects. ■

1. Terada, N., Narumi, Y., Katsumata, K., Yamamoto, T., Staub, U., Kindo, K., Hagiwara, M., Tanaka, Y., Kikkawa, A., Toyokawa, H., Fukui, T., Kanmuri, R., Ishikawa, T. & Kitamura, H. Field-induced lattice staircase in a frustrated antiferromagnet CuFeO2. *Physical Review B* **74,** 180404(R) (2006).

Positive thinking produces better catalysts

Researchers show a new class of positively charged metal clusters to be efficient and selective catalysts in polymerization reactions

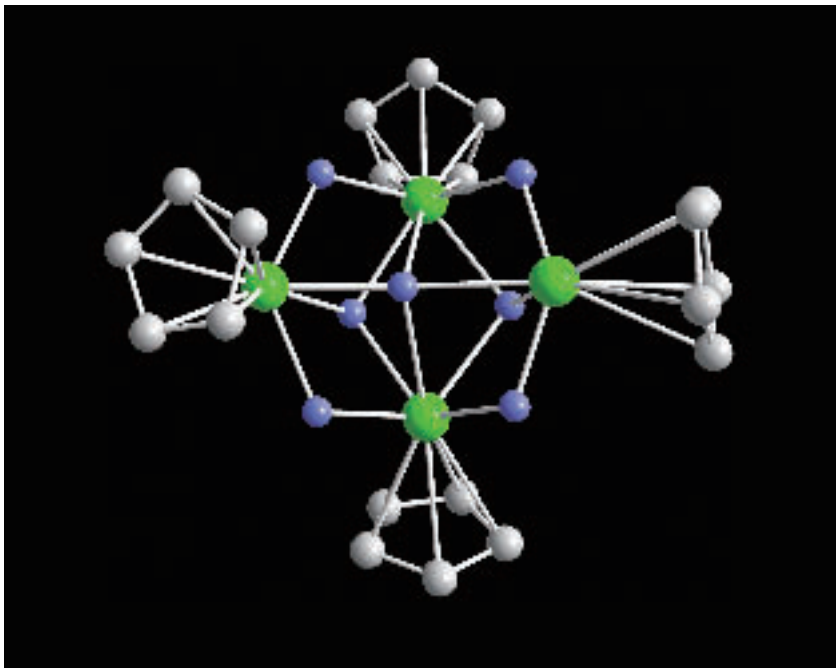

Figure 1: The core structure of the cationic tetranuclear complex based on yttrium (green). The bridging hydrogen atoms are shown in blue and the carbon atoms of the organic ligands are grey.

Catalysts are substances that are added in small quantities to chemical reactions to make them go faster or, in some cases, make them work when they otherwise wouldn't. The development of new catalysts is of fundamental importance in chemical research—new catalysts lead to new reactions that, in turn, can be used to make new and improved products.

Zhaomin Hou and co-workers from the RIKEN Discovery Research Institute in Wako are developing catalysts based on the rare-earth metals—a group of elements that comprises scandium, yttrium and the lanthanides. Recently, they reported[1] a new class of catalysts, called multinuclear polyhydrido complexes, in which four metals are linked together with hydrogen atoms.

Now, they have shown[2] that these neutral compounds can undergo a simple chemical reaction—in which each cluster loses one hydrogen atom and two electrons—to form positively charged (cationic) rare-earth hydrides (Fig. 1), which are the first examples of their kind. These cationic complexes have completely different catalytic activities than their neutral precursors, and can be used to make polymeric materials that have not been made previously.

Polymers are produced when many copies of the same small-molecule, referred to as a monomer, are joined together to form chains, often in the presence of a metal-based catalyst. However, it is difficult to controllably polymerize some monomers, resulting in unwanted mixtures of differently connected products. One such example is 1,3-cyclohexadiene, a compound that can be polymerized to form a string of six-membered carbon rings that can be linked together in many different ways.

Using their cationic catalysts, however, Hou and co-workers have, for the first time, been able to selectively produce a single pure and crystalline polymeric product from 1,3-cyclohexadiene in which all of the rings are connected together in the same way. This regularity improves the mechanical properties of the material when compared with one in which there are different links along the chain.

Hou points out that polymers of this type have many desirable properties such as high thermal stability, good mechanical strength, transparency and chemical resistance. Moreover, as they still contain reactive sites along the chain, they can be used to make high-performance co-polymers by grafting other groups on to them.

But this is just the beginning. "The successful isolation of these cationic complexes has opened a new frontier in metal hydride chemistry," comments Hou, "and could lead to the development of other new catalytic reactions." ■

1. Shima, Y. & Hou, Z. Hydrogenation of carbon monoxide by tetranuclear rare earth metal polyhydrido complexes. Selective formation of ethylene and isolation of well-defined polyoxo rare earth metal clusters. *Journal of the American Chemical Society* **128,** 8124–8125 (2006).
2. Li, X., Baldamus, J., Nishiura, M., Tardif, O. & Hou, Z. Cationic rare-earth polyhydrido complexes: Synthesis, structure, and catalytic activity for the cis-1,4-selective polymerization of 1,3-cyclohexadiene. *Angewandte Chemie International Edition* **45,** 8184–8188 (2006).

How one protein bends cells out of shape

Detailed structural analysis of a protein may have revealed how its shape enables it to induce cell membrane deformation in response to specific signals

The insulin receptor substrate p53 (IRSp53) protein is associated with a number of cellular functions, including cytoskeletal dynamics and membrane ruffling—the formation of small membrane protrusions on the cell surface. Several years ago, Tadaomi Takenawa and colleagues at the University of Tokyo found that IRSp53 is directly involved in mediating ruffling in response to the activation of Rac[1], a protein involved in numerous important signaling pathways. This finding proved intriguing for Shigeyuki Yokoyama of the RIKEN Genomic Sciences Center in Yokohama. "We are interested in this result because the Rho family GTPases, which include Rac, are involved in regulation of cell shape. And this is a central theme in cellular signal transduction," explains Yokoyama.

Earlier work suggests that the Rac-binding domain (RCB) of IRSp53 (Fig. 1) has structural similarities to the Bin-amphilysin-Rvs (BAR) membrane-binding domain, which directly binds to and alters the shape of cellular membranes. Now, in an effort to further explore potential functional and mechanistic similarities between the RCB and BAR domains, the groups of Yokoyama and Takenawa have collaborated on a detailed structural and functional examination of the RCB domain[2].

Initial structural data revealed that although the two domains show some structural similarities, RCB is considerably straighter than the crescent-shaped BAR domain, with a lipid-binding surface that is convex rather than concave. Upon incubating small lipid vesicles, or liposomes, with the RCB domain from IRSp53 and activated Rac, the researchers observed the formation of small buds in the membrane; this behavior was not observed in the presence of RCB or Rac alone. The shape of the protrusions formed *in vitro* differs in appearance from those formed by BAR. Yokoyama and colleagues hypothesize that this is due to the distinctively convex lipid-binding surface of RCB. The team obtained similar results in mouse cells, where the expression of fluorescently-tagged RCB led to the formation of membrane 'microspikes'. They also demonstrated that variants of RCB in which the lipid- or Rac-binding regions have been mutated lose their ability to induce microspike formation.

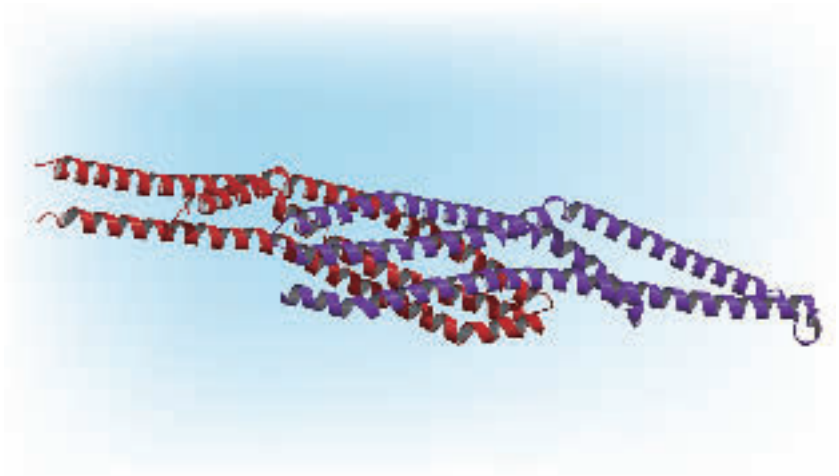

Figure 1: The crystal structure of the RCB domain of IRSp53.

These findings provide new insights into IRSp53 function, but have also generated a host of follow-up questions. Yokoyama and colleagues are now eager to more closely examine the mechanistic foundations of IRSp53-mediated membrane deformation. "The Rac and Cdc42 proteins are known to regulate tubulation," says Yokoyama. "We will study how these GTPases regulate initiation, elongation and termination of tubulation [and] we will try to reveal mechanisms of tubulation by these RCB domains." ■

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2. Suetsugu, S., Murayama, K., Sakamoto, A., Hanawa-Suetsugu, K., Seto, A., Oikawa, T., Mishima, C., Shioruzu, M., Takenawa, T. & Yokoyama, S. The Rac-binding domain/IRSp53-MIM homology domain of IRSp53 induces Rac-dependent membrane deformation. *The Journal of Biological Chemistry* **281,** 35347–35358 (2006).

STIMulating calcium influx

Researchers demonstrate the critical role of a protein in mobilizing calcium ions

Nicolle Rager Fuller/National Science Foundation

Figure 1: An artistic representation of endoplasmic reticulum.

Calcium ions are used by various cell types, including immune cells, bone cells and neurons, to regulate their physiological response. Now, Tomohiro Kurosaki and colleagues from the RIKEN Research Center for Allergy and Immunology, Yokohama, and other academic institutions in Japan, provide new details of how the cell senses low intracellular calcium levels, potentially leading the way to future drug therapies[1].

Typically, calcium is sourced from stores in an intracellular compartment known as the endoplasmic reticulum (Fig. 1). When these stores are depleted, the cell must transport more from the extracellular milieu. Important in this process is a protein in the endoplasmic reticulum called STIM1. Upon calcium depletion, STIM1 is transported to the region underneath the plasma membrane, where it communicates with calcium transport channels residing in the plasma membrane, thereby facilitating calcium influx into the cell[2].

Kurosaki and colleagues show for the first time that STIM1 normally resides in a special sub-compartment of the endoplasmic reticulum. Also, while confirming earlier studies demonstrating that calcium depletion stimulates STIM1 to translocate to regions near the cell's surface, the team shows that it is not inserted into the plasma membrane. They suggest that STIM1 must trigger calcium influx indirectly by activating calcium transport channels within the plasma membrane. How this actually occurs will be an important issue for future studies, says Kurosaki.

By removing specific portions of the protein's structure through genetic engineering, the team further shows that several different regions of STIM1 are required for calcium influx to occur. Interestingly, STIM1 appears to use these regions in different ways to facilitate its movement to the region underneath the plasma membrane. For example, removal of one region blocked the first step in the transport of STIM1, while removal of others either blocked subsequent transport steps or slowed its kinetics. But in all cases, none of the mutated protein made it to the region underneath the plasma membrane in appreciable amounts to properly trigger calcium influx.

Kurosaki points out future studies are needed to "clarify the mechanism of how low intracellular calcium levels stimulate STIM1 to leave the endoplasmic reticulum since the protein's movement is one of the critical points of its regulation". He also believes that some day drugs that target STIM1 may be therapeutic since many cell types, including immune cells and bone-degrading cells, depend on proper calcium oscillations for their activation and function. However, he notes that since STIM1 is expressed widely, these drugs would have to be well-targeted to avoid unwanted side-effects. ■

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Dividing cells stopped in their tracks

A novel compound starves tumor cells and immune lymphocytes

Cells of the body require energy to divide. This is particularly evident in rapidly dividing cells, such as immune lymphocytes responding to infection. Now, Takeo Usui and colleagues, at the RIKEN Discovery Research Institute, Wako, shed light on a drug that halts dividing cells by literally starving them[1].

In addition to immune lymphocytes, cancer cells also divide rapidly. To accommodate such rapid division, both types of cells harvest nutrients—especially amino acids for building proteins—from their surroundings.

Usui and colleagues work with the novel compound Brasilicardin A (BraA), a natural product of the bacterium *Nocardia brasiliensis* (Fig. 1). "At first, we noticed that BraA inhibited protein synthesis," recalls Usui. "We speculated that BraA might block cell division by inhibiting uptake of amino acids into cells." Direct measurement of amino acid uptake confirmed that several essential amino acids could not enter BraA-treated cells.

Usui and colleagues turned to the molecular structure of BraA for further clues. It was known that part of BraA structurally resembles natural amino acids. "We found that the 'amino acid-like' component is quite important for BraA's ability to inhibit cell division," says Usui.

How? When lymphocytes and cancer cells rapidly divide, internal biosensors monitor when nutrients available to the cell are low. In response, cells produce specialized 'transport proteins' that enable uptake of nutrients such as amino acids, sugars and fats.

Most likely, BraA directly blocks the function of one class of amino acid transport proteins and thus "disturbs amino acid metabolism" within the cell, notes Usui. The result is cells that are starved and would die except for their intrinsic 'stress response' that staves off death.

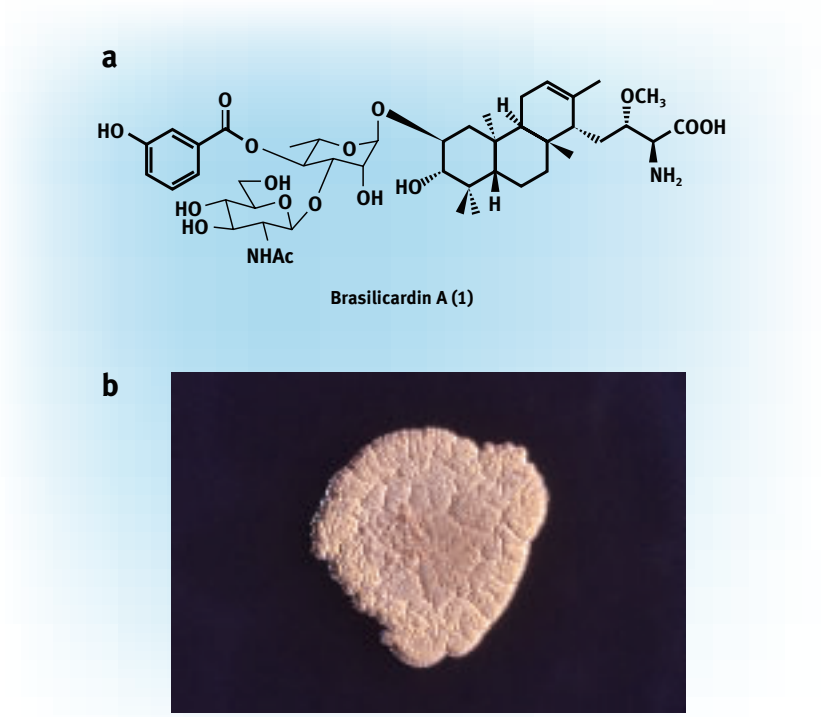

Figure 1: a) Molecular structure of Brasilicardin A. b) Brasilicardin A is a natural product isolated from cultures of the bacterium *Nocardia brasiliensis*.

In support of those conclusions, Usui and colleagues find that BraA-treated cells contained an activated form of GCN2—a protein that is involved in the stress response that protects cells from amino acid starvation.

"Because all cells require amino acids to live, high concentrations of BraA [if used to treat people] should be toxic. However, dividing lymphocytes and cancer cells have extraordinary amino acid requirements," says Usui, suggesting that delivery of BraA to dividing cells might not result in toxicity.

The team also has ideas for improving the inhibitory activity of BraA. "We hope that we can obtain more potent, and clinically applicable, inhibitors that will block unhealthy cell division."

Understanding more about the mechanism by which BraA suppresses cell division may help to design other drugs to prevent organ transplant failure or malignant cancer. ■

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Screening for genes that regulate chromosomal duplication

Using the mustard plant, *Arabidopsis*, scientists identify a new gene involved in regulating endoreduplication

Many plants have multiple copies of their chromosomes in their cells. This condition is referred to as polyploidy and occurs through a process called endoreduplication in which the chromosomes are replicated, but the cell does not divide. Given their polyploidy, plants offer scientists a way to identify key cellular factors that control endoreduplication, which is tightly linked to the control of the endocycle—a variation of the cell cycle. Regulators of the cell cycle are implicated in human diseases, such as cancer, that result from abnormal cell growth. The hope then is that such insights, while illuminating important aspects of plant biology, may also have direct relevance to animal biology and human health.

Researchers at the RIKEN Plant Science Center in Yokohama, in collaboration with other Japanese colleagues, have developed a genetic screen using the plant *Arabidopsis* to identify such cellular factors[1]. In this way, the researchers identify six dominant mutations that increase polyploidy, but focus their attention on one of these, a protein they call ILP1 (Fig. 1).

The team shows that the gene for ILP1 is related to a portion of a human gene, as well as to genes in mice, flies, and worms. They also show ILP1 localizes in the cell nucleus where it inhibits the gene expression of A2-type cyclins (*CYCA2s*), which are important cell cycle regulators. Using mouse cells, the team shows that mouse ILP1 also interferes with the expression of *Ccna2* (the A-type cyclin gene in mice).

Returning to *Arabidopsis* cells, the team then shows that interference with the normal expression of *CYCA2* by the mutant of ILP1, identified in their screen, is the main cause of the high level of polyploidy. They show this by knocking out *CYCA2* expression in a wild type *Arabidopsis* plant and seeing a similar effect on polyploidy. Furthermore, the effect of *CYCA2* deficiency on polyploidy confirms an earlier study showing that another variant of *CYCA2* is a key regulator of polyploidy in plants[2].

The team is yet to determine whether ILP1 has cellular targets other than *CYCA2*. Minami Matsui, the team leader at RIKEN, points out that their preliminary results "indicate that ILP1 does not bind DNA directly, so it must be interacting with other proteins to affect *CYCA2* expression". The team is now setting up biochemical experiments to identify what those cellular targets are and how they affect plant biology. ■

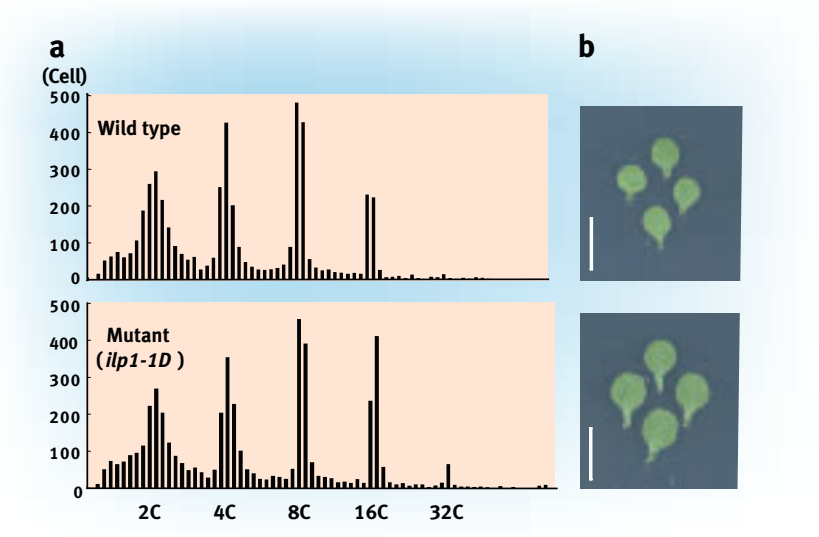

Figure 1: Wild type and mutant *Arabidopsis* plants. a) Differences in ploidy levels in wild type (top) and mutant (bottom) *Arabidopsis* cotyledon cells. X- and Y-axes indicate nuclear ploidy and cell numbers, respectively. b) Wild type *Arabidopsis* cotyledons (top) are 30% smaller than the *ilp1-1D* mutant (bottom). Bar, 5 mm.

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2. Imai, K.K., Ohashi, Y., Tsuge, T., Yoshizumi, T., Matsui, M., Oka, A. & Aoyama, T. The A-type cyclin CYCA2;3 is a key regulator of ploidy levels in *Arabidopsis* endoreduplication. *The Plant Cell* **18,** 382–396 (2006).

Not just counting sheep

Research has revealed neuronal firing patterns that could help explain how the brain makes memories during deep sleep

Most people think of a good night's rest as a refreshing and relaxing experience, but even with closed eyes and a still body, the brain continues to work away. In fact, many neuroscientists believe that the brain uses certain stages of sleep—so-called 'slow-wave' sleep—as an opportunity to process the day's events.

A key component of this process is communication between the hippocampus, the brain's primary memory center, and the neocortex, which handles a wide range of brain functions, including sensory processing. "Memory information may be consolidated in the hippocampus and transferred to the neocortex during sleep, especially slow-wave sleep," explains Yoshikazu Isomura of the RIKEN Brain Science Institute in Wako. "In fact, neuronal firing patterns experienced in a waking and behaving state are often reproduced during slow-wave sleep."

Previous research has shown that neocortical neurons exhibit slow oscillatory patterns of activity during slow-wave sleep, between a highly active 'UP' state, and a hyperpolarized quiescent 'DOWN' state. However, it has been unclear exactly how this stage affects the behavior of neurons in the hippocampus and adjacent parahippocampal regions. To investigate this further, Isomura and colleagues at Rutgers University in New Jersey performed a series of experiments to characterize the firing patterns of single neurons and groups of neurons in anesthetized and naturally sleeping rats[1].

They uncovered evidence that although parahippocampal neurons, such as those in the entorhinal cortex and subiculum (Fig. 1), undergo slow oscillations that synchronize with those occurring in the neocortex, most hippocampal neurons do not. Nevertheless, hippocampal neurons were influenced by the activity of entorhinal neurons in the 'UP' state, and even exhibited subarea-specific activity when entorhinal and neocortical neurons were in the 'DOWN' state. Isomura and his colleagues postulate that this communication from neocortex to hippocampus may trigger the 'sharp-wave-ripple' hippocampal oscillations that signal back to the neocortex.

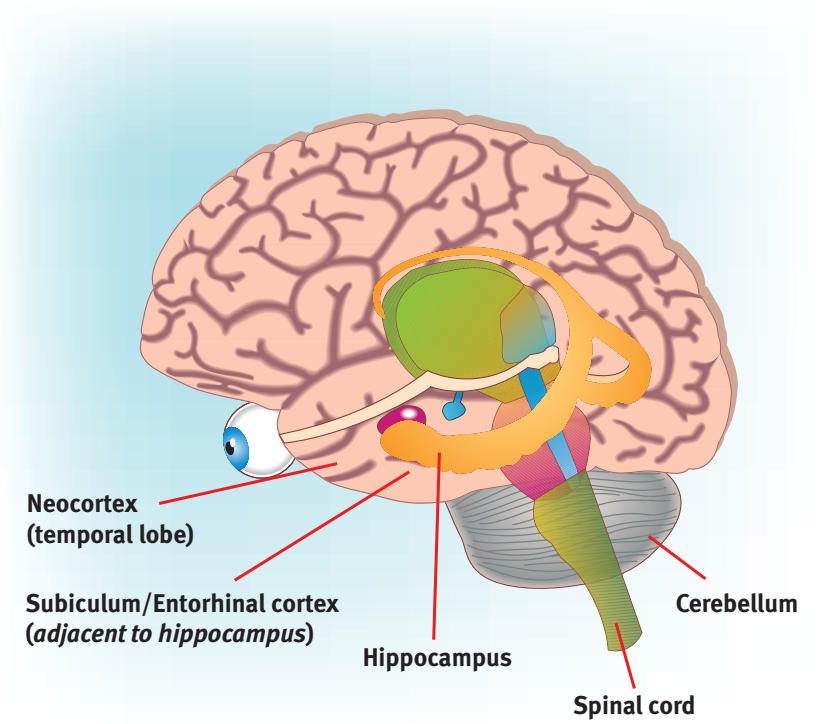

Figure 1: Regions of the brain.

"Researchers in this field believe that sharp-wave-related ripple oscillations may contribute to memory consolidation in the hippocampus or memory transfer from the hippocampus to the neocortex," Isomura says.

These findings could provide an important piece in the puzzle of understanding memory processing during deep sleep, but there are still many unanswered questions. In particular, although the entorhinal cortex appears to play a role in this memory-building process, the specifics remain unclear. "The entorhinal cortex is not so well understood, even in its basic physiological properties," Isomura says, "and we will focus on its functional role in relation to hippocampus–neocortex interaction in the near future." ■

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PIERO CARNINCI
New concepts for genomics

A RIKEN biologist has contributed to renewing the conventional concept of genome science with state-of-the-art techniques for sequencing and analyzing genes

A RIKEN group led by geneticist Yoshihide Hayashizaki has been at the forefront of the world's post-genomic research—providing insights that have redefined the concept of what genes are, uncovering some surprising functions of RNA, and reinterpreting gene-expression systems. None of these striking outcomes would have been achieved without an energetic Italian biologist, Piero Carninci.

Now that the major genomes have been sequenced, researchers are eager to find a systematic way of unlocking the functions of genes and proteins. Central to this is an understanding of how transcription works—how DNA is copied into RNA to translate the transcript into proteins. With that knowledge, scientists will be able to manipulate gene expression, and design drugs effectively.

In the mid 1990s, RIKEN researchers started a project to identify tens of thousands of full-length mouse cDNAs (cDNA is complementary to RNA and can be considered as a gene itself). Since joining RIKEN in 1995, Carninci has developed all the key technologies behind RIKEN's cDNA research, and invented new methods of analyzing an organism's entire set of RNAs (the 'transcriptome').

"These success stories at RIKEN are due to Piero's technologies. He is very persistent and has developed what we had thought impossible theoretically," says Yasushi Okazaki, Carninci's former senior colleague at RIKEN and now professor at Saitama Medical University.

Out of Italy

A senior scientist at both the Discovery Research Institute in Wako and the Genome Sciences Center in Yokohama, Carninci's interests range from technology development to analyzing genome networks in the brain.

Born in Trieste in northeastern Italy, Carninci gave up a promising career as a soccer player to pursue science instead. In 1989, he obtained his doctorate in biological science at the University of Trieste.

In the same year, he spent six months as a research associate at Trieste's International Center for Genetic Engineering and Biotechnology, where he developed more efficient way of sequencing DNA. After that came a year of military service, in which he served as a health assistant. It was, Carninci recalls, the most enjoyable time of his career—working in the army during the day and then rushing into his university laboratory to do research until midnight.

With his military service over, Carninci became a researcher at a biotech company called TALENT, spun out of his supervisor's lab, and developed easy-to-use methods to extract and sequence high-quality DNA.

Carninci was also involved Italy's small genome project, but was suffering from small budgets and insufficient support. So when he met Hayashizaki at a conference in Germany in 1994, they found a mutual interest: Carninci was looking for a place to improve genome analysis techniques; Hayashizaki had just been recruited by RIKEN to launch a genome project and needed technical supports.

An encyclopedia of the mouse

In 1995, Carninci joined the Genome Science Laboratory in Tsukuba just as Hayashizaki was launching the Mouse Encyclopedia Project to collect and sequence full-length mouse cDNAs as a model for human diseases. Carninci's first mission was to develop technology to clone full-length mouse messenger RNA in the form of cDNAs. Conventional technology often failed to clone the gene from the beginning to end, missing an important 'cap' where protein translation is initiated. After eight months during which he often saw the dawn from his lab, Carninci completed his 'cap trapper' technology—capturing the cap and picking up full-length genes while eliminating broken ones[1].

Carninci also developed a way of stabilizing an enzyme that stimulates the conversion of long messenger RNA into cDNA. A few years later, he improved the technology to enable it to remove copies of highly expressed RNAs and collect only rare ones, helping RIKEN stand out from its competitors.

By 2000, Carninci's technologies had created a database with

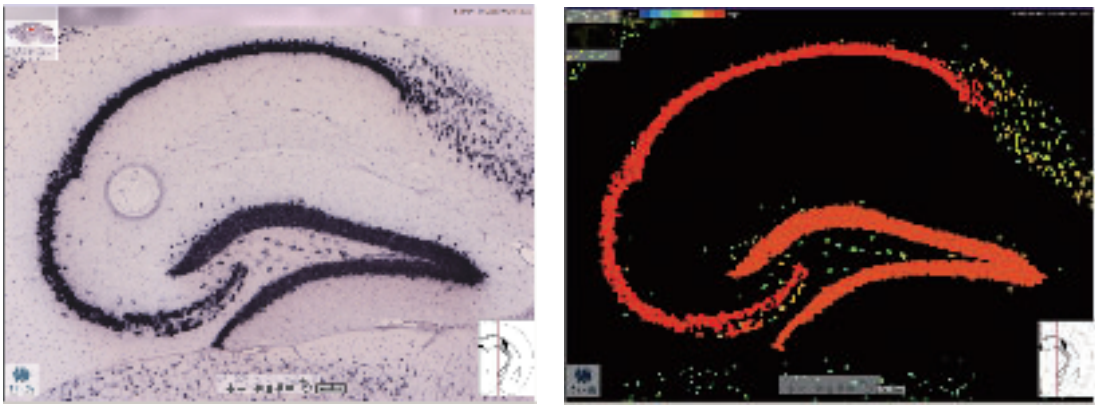

Figure 1: Expression analysis of genes expressed in the hippocampus, a brain region responsible for the formation of memory. Left panel, in-situ hybridization. Right panel, pseudo-color coded analysis of the in-situ hybridization. The intensity of the expression varies from very intense (red) to progressively less intense expression (orange, yellow, green and blue). In-situ expression produced by S. Sunkin of the Allen Institute based on the expression of transcript analyzed by CAGE.

21,000 full-length cDNAs. But it was just the start. "We had a very long list of genes, but two-thirds of them were unknown. And we had piles of printed papers of data, but there was no clue to their biological patterns."

Massive efforts to interpret genes

Driven by this frustration, the team led by Hayashizaki and Okazaki convened an ambitious project to annotate all the cDNAs. They called the project FANTOM (Functional Annotation of Mammalians) and organized a meeting in the summer of 2000, inviting 50 Japanese and overseas researchers. It was "annotation prison camp", Carninci jokes, with guests either in a hotel in a rice field in Tsukuba or in RIKEN for the entire fortnight.

The results[2] "helped us to understand the first 21,000 cDNAs, yielding the first view of transcriptome functions," Carninci says.

FANTOM-2 in 2002, which looked at a larger transcript of another 39,000 cDNAs, revealed that several RNAs don't encode proteins but have other functions[3].

Carninci continued to improve technologies, and invented methods such as CAGE (Fig.1) and GSC, which were designed to collect the short sequence tags that identify where transcription sites start and end. Those new methods were used in FANTOM-3, which ran from the end of 2002 through 2006. Out of two million mouse cDNAs, researchers analyzed 103,000 in detail. The result? "We completely changed a genetic philosophy," Carninci says.

For example, it had been believed that only 2% of the genome are important in encoding for human (and mouse) proteins, but the researchers found that about 63% of the genome play a critical role by being actually transcribed into RNAs. They also found the percentage of RNAs that aren't coding proteins (non-coding RNAs)[4] constitutes even more than half of RNAs, and these non-coding RNAs are often transcribed in the opposite orientation of protein coding genes[5]. They also found the starting point of a gene's activity, called a promoter, was scattered in broader sites than had been predicted and that these sites are poorly conserved between species, suggesting that gene expression became more important than sequence conservation as evolution progressed[6].

The series of achievements at FANTOM projects are largely attributed to Hayashizaki's leadership and ideas, but, says Okazaki, "behind all these was Piero's ability to develop technologies by looking at both a big picture and details".

An appetite for discovery...and food

Carninci and other researchers are preparing to start FANTOM-4, partly to deepen analysis of the relationships between promoter elements and transcriptional functions. "Dr Carninci has an incredible amount of energy. He knows what is not written in books," comments Charles Plessy, a junior colleague of Carninci. And "his great appetite is not only for discovery, but also for food," says the French scientist, adding he shares with Carninci the culture that a good lunch is important in life.

Over the past few years, Carninci has been drawn into the brain science, so in addition to working for FANTOM projects, he collaborates with neuroscientists at RIKEN and elsewhere to study transcriptional patterns in neurons. Carninci says RIKEN is a good place for non-Japanese young researchers to work, because it is trying to bring Japan's research system to a new level. "I recommend they consider coming to RIKEN. Here, my dreams have come true," Carninci says.

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2. Kawai, J. *et al.* Functional annotation of a full-length mouse cDNA collection. *Nature* **409,** 685–90 (2001).
3. Okazaki, Y. *et al.* Analysis of the mouse transcriptome based on functional annotation of 60,770 full-length cDNAs. *Nature* **420,** 563–73 (2002).
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5. Katayama S. *et al.* Antisense transcription in the mammalian transcriptome. *Science* **309,** 1564–66 (2005).
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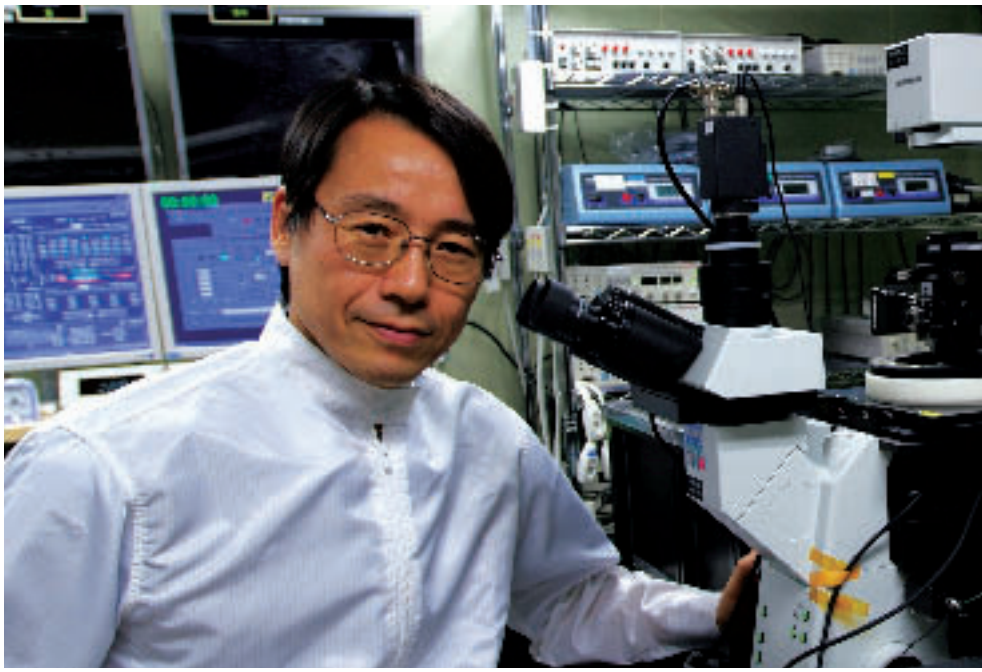
About the researcher

Piero Carninci was born in Italy in 1965 and earned his doctorate in biological science at the University of Trieste in 1989. For six months from April 1989, he worked as an research associate at the International Center for Genetic Engineering and Biotechnology, and then did his military service as a health assistant in the Italian army. In 1990, he became a researcher at a biotech company called TALENT, and then moved to RIKEN as a researcher in 1995. Since 2003, he has been working as a senior scientist at the Genome Science Laboratory at the RIKEN Frontier Research Institute and at the RIKEN Genomic Sciences Center.

Pioneering a new field through intracellular single molecule imaging

Makio Tokunaga

Unit Leader
Research Unit for Single Molecule Immunoimaging
Research Center for Allergy and Immunology
RIKEN Yokohama Institute



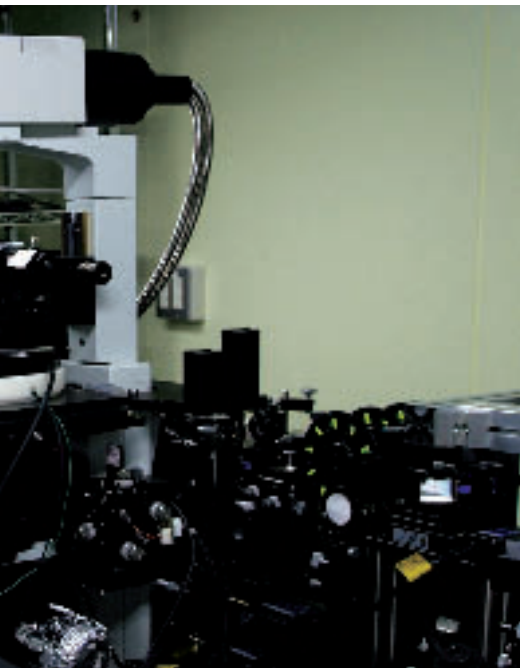
Visualizing single molecules in living cells—this was achieved by Makio Tokunaga, Leader of the Research Unit for Single Molecule Immunoimaging at the RIKEN Research Center for Allergy and Immunology (RCAI). Essentially, biological phenomena cannot be understood fully without directly examining individual molecules in living cells. Single-molecule imaging is a technology that is drawing considerable attention from researchers in the life sciences. Tokunaga developed an intracellular single-molecule imaging microscope with the world's highest performance. We interviewed him about the past, present, and future of single-molecule imaging.

Visualizing single molecules using evanescent light

The microscopic system that is drawing the attention of the world's life scientists is installed in a small room covering an area of about six tatami mats (about 9.9 square meters) in the innermost area of the laboratory. "Using this system, we can obtain direct, real-time observations of molecular dynamics and the interactions of individual molecules in living cells," says Tokunaga. Arranged in the room are an optical microscope, laser generator, control computer, monitor display, and other equipment. "Most of these are handmade with various refinements here and there. This is our intracellular single-molecule imaging microscope, which is capable of taking images that cannot be captured by any other microscopes."

With its ability to visualize individual molecules in living cells, single-molecule imaging is a powerful tool toward the understanding of biological phenomena. To this end, competitive attempts at technical developments have been made worldwide since the 1990s. Initially, their primary goal was to visualize active molecules in vitro. In 1995, single-molecule imaging in aqueous solution was achieved independently, for the first time in the world, by the Yanagida Biomotron Project (supervised by Toshio Yanagida of Osaka University's Graduate School), ERATO, Japan Science and Technology Agency, which Tokunaga had joined, and the research group of Kazuhiko Kinosita of Keio University (now at Waseda University).

In single-molecule imaging, subject molecules labeled with fluorescent dye are excited by a laser beam. The resulting fluorescence is examined using an optical microscope. There are three keys to successful imaging of single discrete molecules: reducing background light, increasing camera sensitivity, and using bright fluorescent dyes. "We had had the greatest difficulty in reducing the background light," says Tokunaga. Usually, a laser beam is applied at right angles with respect to the glass plate carrying the specimen. However, because this allows the laser to reach deep into the specimen, fluorescent dyes at many points throughout the sample become



excited to the extent that the brightness is diffused over the entire specimen; the fluorescence from the individual molecules cannot be distinguished from each other. "Hence, we attempted to apply a new method known as total-reflection illumination," says Tokunaga. (See left panel in Fig. 1.) When applied obliquely to the glass plate carrying the sample, the laser beam is totally internally reflected, producing near-field light (evanescent light), which emanates shallowly from the sample's surface. Because the distance the near-field light can travel is only up to 200 nm from the specimen surface, the fluorescence from each molecule can be clearly visualized against the background, which remains dark.

Then, why has Japan become the leader in this field? "Dr Fumio Oosawa [Professor Emeritus of Nagoya and Osaka Universities], the founder of biophysics in Japan, pointed out the importance of research into single molecules as early as around 1980," explains Tokunaga. "Hence, Japan is ahead of other countries. At present, Japan is the world leader in single-molecule research."

Going beyond fixed ideas

After completion of the Yanagida Project, Tokunaga transferred to the National Institute of Genetics and succeeded in developing a microscope system that enabled single-molecule imaging on the cell surface based on total-internal-reflection illumination technology. He then set down his next target as intracellular single-molecule imaging. "Exploration on the cell surface is not enough. It is absolutely necessary to visualize single molecules in cells to understand biological phenomena." And how can this be done? "We use the thin-layer oblique-illumination technique," Tokunaga replies. The laser beam is slightly inclined compared with the angle used for total-reflection illumination to reduce the thickness of the exposure field. Thus, the laser strikes an extremely narrow area in the cell. "It's quite easy." (Fig. 1.) Tokunaga smiles as he answers. This method had previously been described as theoretically impossible owing to light diffraction. "I had realized the theoretical difficulty. But as I learned from Setsuro Ebashi of the University of Tokyo, who died last July, it is important to try."

Setsuro Ebashi discovered the role of calcium ions in cells and made remarkable discoveries. "Although I spent only two months studying under Dr Ebashi at my graduate school, my experience there totally changed my life as a scientist," says Tokunaga. " Dr Ebashi used to say, 'Thoughts that come into our head are often incorrect. Move the parts under your head and carry out experiments!'" Tokunaga adds that from Ebashi, he also learned the importance of having the spirit to challenge accepted wisdom. "It is theoretically impossible; established authority says don't do it—these kinds of remarks have no relevance. The most important thing is to do what you think is needed. 'Try, believing that you will succeed'; this will result in you putting in the maximum effort." Accordingly, Tokunaga accomplished the goal of developing the intracellular single-molecule imaging microscope, a task that had reportedly been difficult to carry out.

In 2004, Tokunaga established the Research Unit for Single Molecule Immunoimaging at RCAI. He says, however, "I am not a specialist in immunology". Then, why did he join RCAI? "Immune responses are highly suitable subjects for single-molecule imaging because they involve the action of individual immunocytes or occur in the form of cellular interactions," Tokunaga replies. "I thought that we would be able to do original research and make groundbreaking discoveries, making use of the great potential of single-molecule imaging." He continues to explain how first, he refined all parts of the single-molecule microscope he had developed previously as far as possible, and thus developed a new microscope, specially designed to explore immunocytes.

Tokunaga and his collaborators also developed a method for distinguishing between three or more kinds of molecules by simultaneous real-time multicolor examination, and improved the ease of operation by systematizing individual instruments on a personal computer. Many other refinements were made. "Development of automatic-focusing technology was groundbreaking," says Tokunaga. What microscopy examines is the submicrometer world. Even a very

small temperature change alters the sample position, resulting in an unwanted shift of the focusing point. Hence, Tokunaga developed automatic focusing technology, which constantly monitors the specimen depth using a guide laser beam. "This autofocus apparatus is expected to become a standard device for microscopes. It should be an essential technology in the future."

The starting point of immune responses visualized

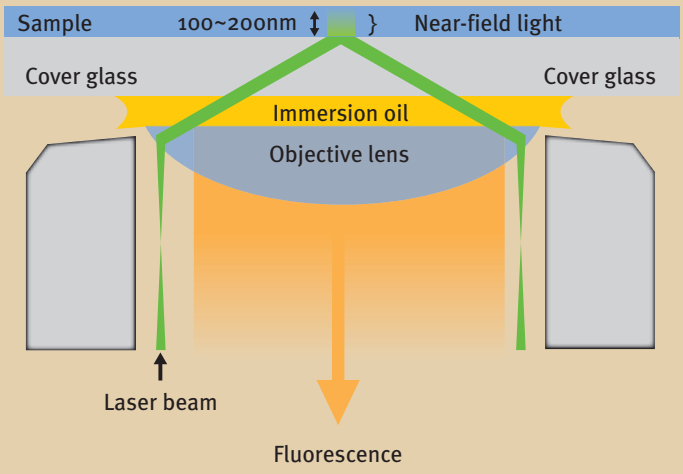
Described below is an achievement recently produced using the single-molecule microscope. This involves a joint project with the RCAI Cell Signaling Group, an internal research group conducting T cell research under director Takashi Saito. T cells are a type of lymphocyte that regulates the immune system to protect the body against foreign substances. When activated to excess, T cells cause allergic disease and autoimmune disease. Elucidating the mechanisms behind T cell activation represents an important target in immunology research.

T cells detect and adhere to antigen-presenting cells, presenting information on foreign matter entering the body on their surfaces. Investigations that have been conducted so far have shown that antigen-recognizing T cell antigen receptors gather at the center of the adhesion interface, between T cells and antigen-presenting cells, and are surrounded by adhesive molecules. Conventionally, it was hypothesized that immune responses are initiated on antigen recognition, resulting in T cell activation. The place where T cell antigen receptors gather is called the immune synapse because immune cells exchange information there. However, the intracellular single-molecule imaging microscope visualized a phenomenon that upset this traditional hypothesis.

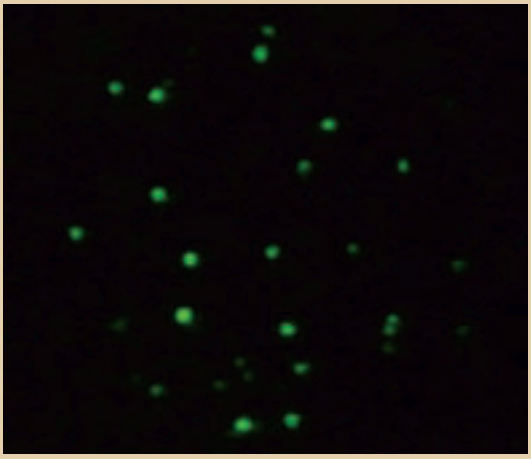
"We prepared an artificial lipid-bilayer membrane mimicking an antigen-presenting cell on a glass plate, dropped T cells onto it, and examined it using the single-molecule microscope from the moment of the reaction by the 'time-zero method'," explains Tokunaga. Small brilliant dots appeared one by one, on the adhesive interface just after the adhesion, and moved toward the center. "We were surprised at the dynamic movements."

"About 100 molecules of T cell antigen receptors and intracellular transduction molecules emerged, and we named them microclusters," continues Tokunaga. Extensive analysis showed that antigen recognition and cell activation occurred in microclusters. Even after immune synapses were formed, microclusters continued to be formed to sustain cell activation. Hence, the immune synapse, which had been thought to be the place for antigen recognition and activation, proved to be a site where things gather after the reaction. The microclusters were found to be the starting point of immune responses, and also to be responsible for their maintenance (Fig. 2).

"We had at least two competitors in our research into this theme. They presented papers at nearly the same time as ours but their papers were not accepted," says Tokunaga. The low quality of the images and the inability to follow the course of the reaction from the beginning seem to be the reasons why their papers were rejected. "We were able to visualize the reaction right from its start by means of the unique technology developed through a trial-and-error process by scientist Dr Kumiko Sogawa, one of the members of our unit," Tokunaga continues. "Our images were much clearer than our competitors." He adds that they could not have achieved the present discovery without the unified efforts of Tadashi



For exploration of the cell surface, very confined near-field light is produced by total-reflection illumination on the surface of the sample, on a glass plate. To examine the inside of the cell, the thickness of the field of exposure is minimized by oblique illumination.



Molecular transport between the cytoplasm and the nucleus was examined under thin-layer oblique illumination. Interactions with nuclear pores are visualized. Each dot corresponds to one molecule.

Figure 1 : Single molecule imaging by objective-type total reflection illumination and thin-layer oblique illumination

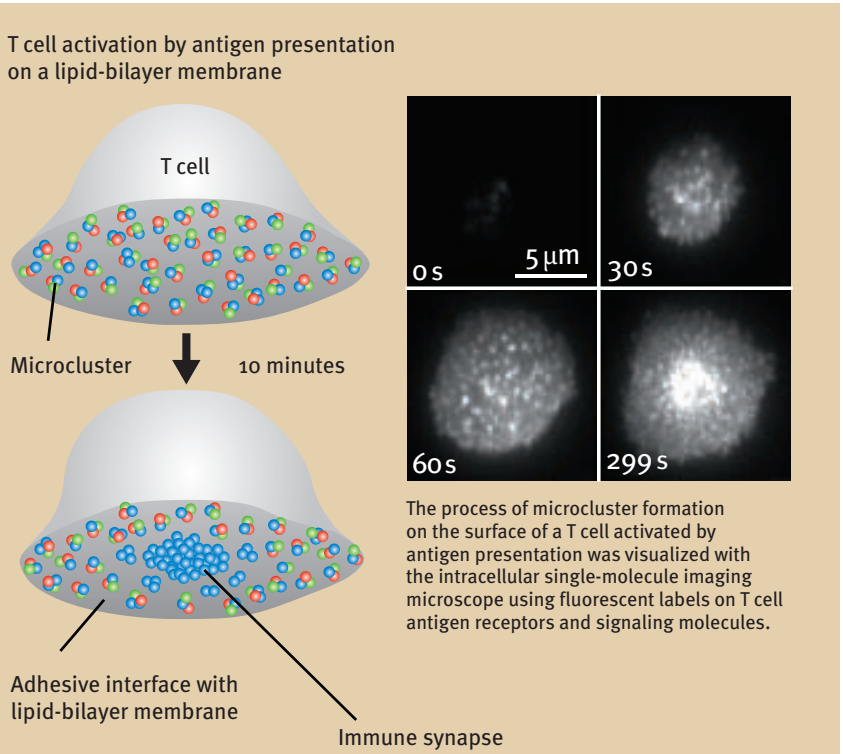
Yokosuka and other members of the Cell Signaling Group working to prepare good samples, as well as members of their unit engaged in developing the microscope and taking the images.

From molecules to a system

Keys to future development in single-molecule imaging are quantification of biological phenomena and multicolor, three-dimensional imaging for simultaneous examination of many kinds of molecules.

"Currently, systems biology, a discipline aimed at reproducing biological phenomena on computers, is drawing a lot of attention," says Tokunaga. This involves reproducing the biological phenomenon being investigated on a computer on the basis of experimental data, feeding the results back from the reproduction to the laboratory, and then reproducing the phenomenon on the computer again. "Without this process, an understanding of the biological phenomenon would not be obtained," says Tokunaga. It should be noted, however, that the actual cell status poses a difficult problem in quantifying a biological phenomenon into computer-processable numerical values. "So, single-molecule imaging is necessary," says Tokunaga. "Because single-molecule imaging enables us to determine the intensity of the fluorescence from each molecule, we can obtain numerical quantitative values of the number of molecules that have gathered, the number of molecules required for the reaction, and the intensity of the interactions." It is Tokunaga's opinion that integration of single-molecule imaging and systems biology will become a key pillar for the life sciences in the 21st century.

What is Tokunaga's dream? "I am planning to watch how reactions on the cell surface are transmitted to the inside of the cell, and then into the nucleus, and what happens in the nucleus," he replies. "Hence, I want to elucidate these processes as an integral system."



The process of microcluster formation on the surface of a T cell activated by antigen presentation was visualized with the intracellular single-molecule imaging microscope using fluorescent labels on T cell antigen receptors and signaling molecules.

Figure 2: **Microcluster formation**

Tokunaga is also working to visualize molecular transport between the cytoplasm and the nucleus by single-molecule imaging in cooperation with Naoko Imamoto, Chief Scientist at the Cellular Dynamics Laboratory, Discovery Research Institute (see right panel in Fig. 1). "We examined molecules passing through the nuclear pores and found that the speed of passage was slower than expected from conventional theory," Tokunaga says. "Although there is strong opposition, we believe that our finding is right because other phenomena can also be explained reasonably by reproducing them on a computer, based on quantitative data on the number of molecules involved, the reaction time, binding strength, and other factors." It is Tokunaga's opinion that this serves as a good model for integration of single-molecule imaging and systems biology, although the scale is small. "I am also planning to apply the single-molecule imaging technique to simultaneously examining three or more molecules at arbitrarily chosen positions," continues Tokunaga. "Is it really possible? Yes, we can do it if we have a strong desire to do so."

About the researcher

Makio Tokunaga was born in 1959 in Ehime, Japan. In 1982, he graduated from the University of Tokyo, where he majored in physics. He earned his masters in physics in 1984 and his doctorate in physics in 1987, both from the same university.

In 1987, Tokunaga became a research fellow at the Japan Society of the Promotion of Science. In 1988, he was appointed as an assistant professor of physics at the University of Tokyo, and then in 1992 became the group leader of the Yanagida Biomotron Project at the Japan Science and Technology Corp.

In 1997, Tokunaga was named as an associate professor of the Structural Biology Center at the National Institute of Genetics, and has been serving as a professor since 2000. Concurrently, he has been working as a senior scientist at the RIKEN Research Center for Allergy and Immunology since 2002. He has two other positions at present-he's a visiting professor at the Department of Applied Physics, the University of Tokyo and a professor at the Graduate University of Advanced Studies.

RIKEN CDB hosts joint meeting on developmental neurobiology

The RIKEN Center for Developmental Biology (CDB) hosted a joint symposium on Development and the Emergence of Function in the Nervous System, co-sponsored by the United Kingdom's Foreign Commonwealth Office and the Asia–Pacific Developmental Biology Network (APDBN; www.apdbn.org) from February 8 to 10. The joint UK–APDBN effort was the first of its kind in bringing together researchers and students from the UK and the Asia–Pacific region for an intensive three-day meeting to promote discussion and the exchange of findings, and to help develop the potential for further collaborations between scientists from these areas of the world.

The meeting featured more than 20 talks by British, Asian and Australian scientists working on fundamental questions in fields including neurodevelopment, brain patterning, neural cell biology, behavior, and evolutionary developmental biology. Co-organized by K. Vijay Raghavan (NCBS, Bangalore, India) and James Briscoe (NIMR, Cambridge, UK), the program introduced recent work from the laboratories of eminent researchers such as Mu-Ming Poo (University of California, US/Shanghai Institutes of Biological Sciences, China), Graham Collingridge (MRC Center for Synaptic Plasticity, UK), Paul Whitington (University of Melbourne, Australia), and Andrea Brand (Cambridge University, UK). Involving fundamental neuroscience and developmental biology (including stem cell biology), neurodevelopment has attracted significant scientific interest for its great potential to advance our understanding of how a simple, undifferentiated collection of cells (the early embryo) is capable of giving rise to an organ as extraordinarily complex as the brain.

The lively poster sessions following the talks at the end of each day were a particular highlight, with nearly 60 presentations by graduate and postdoctoral students from the UK and the Asia–Pacific region. The turnout was bolstered by the mutual decision by the UK government and the APDBN to provide additional funding for each speaker to bring a student from their laboratory to the meeting. This provided a unique opportunity for young scientists to meet their peers from other parts of the world.

On the final day of the event, the invited speakers gathered for a roundtable discussion on the challenges, opportunities and best practices in developing inter-regional scientific collaboration. Emphasizing the lack of a single comprehensive information resource on sources of funding for such cross-border programs, the scientists called for additional support for student travel as a means of kick-starting interaction between laboratories. ■

Annual party held to facilitate industry collaboration

The RIKEN Friendship Society, an organization consisting of companies who want to exchange information with RIKEN, held an annual exchange party in the middle of February.

These days, the number of venture businesses in Japan is increasing; however, RIKEN started venture businesses almost 90 years ago in the 1920s. In its heyday in 1939, the number of RIKEN venture businesses rose to 63 and and were collectively called 'RIKEN *konzern*'. These businesses were engaged in the production of the piston ring, vitamin A, pesticides, sensitized paper, synthesized-alcohol and absorbents. Following the outbreak of World War II in 1941, the *konzern* was broken up. However, many of those companies remained and kept on developing, and still support Japan's economic growth today.

The RIKEN Friendship Society was organized by companies involved with RIKEN. Activities of the Society include the annual exchange party, seminars and visits to RIKEN. The annual exchange party, where the minister of the Ministry of Education, Culture, Sports, Science and Technology (MEXT) gave the opening speech, is regarded as an important meeting by politicians.

Research outputs from each RIKEN research center and activities of RIKEN venture businesses, which were set up from 1998, were presented in poster sessions. More than 400 businessmen exchanged useful information with RIKEN staff. At the party, RIKEN's historical synthesized-alcohol 'Golden Rikyu' was served to everyone. ■

The Molecular Imaging Research Program kicks off

The Molecular Imaging Research Program (MIRP) of the RIKEN Frontier Research System held its opening ceremony on January 18 at their research base in Kobe. On February 15, MIRP received permission from the Ministry of Education, Culture, Sports, Science and Technology to use its RI facility and started full-scale experiments.

The Program is aimed at speeding up the process of designing new drugs using a novel method—molecular imaging—to analyze the molecular dynamics of drugs *in vivo*. Molecular imaging is an important technology used in positron emission tomography (PET). (Also see 'Molecular imaging program is ready for full-scale operation', Roundup, *RIKEN RESEARCH* **1**(2), 17(2006)).

The MIRP has two small cyclotrons to produce various kinds of RI, and five hot cells—a heavily shielded room in which radioactive materials can be handled remotely using robotic, or otherwise remote manipulators, and viewed through shielded windows. This equipment allows researchers to synthesize labeled chemical compounds efficiently. According to the program director, Yasuyoshi Watanabe, previously it has taken university laboratories one whole month to synthesize labeled chemicals and develop novel chemical pathways, however, MIRP researchers cut it to just one day. Consequently, MIRP is now expected to become the core research facility in Japan to promote so-called 'Evidence Based Medicine (EBM)'.

Around 1,000 people attended the opening ceremony and an international symposium, held on the 18th and 19th of January, which shows that this program is already attracting considerable attention. ■

Japan's new era of life science research – Genomics at RIKEN, Part I

RIKEN has strategically nurtured its strengths in genomic research and played an essential role in huge international projects

Since the 1990s, RIKEN has played a central role in Japan in genome research—or the science of understanding the genetic blueprint of humans. Established in 1998 in Yokohama, the RIKEN Genomic Sciences Center (GSC) has contributed to international genome sequence projects, and effectively analyzed the structures and functions of key genes and proteins. The abundance of results has brought new insights to the life sciences and could lead to the development of effective therapeutic drugs.

Genomics is rooted in the 1953 discovery of DNA's double-helix structure by James Watson and Francis Crick. In Japan in 1972, RIKEN started a DNA research project with participation from 18 laboratories. Around that time, biophysicist Akiyoshi Wada, now a special adviser to RIKEN, believed Japan's technological prowess could make it the world's front-runner in the life sciences and biotechnology.

In 1981, the government established a project to develop technologies to extract, analyze and synthesize DNA, and asked Wada to help promote the project. Thanks largely to Wada's support, a prototype of an automated DNA sequencer was developed in Japan in 1982.

Interest in genomics continued to heighten worldwide through the 1980s. In 1989, researchers from the US, Europe and Japan formed the Human Genome Organization (HUGO) to collaborate on the Human Genome Project, which kicked off in 1990. Many leading Japanese researchers, including Wada and Yoshiyuki Sakaki, current director of the GSC, lobbied the government to concentrate personnel and funds on this project to stay at the forefront of research.

In the early 1990s, researchers at RIKEN were keen to analyze structures and functions of important proteins and genes in addition to their work in the Human Genome Project. In 1992 RIKEN recruited geneticist Yoshihide Hayashizaki for the human genome project promotion center, and appointed him as a chief scientist in 1994. Hayashizaki, renowned for his genome scanning techniques, believes it is critical to analyze cDNA, a copy of DNA molecules of messenger RNA, to understand the characteristics of genes. Hayashizaki established the Mouse Encyclopedia Project to fully sequence full-length mouse cDNA, and helped create an international consortium called FANTOM (Functional Annotation of Mouse cDNA).

In 1993, RIKEN also appointed Shigeyuki Yokoyama, a biologist at the University of Tokyo, as chief scientist of the laboratory for control and design molecules. He proposed systematically studying the basic structures of proteins using two powerful tools—RIKEN's huge nuclear magnetic resonance (NMR) facilities and the SPring-8 synchrotron facility.

Figure 1: The RIKEN Genomic Sciences Center, established in 1998, is equipped with cutting-edge nuclear magnetic resonance (NMR) facilities to study the basic structures of proteins.

In 1998, RIKEN established the GSC to consolidate its various genome research projects (Fig. 1). Four years later, a team led by Yokoyama launched the Protein 3000 project to solve 3,000 out of 10,000 basic protein structures. The project is expected to finish in March 2007 with some 2,500 structures solved. Meanwhile, using ultra fast-speed sequencers, Hayashizaki and colleagues have accelerated research into mouse cDNA and unveiled some surprising results. For example, many of the full-length mouse cDNA sequences were found to be non-coding RNA. Meanwhile, other researchers have successfully analyzed about 18,000 sequences of full-length cDNA of Arabidopsis.

In 2000, the Human Genome Project ended with a draft sequence of the human chromosome 21. RIKEN was one of the six major research centers in the project, representing much of Japan's contribution. Many of the RIKEN researchers have since become pioneers in fields such as comparative genome analysis.

With the dawn of the post-genome era, the US started a research consortium called ENCODE (the Encyclopedia of Human DNA Elements) in 2003. In 2004, led by Hayashizaki and Sakaki, Japan launched the Genome Network initiative to create a new research system needed for the 21st century. ■

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