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Biology

Raising an antitumor army

Reprogrammed immune cells might give doctors an edge in rallying the body's defenses against tumor growth

Genetic abnormalities accrued by tumor cells lead to inappropriate production of proteins at the wrong time or place, or even the synthesis of unusual hybrid proteins not found in normal cells. Such abnormalities can serve as 'red flags' that alert the immune system that something has gone awry, triggering proliferation of cytotoxic T lymphocytes (CTLs) that can recognize and destroy defective cells based on these protein signatures.

Unfortunately, cancers ultimately deploy defensive strategies that render the body's natural immune response incapable of stopping cancerous growth, and scientists have encountered only limited success with vaccines and other strategies that help 'super-charge' the anti-tumor immune reaction. Now, new stem cell research by Hiroshi Kawamoto and colleagues at the RIKEN Research Center for Allergy and Immunology promises to greatly bolster the effectiveness of such approaches[1].

Although the concept of vaccines based on tumor-specific antigens is sound, the generation of a CTL response sufficient to overwhelm a cancer's defenses has proved elusive. "The number of potent anti-cancer cells is small and even if they are efficiently activated, their life span is short," explains Kawamoto. "With these methods, effects can be seen in 10–30% of cases, but this does not necessarily mean a cure—just slightly prolonged survival." To address this challenge, Kawamoto and his colleagues pursued a strategy for producing far greater numbers of patient-specific antitumor CTLs.

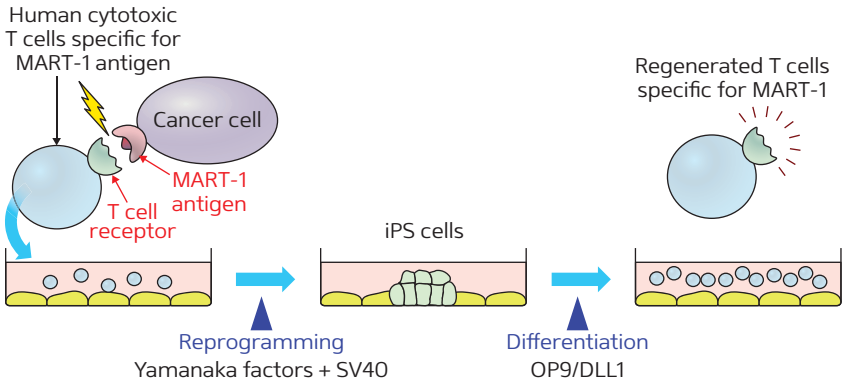

Figure 1: Scientists reprogrammed purified CTLs into embryonic stem cell-like iPSCs by forcing them to express genes known as the 'Yamanaka factors'. After allowing these reprogrammed cells to proliferate, they then cultivated these iPSCs alongside specialized cells (OP9/DLL1) that promote their maturation into transplantation-ready, MART-1-specific CTLs.

Made to order

Stem cells represent an ideal resource for the production of patient-specific anti-tumor CTLs, but the production process poses special challenges. To deal with the vast array of potential threats that might endanger our health, the immune system uses a complicated gene-shuffling process called recombination to generate armies of cells that each express receptors capable of recognizing a different target. This means that CTLs produced from either embryonic or adult stem cells would hardly retain the target-recognition capabilities needed to kill a tumor.

Kawamoto's team therefore pursued an alternative approach involving the use of a genetic reprogramming technique to transform mature human CTLs into induced pluripotent stem cells (iPSCs), which closely resemble embryonic stem cells in terms of their developmental flexibility. From this undifferentiated state, iPSCs can be cultivated under conditions that favor their development into mature CTLs (Fig. 1). Importantly, as the genes of the parental cells are pre-shuffled, the end result should be a large quantity of targeted CTLs. "All of these lymphocytes will come to express the same antigen receptor as the original T cells," says Kawamoto. "The technique allows us to regenerate antigen-specific T cells with very high efficiency."

After proving the soundness of this approach using an isolated pool of mixed human CTLs, the researchers applied their method to a specific line of CTLs that selectively recognize MART-1, a protein commonly overexpressed by the skin cancer melanoma. After reprogramming, they obtained two iPSC lines that contained the same recombined MART-1-specific receptor gene found in the parental CTL cell line (Fig. 2). Using one of these as starting material, the researchers were able to produce a 95%-pure pool of new anti-MART-1 CTLs.

Initial experiments using the purified MART-1 confirmed that these iPSC-derived CTLs were producing the specialized receptor required to bind this melanoma antigen. Immune cell activation typically entails the production and release of specialized signaling molecules called cytokines, and Kawamoto and his colleagues confirmed that their CTLs produced appropriate cytokines when cultured alongside human cells that express MART-1 on their surface. This result is encouraging, as the experiment roughly replicates the context in which CTLs might encounter this antigen in a melanoma patient.

Testing the troops

The question of whether this apparent tumor-specific immune response will translate into improved patient outcomes remains to be determined, and this will be a top focus of Kawamoto's team moving forward. "We wish to test whether the regenerated T cells can kill tumor cells in *in vivo* situations using mouse models," he says. "We are also planning to try the same experiments using different tumor antigens to confirm that this method can be generally used."

If the experiments prove successful, this technique could benefit large numbers of cancer patients. In addition to melanoma, scientists have categorized a broad range of potentially useful target antigens from a host of different tumors, including common killers such as breast, lung and colorectal cancer. Experimental vaccines are already in the developmental pipeline for several of these diseases, and their performance could be dramatically enhanced by coupling with techniques that can help doctors to quickly raise vast quantities of patient-specific CTLs. These benefits need not be limited to cancer patients, and Kawamoto notes that their iPSC-based approach could also help patients mount more effective defenses against particularly intractable infections, such as human immunodeficiency virus (HIV).

Several other technical challenges also remain to be addressed. The initial preparation of iPSCs remains a time-consuming and inefficient process, and the isolation of high-quality, tumor-specific 'source' CTLs from patients will likewise require significant effort. However, Kawamoto believes that this could be a valuable clinical proving ground for these cells, which have garnered considerable attention in the research community—including last year's Nobel Prize—but have not yet begun to achieve their perceived potential in the world of human regenerative medicine. "iPSC technology is being applied primarily to the compensation of lost or damaged tissues, but only a relatively limited number of patients require tissue regeneration," says Kawamoto. "If this technology can be applied to cancer therapy, an extremely large number of patients would benefit."

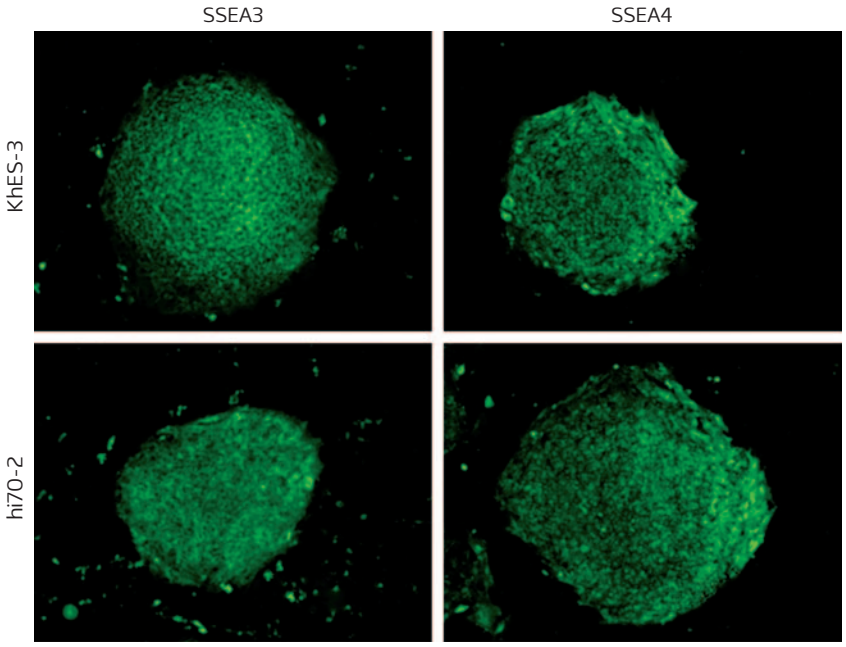

Figure 2: Human embryonic stem cells (KhES-3) express a number of specific genes that enable them to maintain their developmental flexibility, such as *SSEA3* and *SSEA4*. CTL-derived iPSCs (hi70-2) express these genes at levels that are essentially indistinguishable from embryonic stem cells, demonstrating that they also retain the flexibility for subsequent production of mature immune cells.

1. Vizcardo, R., Masuda, K., Yamada, D., Ikawa, T., Shimizu, K., Fujii, S., Koseki, H. & Kawamoto, H. Regeneration of human tumor antigen-specific T cells from iPSCs derived from mature CD8+ T cells. *Cell Stem Cell* **12**, 31–36 (2013).

ABOUT THE RESEARCHER

Hiroshi Kawamoto was born in Kyoto, Japan, in 1961. He graduated from the Faculty of Medicine, Kyoto University, in 1986, and worked as a physician for three years. He started his doctoral course at Kyoto University from 1989, and then joined the Institute for Frontier Medical Sciences from 1994 to 2001 as a visiting researcher. There he commenced research into early hematopoiesis and T cell development while working as a physician at the Kyoto Reformatory Hospital. In 2001, Kawamoto became an assistant professor at the Faculty of Medicine and was later promoted to team leader of the RIKEN Research Center for Allergy and Immunology in 2002. He moved to Kyoto University in 2012. Kawamoto is also focusing on the development of immune cell therapy using regenerated lymphocytes.

A new dimension for particle physics

The production of rare isotope beams with aligned nuclear spins opens up new possibilities for high-energy physics research

Particle physicists routinely and incrementally test the boundaries of what is possible in physics research. Occasionally however, they achieve a breakthrough that reveals an entirely new, uncharted field of study. Researchers at RIKEN's Radioactive Isotope Beam Factory (RIBF), part of the RIKEN Nishina Center for Accelerator-Based Science, have recently made such a breakthrough with their production of a beam of spin-aligned rare isotopes[1].

The nucleus of an atom consists of protons and neutrons, the numbers of which determine the element and the isotopic variant. Every element has a range of possible isotopes, but the vast majority of isotopes do not occur in nature—they can only be produced experimentally by bombarding materials such as thin metallic foils with a beam of ions using a particle accelerator.

Researchers at the RIBF have been producing beams of rare and heavy isotopes to explore the unknown 'nuclear chart' for many years. The beam of isotopes produced by the RIBF has a random alignment of spins, a quantum magnetic property of the isotope related to its angular momentum. By passing the isotope beam through a slit, the beam can be pared down to a narrow range of momentum to afford a beam with enhanced spin alignment. This technique has been applied in the past as a system consisting of two target–slit stages, but the degree of spin alignment achieved had been marginal. In their latest work, the RIKEN researchers were able to improve the spin alignment effect by a factor of 50 by replacing the first slit with a much more effective momentum dispersion matching stage that relies on the divergent spread of isotope trajectories due to their differences in momentum.

"Our method expands research into radioisotope beams and offers new possibilities to conduct microscopic investigations into physical and chemical processes that take advantage of nuclear properties such as spin," says Yuichi Ichikawa from the research team.

Not only does the new system produce a beam with a spin alignment of up to 8%, the ability of the momentum-dispersion matching stage to harvest a larger fraction of the initial isotope beam also greatly enhances the beam's overall intensity. The system can also be applied for a wide range of isotopes. "The RIBF is expected to produce 4,000 species of radioisotope beams," says Ichikawa. The production of such spin-aligned isotope beams is anticipated to open up new opportunities for research on unusual nuclear structures and the quantum dynamics of condensed matter. ■

1. Ichikawa, Y., Ueno, H., Ishii, Y., Furukawa, T., Yoshimi, A., Kameda, D., Watanabe, H., Aoi, N., Asahi, K., Balabanski, D., *et al.* Production of spin-controlled rare isotope beams. *Nature Physics* **8**, 918–922 (2012).

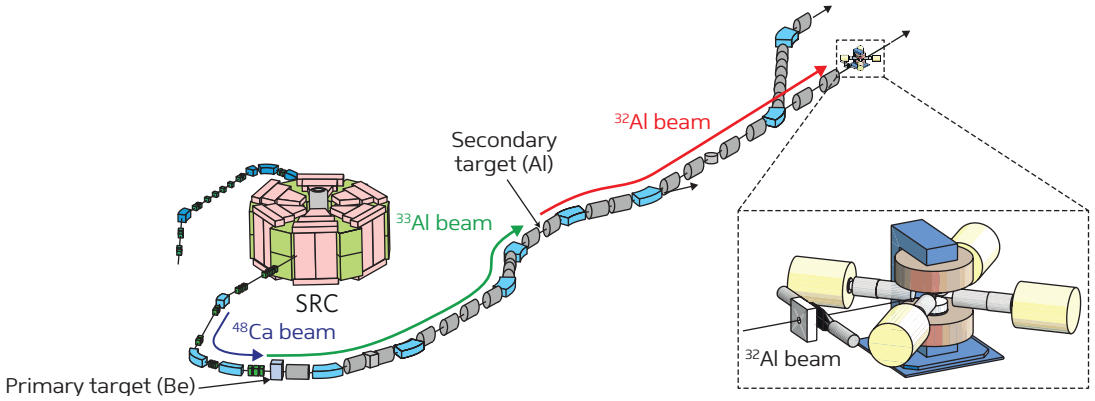

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Figure 1: The RIBF facility. Spin-aligned beams of aluminum-32 (^{32}Al) are produced in two stages by first bombarding a beryllium (Be) target with a calcium-48 (^{48}Ca) beam generated by the superconducting ring cyclotron (SRC) to produce an aluminum-33 (^{33}Al) beam, and then bombarding a secondary aluminum target and selecting a narrow band of momentum to produce a ^{32}Al beam with high degree of spin alignment.

One-way traffic boosts chemical selectivity

'Half-sandwich' rare-earth catalysts provide unprecedented directional control over aromatic insertion reactions

Methoxybenzene, commonly known as 'anisole' because of its aniseed aroma, is an important compound in organic chemistry. Anisole-type frameworks are found in numerous molecules ranging from pheromones to fluorescent dyes. New findings from Zhaomin Hou and colleagues from the Organometallic Chemistry Laboratory at the RIKEN Advanced Science Institute now promise to make the construction of these substances more efficient thanks to unique rare-earth catalysts that direct synthetic reagents to specific sites on anisole rings[1].

One of the simplest ways to modify anisole molecules is through a process known as 'C–H alkylation', where a metal catalyst approaches the methoxy-ether group of the anisole and activates a carbon–hydrogen bond on the aromatic ring. After the metal bonds to the activated position, unsaturated reagents, such as double-bonded alkenes, can insert themselves into this bond, producing an anisole with a new alkyl substituent. Although promising, this procedure has had mixed success thus far due to the weak interactions between metals and the anisole, which results in the generation of a mixture of possible products.

Hou and his team took a different approach to this problem by using 'half-sandwich' rare-earth complexes to catalyze the C–H alkylation. They recently discovered that these distinctly shaped compounds, which contain elements like scandium and yttrium, could polymerize olefins with high efficiency[2]. A key step in this polymerization involved selective alkene insertion into metal–carbon bonds—a process the researchers anticipated could be reproduced with anisole reagents.

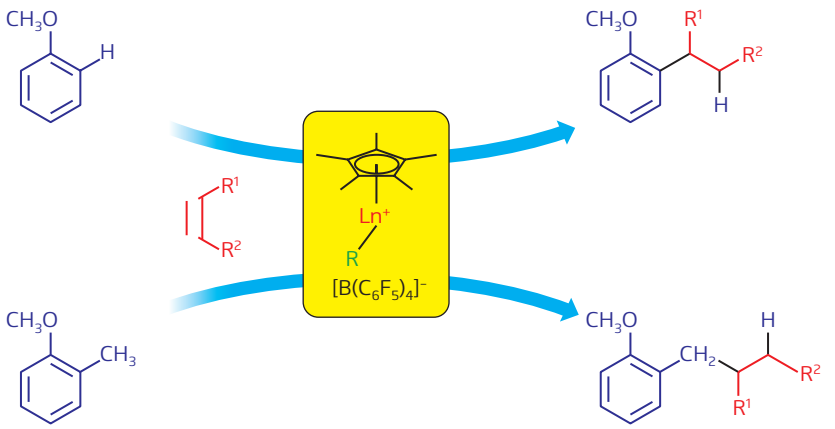

Figure 1: A cationic 'half-sandwich' rare-earth complex (center, yellow) catalyzes the addition of anisoles (blue structures) to alkenes (red structures) with high site-specificity.

The team first attempted the C–H alkylation with a neutral rare-earth complex to encourage the initial activation step. However, this catalyst could not promote the critical alkene insertion step. By switching to a cationic half-sandwich complex—a positively charged material with high affinity for alkenes—the researchers were successful in attaching a new alkyl group to the anisole ring.

The rare-earth complex also proved exceptionally adept at controlling product selectivity. Experiments with several alkene reagents and anisole derivatives revealed that alkylation occurred at a single specific location on the aromatic ring, and the rare-earth catalyst continued to promote alkylation at this site even if already occupied by a methyl substituent.

Hou notes that this site selectivity for anisole alkylation arises from the strong affinity of rare-earth metals towards oxygen. This interaction directs the initial coordination of the catalyst to the anisole, as well as subsequent alkene insertion, to proceed in a specific orientation. The team continues to investigate further applications of this novel process. ■

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2. Nishiura, M. & Hou, Z. Novel polymerization catalysts and hydride clusters from rare-earth metal dialkyls. *Nature Chemistry* **2**, 257–268 (2011).

Rapid cancer detection on a chip

An inexpensive microfluidic device for rapid point-of-care disease detection gets a boost in sensitivity

Early detection is vital for the effective treatment of cancer. In many cases, telltale biomarkers are present in the bloodstream long before outward symptoms become apparent. Early-stage cancers, for example, release tiny quantities of biomolecules called microRNAs into the blood. The development of an inexpensive and rapid point-of-care diagnostic test capable of spotting such early biomarkers of disease could therefore save many lives. A research team in Japan working on developing such a test has now produced their most sensitive microRNA detector yet[1].

The test developed by Kazuo Hosokawa and colleagues at the RIKEN Advanced Science Institute is a self-powered microfluidic chip (Fig. 1) that can perform an analysis for cancer-specific microRNAs in a drop of patient blood in as little as 20 minutes. If enough of the target microRNA is present, the chip produces a fluorescence signal that can be detected using a fluorescence microscope[2].

The team's microfluidic chip is inexpensive to make and relies on an internal pressure gradient to pump the sample through the microchannels, thus eliminating the need for an external power supply—features that make the system highly suitable for practical point-of-care disease diagnosis. Previous versions of the chip, however, could only detect microRNA at concentrations far above those required for early cancer detection.

In their latest work, Hosokawa and co-workers increased the chip's sensitivity by boosting the intensity of fluorescence generated by a positive test. The original chip worked by immobilizing target microRNA on probe DNA in the main microchannel, where each bound site produced a fluorescent signal. In the new chips, the researchers added a fluorescence amplification process that involves passing two amplification reagents over the immobilized microRNA. The reagents—a fluorescent tag and a branched linker—bind to immobilized microRNA to form tree-like dendritic structures that amplify the fluorescence signal by up to 1,000 times. Using this amplification process, the researchers were able to improve the sensitivity of the device to a level approaching that required for early cancer detection.

The next step for the team will be to further simplify the device by eliminating the need for a fluorescence microscope, which will involve replacing the fluorescent tags with some other form of marker. "That is a very important direction for future development," says Hosokawa. "We are planning the use of different labeling materials instead of the fluorescent dye, such as gold particles, which would enable naked-eye detection," he says. The team is also working to improve the sensitivity of the technique. ■

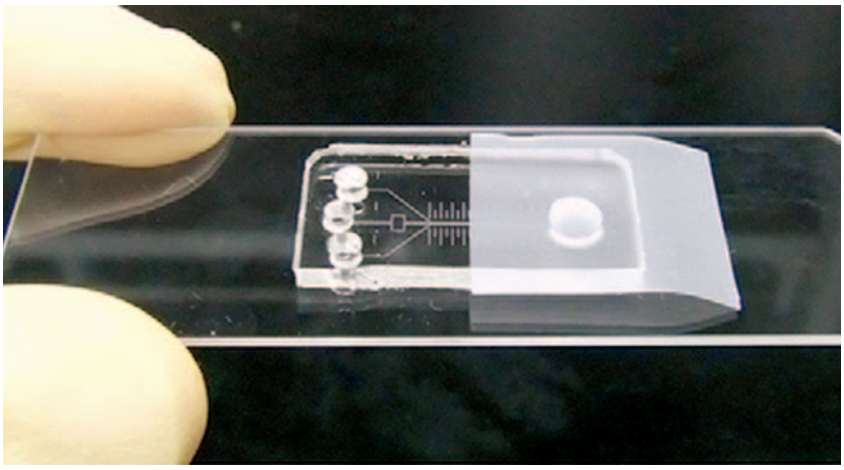

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Figure 1: A self-powered microfluidic chip for cancer biomarker detection. The sample and two fluorescence amplification reagents are added to the three inlet ports. The presence of cancer biomarkers can be detected by fluorescence in the main microfluidic channel.

1. Arata, H., Komatsu, H., Hosokawa, K. & Maeda, M. Rapid and sensitive microRNA detection with laminar flow-assisted dendritic amplification on power-free microfluidic chip. *PLoS One* **7**, e48329 (2012).
2. Arata, H., Komatsu, H., Han, A., Hosokawa, K. & Maeda, M. Rapid microRNA detection using power-free microfluidic chip: coaxial stacking effect enhances the sandwich hybridization. *Analyst* **137**, 3234–3237 (2012).

Brain waves wax and wane

Fluctuations in the size of brain waves contribute to information processing

Figure 1: The researchers used electrode arrays to record brain waves from the rat hippocampus while the animals performed various behaviors, such as running through a maze.

Cyclical variations in the size of brain wave rhythms may participate in the encoding of information by the brain, according to a new study led by Colin Molter of the Neuroinformatics Japan Center, RIKEN Brain Science Institute, Wako[1].

Brain waves are produced by the synchronized activity of large populations of neurons. Low frequency brain waves called theta oscillations are known to support memory formation. Researchers typically examine the frequency of oscillations in a given part of the brain and the timing of oscillations in different brain regions, but know very little about how variations in the size of these oscillations contribute to information processing.

Molter and his colleagues used electrode arrays to record brain waves from the rat hippocampus, a structure known to be critical for memory formation and spatial navigation, while the animals performed various behaviors, such as exploring open spaces, running through a maze and in a wheel, and sleeping (Fig. 1). They observed fluctuations in the size of theta oscillations during all the behaviors—the brain waves did not remain the same size, but rather waxed and waned second by second.

During spatial navigation for example, individual hippocampal neurons called place cells become more active when the animal is in one or a few specific locations compared to the rest of the explored environment. The researchers found that the time of firing of many of the place cells correlated with the fluctuations in the size of the theta waves. During sleep, the activity of most of the cells was timed with the largest theta oscillations.

Even though the size of theta waves is correlated with motor behavior, their cyclic fluctuations at this time scale, observed while the rats ran and explored, were not correlated with the animals' speed or acceleration. The fluctuations are instead likely to be generated by the brain itself, as their presence during sleep also suggests they are intrinsic.

The researchers speculate that this phenomenon could be helpful for the neuronal representation of space, resolving the ambiguity of space coding by place cells that become active in multiple preferred locations. "We are currently working on several new experiments to understand how the spatial location may affect the slow modulation and how the timing of the slow modulation affects behavior," says Molter. "We are also trying to provide a model that incorporates the theta slow modulation to help propagation of activity between cell assemblies." ■

1. Molter, C., O'Neill, J., Yamaguchi, Y., Hirase, H. & Leinekugel, X. Rhythmic modulation of theta oscillations supports encoding of spatial and behavioral information in the rat hippocampus. *Neuron* **75**, 889–903 (2012).

Feeding the flames for fat accumulation

A protein in fat cells that stimulates inflammatory signaling helps put the gears in motion for the onset of diet-induced obesity

Poor diet and lifestyle choices set the stage for obesity and diabetes, but the immune system plays a relatively underappreciated role in accelerating this process. Metabolic changes in fat cells stimulate the release of inflammatory signals known as cytokines, which block insulin signaling at a cellular level, as well as other factors that recruit immune cells into fatty tissue to perpetuate the cycle of declining metabolic function.

Yoshio Hirabayashi, Yeon-Jeong Kim and colleagues from the RIKEN Brain Science Institute's Laboratory for Molecular Membrane Neuroscience have now begun to characterize the mammalian counterpart of a protein related to fat metabolism in fruit flies. Their latest work reveals that this mammalian protein, GPRC5B, is a key promoter of cytokine signalling[1].

Several years ago, Hirabayashi's team identified that the protein BOSS regulates glucose and fat metabolism in fruit flies[2]. In investigating the mammalian counterpart GPRC5B, they determined that this protein is strongly expressed in adipose tissue in mice, and contributes strongly to fat accumulation. After being fed a high-fat diet, mice genetically deficient in GPRC5B gained less weight and showed reduced body fat (Fig. 1) compared with wild-type mice, and generally exhibited a higher metabolic rate.

The researchers found that GPRC5B primarily localizes within cell membrane-based structures called 'lipid rafts', where it interacts with the signaling protein Fyn. Subsequent experiments revealed that the two proteins collaborate closely: Fyn helps to switch on GPRC5B, which in turn enables GPRC5B to strongly stimulate Fyn activity. "Fyn is usually activated by growth factors—including insulin—and cellular stress, which is crucially important for obesity and the inflammatory process in adipose tissue," says Hirabayashi.

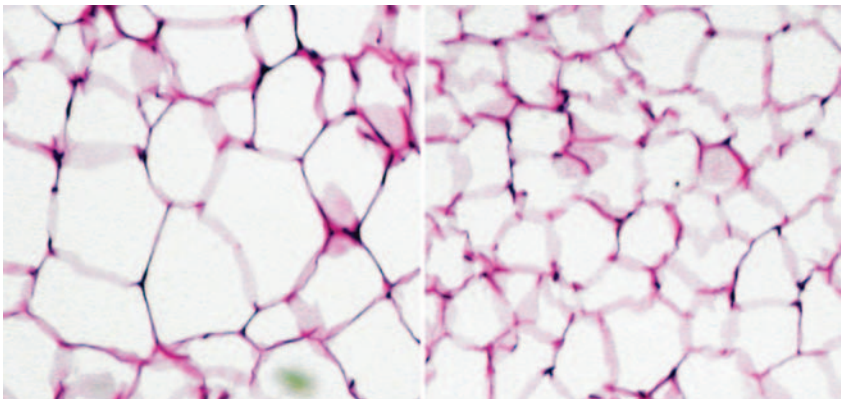

Figure 1: Mice fed a high-fat diet normally display heavy lipid accumulation in the adipocytes that compose fatty tissue (left). In GPRC5B-deficient animals, this diet-induced fat buildup is markedly reduced (right).

The researchers view GPRC5B as a potentially important player in fueling the Fyn-mediated inflammatory response in diet-induced obesity, as evidenced by the reduced pro-inflammatory signaling in the fatty tissue of GPRC5B-deficient animals. A cellular signaling cascade known as the NF-κB pathway is critical in driving production of these cytokines, and Fyn-inactivating mutations sharply reduced NF-κB activation in cultured mouse fat cells, confirming the importance of GPRC5B-Fyn crosstalk. "GPRC5B may contribute to enhanced activation of localized Fyn," says Hirabayashi, "leading to the promotion of inflammatory signaling."

The research team now hopes to fill in the blanks for this signaling pathway. This includes identifying mechanisms that regulate GPRC5B activation and the mechanism by which GPRC5B and Fyn stimulate NF-κB activity to generate an inflammatory response—findings that should help illuminate how physiological stress associated with initial onset of obesity fuels subsequent disruption of metabolic regulation. ■

1. Kim, Y.-J., Sano, T., Nabetani, T., Asano, Y. & Hirabayashi, Y. GPRC5B activates obesity-associated inflammatory signaling in adipocytes. *Science Signaling* **5**, ra85 (2012).
2. Kohyama-Koganeya, A., Kim, Y.J., Miura, M. & Hirabayashi, Y. A. *Drosophila* orphan G protein-coupled receptor BOSS functions as a glucose-responding receptor: Loss of *boss* causes abnormal energy metabolism. *Proceedings of the National Academy of Sciences USA* **105**, 15328–15333 (2008).

Intuition results from training

A game of Japanese chess reveals how experts develop their capacity for rapid problem-solving

The superior capability of experts to rapidly solve problems depends largely on their intuition, and it has long been known that this is related to experience and training. Although many psychological models relating to the development of intuition have been proposed to explain this phenomenon, none have been validated, and the underlying neural mechanisms remain a mystery.

Keiji Tanaka and colleagues from the Cognitive Brain Mapping Laboratory and Support Unit for Functional Magnetic Resonance Imaging at the RIKEN Brain Science Institute have now shown that activity in the basal ganglia of the brain, which is related to the automatic, rapid information processing or intuition characteristic of experts, develops during the course of training[1]. The work provides a first insight into the neural response of the brain to extended training and hints at ways to improve the efficiency of training experts in industry.

In earlier work, another research team led by Tanaka showed that amateur players of the Japanese chess-like game of *shogi* plotted their best next-moves consciously using the human brain's highly developed cerebral cortex. In contrast, they found that in professional players an important part of this process was unconscious or intuitive and had shifted to the head of the caudate nucleus in the basal ganglia, a much older part of the brain. This would leave the cortex free for higher-level strategy, the researchers suggested. Yet it remained unclear as to whether this shift of neural activity was entirely due to training, or dependent to some extent on pre-existing ability.

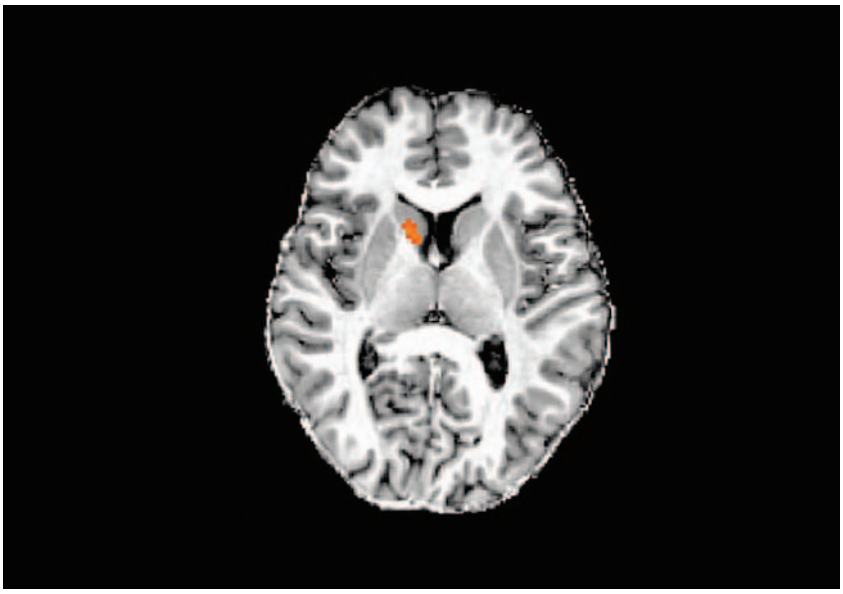

Figure 1: Activation of the caudate nucleus (orange) during intuitive generation of the best next-move, observed after training.

Tanaka's most recent experiments involved training 20 novices for 15 weeks in mini-*shogi*, a simplified version of *shogi*. After about two weeks and again at the end of the 15-week program, the intuition of the volunteers was tested through their ability to come up with the best next-move to end-phase patterns of mini-*shogi* games. To ensure the answers were intuitive, each problem was presented for just two seconds and participants had to respond within three seconds. During this process, brain activity was recorded using functional magnetic resonance imaging (fMRI). The researchers found that activity in the caudate nucleus developed over the training period, whereas activity in the cortex remained unchanged (Fig. 1).

"This work should open a fruitful interaction between the cognitive psychology of expertise development and biological studies of the basal ganglia," says Tanaka. "We now would like to elucidate what computations the caudate nucleus conducts in generating the best next-move." ■

1. Wan, X., Takano, D., Asamizuya, T., Suzuki, C., Ueno, K., Cheng, K., Ito, T. & Tanaka, K. Developing intuition: Neural correlates of cognitive-skill learning in caudate nucleus. *The Journal of Neuroscience* **32**, 17492–17501 (2012).

Secrets of a *t*-haplotype gene finally revealed

A decade-long hunt turns up a key gene involved in early mammalian development

The *t* haplotype in mice—a block of linked genes occupying the proximal half of mouse chromosome 17—is one of the best-studied examples of a selfish genetic element. Through an elaborate sperm-poisoning system, heterozygous males with only one copy of the *t* haplotype transfer the genetic element to over 95% of their progeny, while offspring that inherit two copies of the haplotype typically die during development.

This *t* haplotype is found in all subspecies of house mice around the world and is thought to have existed for more than 2 million years. Yet the lethal mutations contained within the complex that cause mice to perish *in utero* have eluded developmental geneticists for years. Now, after a decade-long search, scientists at the RIKEN BioResource Center have used positional cloning to discover the first one of these mutated genes—the gene responsible for the t^{w5} allele[1]. The findings reveal previously unknown developmental functions of this poorly characterized mammalian gene.

"I used to chat with my colleagues in the lab that cloning t^{w5} is like 'Waiting for Godot' [the Samuel Beckett play]; it may never come," says Kuniya Abe, who led the work. "But finally we found the t^{w5} gene."

Abe's team showed that the t^{w5} gene in mice is a homologue of one found in yeast that is involved in the trafficking of intracellular vesicles. When they deleted this gene, known as *vacuolar protein sorting 52* (*Vps52*), they could recapitulate the hallmark t^{w5} phenotype in mice. Follow-up experiments showed that *Vps52* is expressed in the visceral endoderm, a layer of cells that covers the very early-stage mouse embryo, and that *Vps52* acts to support growth of the pluripotential embryonic ectoderm (Fig. 1). The researchers also found that *Vps52* is involved in blood vessel formation (vasculogenesis) later in development, all through intricate cell–cell interactions.

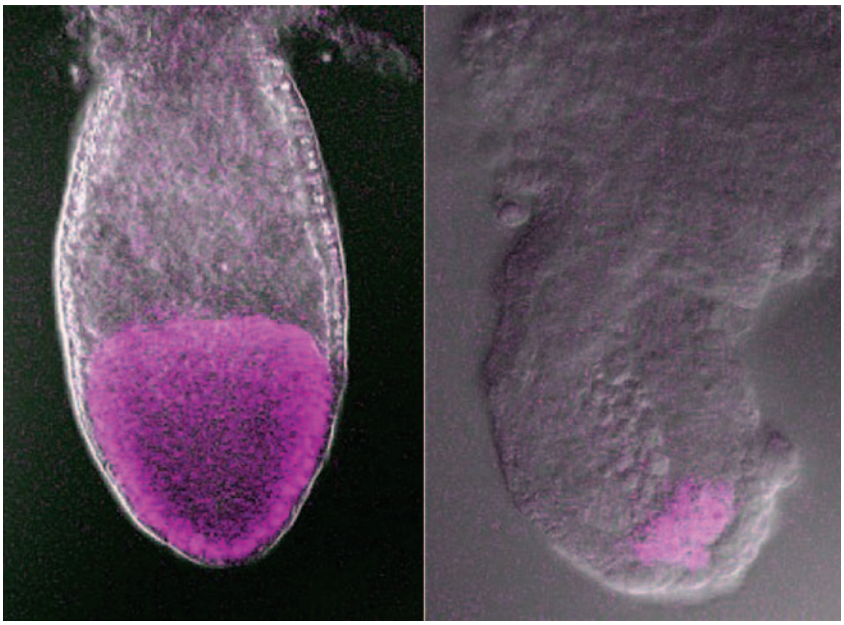

Figure 1: Characteristic defects in the embryonic ectoderm (magenta) of a t^{w5} mutant embryo (right).

"We could clearly show the gene is essential for at least two important developmental steps," Abe says. "We were intrigued by the results because a link between *Vps52* and development was not expected."

Since mutations in *Vps52* or in a related gene called *Vps54* lead to defects in vasculogenesis, heart development and motor neuron degeneration, at least in mice, Abe suggests further exploration of the roles of these genes in cardiovascular and neurological diseases in humans should be pursued. "Elucidation of the *Vps52* gene functions should provide insights into the etiology of these disease conditions, and eventually therapeutic applications as well," he says. ■

1. Sugimoto, M., Kondo, M., Hirose, M., Suzuki, M., Mekada, K., Abe, T., Kiyonari, H., Ogura, A., Takagi, N., Artzt, K. & Abe, K. Molecular identification of t^{w5}: *Vps52* promotes pluripotential cell differentiation through cell–cell interactions. *Cell Reports* **2**, 1363–1374 (2012).

The complexity of regulated development in plants

The herculean task of defining the physical interactions and locations of hundreds of *Arabidopsis* proteins involved in targeted protein degradation is underway

In most living organisms, growth and development are controlled by selective modification of the lifespans of particular proteins. This mechanism is especially prevalent in plants, allowing rapid moderation of gene expression. Even the relatively streamlined *Arabidopsis* genome encodes more than 1,400 components of ubiquitin ligase complexes—molecular machines that are each able to single out a specific type of protein for degradation while sparing tens of thousands of others. However, the selectivity with which these hundreds of components assemble to form complexes has yet to be defined.

A team led by Minami Matsui from the Plant Functional Genomics Research Group at the RIKEN Plant Science Center has now begun to explore the diversity of the largest group of plant ubiquitin ligases—the SCF complexes—in *Arabidopsis*. Each plant SCF complex comprises four canonical components, two of which are an ASK protein and an FBX protein. The *Arabidopsis* genome encodes an estimated 897 FBX and 21 ASK proteins. In their most recent work, the researchers investigated the locations of selected FBX and ASK proteins and their ability to interact with each other[1].

Knowing the locations of proteins is equally as important as understanding their interaction, says Yuki Yanagawa from the research team. "After all, even if two proteins are capable of physical interaction, that's irrelevant in physiological terms if the proteins are found in different tissues or subcellular compartments."

The team used a yeast-based assay to map the physical affinities of each of the 341 *Arabidopsis* FBX proteins for each of the 19 ASK proteins. More than half the FBX proteins didn't interact with any of the ASK proteins tested, suggesting other components, SCF complexes or post-translational modifications might be needed to facilitate certain FBX–ASK interactions. A complementary experiment suggested, however, that the original assay may also have underestimated the capacities of certain FBX proteins to interact with ASKs.

The authors then analyzed a database of gene expression data collected from 79 *Arabidopsis* tissue types to identify groups of FBX and ASK proteins with similar tissue-specific expression profiles. This revealed that SCF complexes are particularly likely to be active in reproductive tissues. They subsequently examined the subcellular locations of 17 FBX proteins by fluorescent tagging (Fig. 1).

"This is a promising start to understanding how many distinct SCF complexes can be formed from different combinations of FBX and ASK proteins," says Matsui. "Eventually, we'll be able to pinpoint the roles of hundreds of SCF complexes in plant growth and development." ■

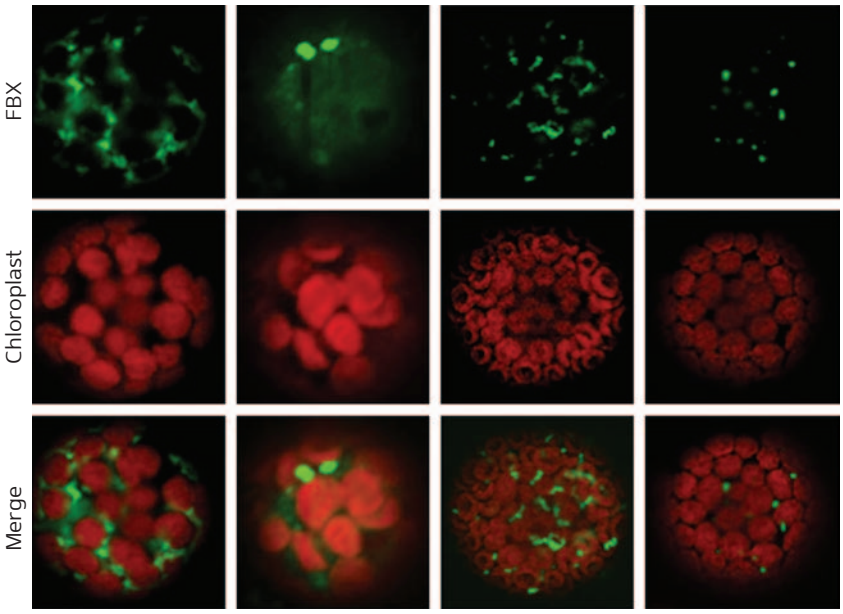

Figure 1: Four *Arabidopsis* FBX proteins (green, top and bottom rows) preferentially accumulate in speckled structures in the cytoplasm of protoplasts in leaves, outside of chloroplasts (red, middle and bottom rows).

1. Kuroda, H., Yanagawa, Y., Takahashi, N., Horii, Y. & Matsui M. A comprehensive analysis of interaction and localization of *Arabidopsis* SKP1-LIKE (ASK) and F-Box (FBX) Proteins. *PLoS One* **7**, e50009 (2012).

How plants halt hair growth

A developmentally timed molecular pathway controls cell size in *Arabidopsis*

Normal development and function in multicellular organisms relies on tight control of cell growth, yet surprisingly little is known about how such control is achieved. Although some promoters of growth have been identified, very few growth suppressors are known.

Keiko Sugimoto and colleagues from the RIKEN Plant Science Center have now uncovered new evidence that the endocycle—a growth mechanism particularly prominent in large cells such as neurons and hairs—is actively terminated through a developmentally timed pathway[1].

New cells in multicellular organisms are generated by mitosis—cell division preceded by the duplication of the cell's genetic material. Endocycling is a mechanism by which cells grow in size through the same genetic duplication, increasing their nuclear gene content or 'ploidy', without triggering cell division. Ploidy-dependent growth is in fact so prevalent, in animals as well as plants, that it is estimated to account for up to half of all biomass on Earth.

Sugimoto's team studied the gene *GTL1* in the leaf hair cells of the model plant *Arabidopsis*. *GTL1* is expressed only in fully grown hair cells (Fig. 1) and codes for the protein GTL1, a transcription factor, which modifies the action of other genes. Using a genome-wide survey, they identified around 3,900 genes bound by GTL1. Microarray analysis of thousands of genome fragments also located genes responsive to GTL1. Cross-referencing the two lists gave 182 potential GTL1 targets, of which two were already implicated in the control of cell growth. The team focused on one of these, *CCS52A1*, which is directly bound and repressed by GTL1. *CCS52A1* is known to activate an enzyme complex known as APC/C, which is central to the endocycle.

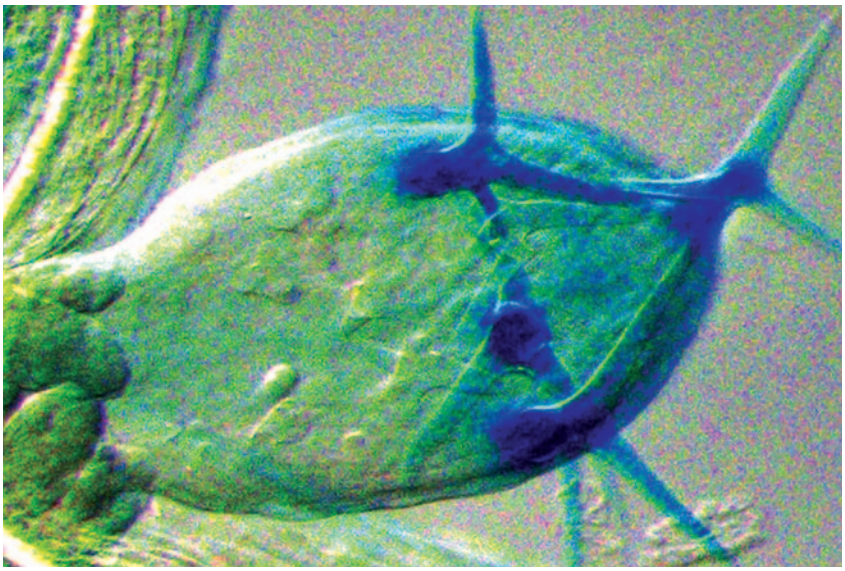

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Figure 1: A developing *Arabidopsis* leaf showing regions of *GTL1* activity. Although *GTL1* activity is absent in leaf hairs at early stages of development (closer to the stem), it becomes strong in mature leaf hairs (stained blue).

The researchers demonstrated the importance of *CCS52A1* using mutant plants defective in *CCS52A1*, which produced smaller leaf hairs with lower ploidy. Mutants with increased *CCS52A1* activity, on the other hand, bore larger, higher-ploidy hairs. The results prove that *GTL1* is both necessary and sufficient to stop the endocycle.

Sugimoto's study also demonstrates the importance of *CCS52A1*, APC/C and the endocycle itself in plant cell growth. In late leaf hair development, GTL1 down-regulates *CCS52A1*, reducing APC/C activity, halting the endocycle and stopping cell growth.

"The endocycle is often referred to as an aberrant form of mitosis," says Sugimoto. "We should instead start considering it an equally important alternative. Now we know that plants have a brake, we want to investigate how they use it in development and in response to environmental change." ■

1. Breuer, C., Morohashi, K., Kawamura, A., Takahashi, N., Ishida, T., Umeda, M., Grotewold, E. & Sugimoto, K. Transcriptional repression of the APC/C activator *CCS52A1* promotes active termination of cell growth. *The EMBO Journal* **31**, 4488–4501 (2012).

Decoding developmental differences

Differences between frog species reveal how developmental patterns are related to species diversity

The development of embryos follows different patterns in different species, with specific events taking place at different times in relation to each other. Such differences can provide insight into how processes in development fit together, and how developmental patterns relate to reproductive adaptations.

Christian Mitgutsch from the Laboratory for Evolutionary Morphology at the RIKEN Center for Developmental Biology, working with researchers Eugenia M. del Pino and Natalia Sáenz-Ponce from the Pontifical Catholic University of Ecuador, has now investigated developmental timing across different species of frogs[1]. The research team demonstrated differences according to the overall rate of development, linking developmental patterns to reproductive adaptations.

Different developmental rates are likely to be evolutionary responses to reproductive strategies and environmental conditions. "For example, development is accelerated in frogs that require rapid development of a free-living tadpole," says Mitgutsch. In contrast, embryos of the marsupial tree frog *Gastrotheca riobambae* (Fig. 1) develop inside a dorsal pouch of the mother where they are protected and can develop slowly.

Six frog species with different developmental rates were chosen for investigation. The rates were defined as the time required to progress from fertilization to the end of gastrulation. Three of the frog species that were investigated develop rapidly, completing gastrulation in just a few hours. The other three develop slowly, taking several days to reach the same stage.

Figure 1: A brooding female of the marsupial frog *Gastrotheca riobambae*. The embryos develop slowly inside a pouch derived from the dorsal skin of the female. The outlines of the large embryos enclosed in the maternal pouch are visible.

"Gastrulation is a developmental process in multicellular animals during which germ layers are formed," says Mitgutsch. "These layers are groups of cells that give rise to the different tissues and organs."

Elongation of the embryo is a major developmental event, occurring when the earliest form of the spinal column, called the notochord, forms and extends. The researchers investigated elongation in their six frog species using protein labeling to watch the development of the head and precursors of the trunk. They found distinct differences in timing between the slowly and rapidly developing species.

"In frogs that develop rapidly, body elongation is superimposed upon the process of gastrulation, and there is acceleration of head development," says Mitgutsch. "In our slowly developing frogs, body elongation occurs after completion of gastrulation, with a relative delay in the development of trunk structures."

Mitgutsch explains that differential timing of developmental processes in different species can be related to growth and life history modes. "Learning about the differences provides information about how amphibian species-specific development evolved. We can generally better understand early development and learn how the diversity of frog species is realized developmentally." ■

1. Sáenz-Ponce, N., Mitgutsch, C. & del Pino, E.M. Variation in the schedules of somite and neural development in frogs. *Proceedings of the National Academy of Sciences USA* **109**, 20503–20507 (2012).

TAKASHI NISHIMURA

Team Leader
Laboratory for Growth Control Signaling
RIKEN Center for Developmental Biology

Exploring the relationship between nutrition and growth using *Drosophila*

How do organisms grow and what determines their body sizes? Takashi Nishimura, team leader of the Laboratory for Growth Control Signaling in the Center for Developmental Biology, RIKEN Kobe Institute, and his colleagues are seeking an answer through *Drosophila* (fruit flies). "Merely taking a large amount of nutrition is insufficient for the *Drosophila* to grow. Hormones that promote its growth must also be produced," says Nishimura. His team is working to elucidate in full the mechanism between nutrition and growth by utilizing powerful genetic techniques in *Drosophila*, including selective suppression of gene expression in specific cells and tissues during particular periods.

Focusing on growth

"I've always wanted to undertake research targeting the whole body of an individual organism. When I was a child, I used to love catching mantises and locusts and keeping them in my home," says Nishimura. When he was a high school student, he became interested in marine organisms. In 1995, he entered the School of Fisheries Sciences at Hokkaido University. "In my graduate study, I investigated the sex hormones of rainbow trout. In those days, the adverse biological effects of endocrine disruptors were creating quite a stir in the media."

When Nishimura was an undergraduate student, he was inspired by the book *Seishin To Busshitsu* (*Mentality and Materials*) by journalist Takashi Tachibana and Nobel laureate and current director of the RIKEN Brain Science Institute (BSI), Susumu Tonegawa. Their book made a great impression on Nishimura and motivated him to become a researcher. "I was very interested in the process through which Dr. Tonegawa reached the solution to a long-standing question within immunology by making the best use of the then-available techniques. His work influenced me so much that I decided to become a research scientist."

Eager to conduct research into the whole body of an individual organism in the future, Nishimura commenced his research at the Nara Institute of Science and Technology (NAIST). "First, I decided to learn about cells and joined a laboratory where they were studying intracellular signaling using mammalian cells in culture. The educational curricula of NAIST were very substantial and informative, and that was very helpful to me in enriching my scarce knowledge of molecular cell biology."

Nishimura then obtained his doctoral degree from Nagoya University and joined the Institute of Molecular Biotechnology (IMBA) in Austria. "At IMBA, I studied the *Drosophila* cell division process that yields two different types of cells. It was during this time that I became really interested in heading up a laboratory for studying the whole body of an individual organism. In those days, I used to converse with my Japanese colleague at the same IMBA laboratory and we talked about themes that may be interesting to study. My colleague introduced me to various research projects, including work by team leader Yoshio Hirabayashi at RIKEN's BSI and his

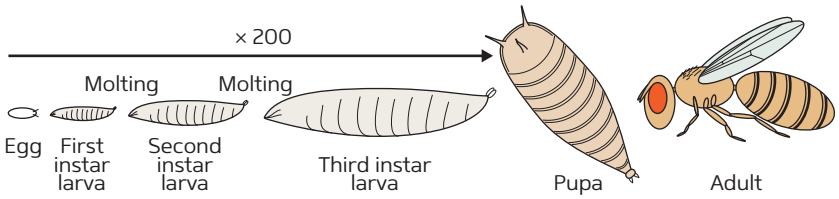


Figure 1: *Drosophila* growth process

The *Drosophila* feeds often and grows in the larval stage, during which its body size increases about 200 times compared with its size in the egg.

colleagues, concerning metabolic interactions of neurons and glial cells mediated by the amino acid serine. I became interested in the key phrase 'recognition of nutrients' and the riddle 'How do organisms grow and how are their body sizes determined?'"

In July 2009, Nishimura established the Laboratory for Growth Control Signaling in the RIKEN Center for Developmental Biology (CDB). "My first research theme was to study the growth of organisms using *Drosophila*. The CDB offers an excellent research environment and cutting-edge equipment, and this encouraged me from the start to take on new areas in our research."

Between nutrition and growth

After hatching from an egg, the *Drosophila* larva feeds and grows, undergoes two molting events, and becomes a pupa in about 5 days. During this larval stage, the body size increases about 200 times compared to its size in the egg. Subsequently, the larva becomes a pupa, from which an adult emerges in about 4 days (Fig. 1).

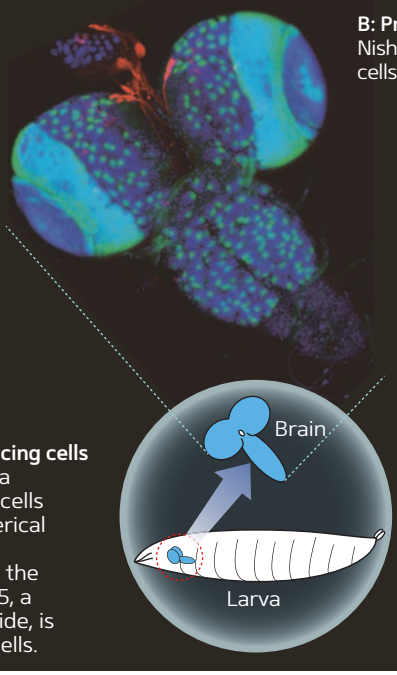
"When the *Drosophila* eats more food in the larval stage, it grows into a bigger adult," explains Nishimura. "However, merely eating a lot of food is insufficient for its growth. After feeding, hormones that promote growth must be produced in the body."

In human beings, peptide hormones similar to insulin (and hence known as 'insulin-like growth factors') control growth. In 1984, a joint research group from Nagoya University and the University of Tokyo discovered an insulin-like peptide in the silkmoth, demonstrating that the same peptide hormone is used by both insects and human beings, who are remote from each other in terms of evolution.

Naoki Okamoto, a special postdoctoral researcher at the Laboratory for Growth Control Signaling, was a graduate student at a laboratory in Nagoya University that had developed from this joint research group. "My research interests included the biochemical properties of the insulin-like peptides extracted from the silkmoth," explains Okamoto. "Then, in the middle of my doctoral course, I commenced research to examine the functions of insulin-like peptides in insects using both the silkmoth and *Drosophila*. In those days, I was the only researcher in Japan who was conducting such research."

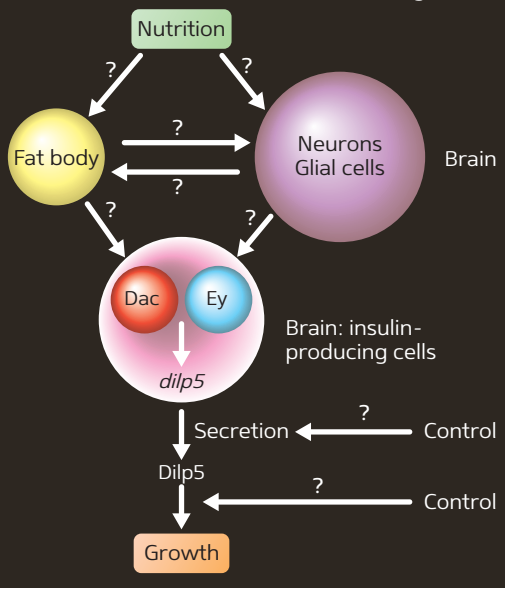
A protein comprises a sequence of various amino acids arranged in the form of a chain according to genetic information. Peptides, like proteins, have amino acids arranged by their genetic information, but their chains are shorter than those of proteins. One useful method to examine the function of an insulin-like peptide in the body is to suppress (or 'knock down') the expression of the relevant gene—which prevents the insulin-like peptide from being produced in particular cells—and then determine its effects. In *Drosophila*, the development of a gene knockdown technology known as RNA interference is remarkably advanced.

Figure 2: Between nutrition and growth



A: *Drosophila* larva and insulin-producing cells
The right image shows the brain of a *Drosophila* larva. Insulin-producing cells (red) are present between two spherical portions (intercerebral area), which correspond to the cerebrum. When the larva takes nutrients (proteins), Dilp5, a growth-promoting insulin-like peptide, is produced in the insulin-producing cells.

B: Predicted mechanism connecting nutrition and growth
Nishimura predicts that many factors, including Dilp5, and cells/tissues, are involved between nutrition and growth.



"I wanted to continue to study insulin-like peptides using *Drosophila* after obtaining my degree, and was seeking a post that would allow me to do such research. At that time, I stumbled upon an advertisement recruiting a researcher for the Laboratory for Growth Control Signaling. I was astonished to find such a laboratory in Japan."

Okamoto joined the Laboratory in April 2010. "The best person in Japan joined my laboratory that day," says Nishimura.

Essentially, *Drosophila* have long been used as laboratory organisms, exerting major impacts on biology as an experimental material for genetic, developmental, and other studies. In 2000, the *Drosophila* became the first insect whose genome was completely decoded.

"The genome decoding showed that seven insulin-like peptide genes are present in the *Drosophila*. These insulin-like peptides work as hormones to regulate the insect's metabolism and growth. Three of them (Dilp2, Dilp3, Dilp5) are produced in insulin-producing cells in the brain (Fig. 2, part A). It was known that if the larva is deprived of insulin-producing cells, it is no longer able to grow even when it feeds. When Okamoto was a graduate school student, he examined the developmental changes in the expression levels of genes for all seven insulin-like peptides. He found that only the expression level of the *dilp5* gene rose in first instar larvae, just after hatching (Fig. 3). As the larva feeds, the expression level of the *dilp5* gene rises."

Nishimura and his colleagues decided to investigate the relationship between nutrition and growth with a focus on the *dilp5* gene. Firstly, to identify the nutrients related to the expression of the *dilp5* gene, they conducted experiments in which *Drosophila* larvae were fed various diets containing sugar or protein alone.

"In the larvae that had eaten only the sugar diet, the *dilp5* gene was not expressed," explains Nishimura. "They survived but were unable to grow. Hence it was found that feeding on protein causes the *dilp5* gene to be expressed and allows the larvae to grow bigger. In addition, when larvae are temporarily starved, the expression level of the *dilp5* gene decreases, but when protein is given again, the normal expression level is restored."

Then, to determine how the *Drosophila* body transmits the signal indicating it has fed on protein, so as to express the *dilp5* gene in the insulin-producing cells in the brain, Nishimura and his colleagues searched for transcription factors that switch on the expression of the *dilp5* gene. "We knocked down the genes for a wide variety of transcription factors one by one to identify the ones whose knockdown dramatically reduced the expression of the *dilp5* gene," says Nishimura. "Another researcher had previously shown that individuals deprived of the *eyeless* (*ey*) gene (knockout) had a decreased level of *dilp5* gene expression, and we confirmed this fact. We further discovered that the expression of the *dilp5* gene also decreases significantly when the *dachshund* (*dac*) gene is knocked down. Hence, when any one of the *dac* and *ey* genes is lacking, the expression of the *dilp5* gene greatly decreases. With these findings in mind, we hypothesized that Dac and Ey bind together to form a complex that switches on the expression of the *dilp5* gene, and we demonstrated our hypothesis through biochemical experiments (Fig. 4)."

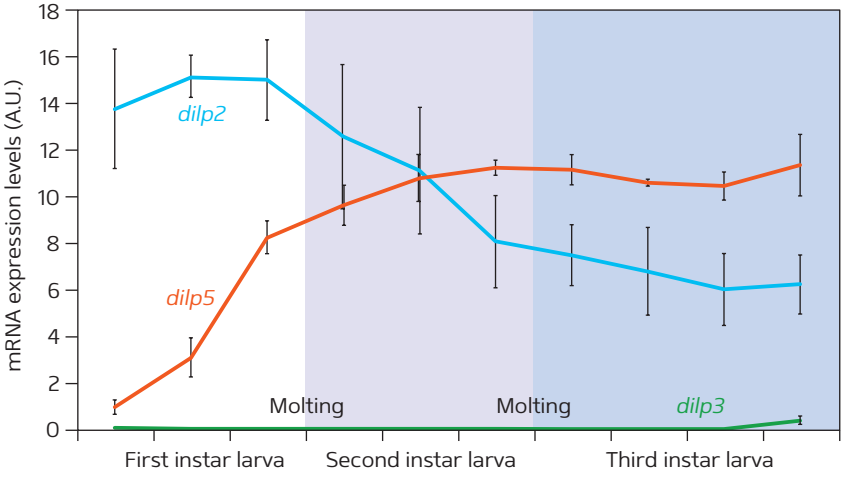


Figure 3: Expression of *dilp5* gene in larval stage
As the larva begins feeding, the expression level of the *dilp5* gene rises in insulin-producing cells.

Elucidating cell–cell and tissue–tissue interactions

To understand how an organism grows and how its body size is determined, Nishimura and his team explore cell–cell and tissue–tissue interactions that create the bridge between nutrition and growth in the whole body of the individual organism. "It is much more difficult to examine remote interactions between cells/tissues than to examine intracellular phenomena," says Nishimura. "I think, however, that the *Drosophila* will enable us to do so."

When the larva is starved, the expression level of the *dilp5* gene falls. "Despite this, Dac and Ey continue to be present," says Nishimura. "Hence, feeding on protein does not induce Dac and Ey production, nor does it switch on *dilp5* gene expression. It can be hypothesized that another factor controls the switching function of Dac and Ey. We are searching for promising candidates that play a role between the protein feeding and expression of the *dilp5* gene (Fig. 2, part B)."

"I suppose that the protein is recognized not by insulin-producing cells in the brain where the *dilp5* gene is expressed, but by other cells or tissues such as neurons and surrounding glial cells in the brain," says Nishimura. "Furthermore, the tissue known as the fat body—the functional equivalent of liver and adipose tissues in vertebrates—may also recognize protein. I think that another hormone is very likely to transmit the signal, after the *Drosophila*

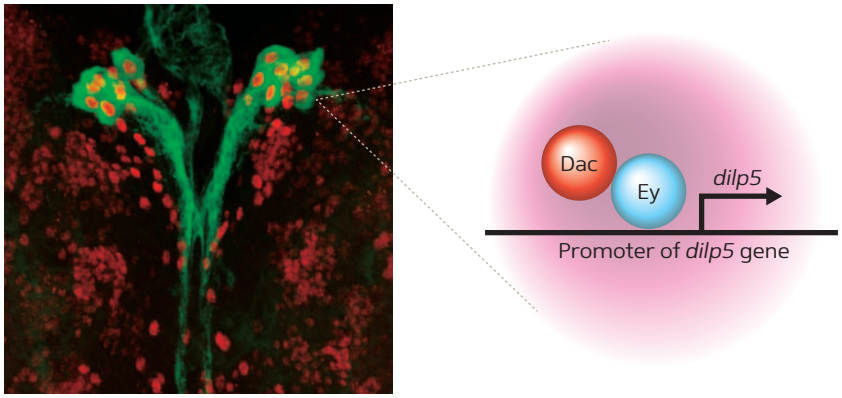


Figure 4: Mechanism for *dilp5* gene expression
It is conjectured that a complex is formed by Dac (red) and Ey in insulin-producing cells (green) and binds to a promoter to express the *dilp5* gene.

body has fed on protein, from such cells and tissues to insulin-producing cells."

After insulin-like peptides are produced, they are secreted extracellularly and act on other cells and tissues to allow the larva to grow. "There should be another factor that controls the secretion of Dilps," says Nishimura. "It is also thought that the larval body possesses mechanisms to regulate Dilps even after their secretion. It is therefore necessary to elucidate the mechanism that connects Dilp secretion and function with larval growth."

The final adult size depends not only on the amount of nutrition taken in by the larva, but also on the duration of the larval feeding period. "When the transformation from larva to pupa is delayed and the larval stage lengthens, a bigger adult emerges. We are also working on the mechanism for determining this developmental timing. It is known that a steroid hormone called ecdysone regulates the time at which the larva becomes a pupa. Other data suggest the involvement of nutrition and insulin-like peptides in the mechanism by which ecdysone is produced and secreted."

Easy gene knockdown in *Drosophila*

One experimental approach that allows researchers to effectively examine cell–cell and tissue–tissue interactions is to list up candidate factors that may be involved in the interactions that occur between certain cells or tissues in a particular period, knock them down one by one, and evaluate their influences.

"In *Drosophila*, the foundations for scientific research that allow more than 90% of the genes in the whole genome to be freely knocked down were established three to four years ago," explains Okamoto. "A number of research organizations across the world, including the IMBA in Austria and Japan's National Institute of Genetics, produce and supply more than 20,000 strains for gene knockdown experiments. If we want to knock down a gene of interest only in a particular tissue, we can obtain the selected strain from such a research organization, conduct experiments, and obtain results within one month. Using *Drosophila*, it is possible to implement experiments that could not be performed with any other laboratory animal."

In experiments involving genetic modifications, such as gene knockout and knockdown, the test procedure can be expedited in organisms with short lifespans. In *Drosophila*, it takes about 10 days for the embryo to grow into an adult, but in the mouse, a representative mammalian laboratory animal, the generation time is from 2 to 3 months. *Drosophila* is therefore a valuable model organism.

"The growth research field is one of the most competitive areas in research concerning *Drosophila*. Additionally, robust foundations for scientific research into gene knockdown were established early on," explains Okamoto. "Past studies discovered some growth-related factors for the first time in *Drosophila* and later revealed similar factors in human beings. Research into growth using *Drosophila* is thought to also be important in understanding human growth. Influential laboratories at institutes around the world have also been actively conducting extensive studies in growth using *Drosophila*.

"Rival laboratories overseas have long been using *Drosophila*," says Nishimura. "At the same time, we have researchers from diverse backgrounds working in our laboratory. I want to conduct innovative research using a combination of experimental methodologies available through individual members' fields of expertise, specifically cell biology, biochemistry, insect physiology and endocrinology."

Over the next decade, growth research focusing on *Drosophila* is expected to further deepen our understanding of the growth and development of living organisms, as well as ourselves. ■

1. Okamoto, N., Nishimori, Y. & Nishimura, T. Conserved role for the Dachshund protein with *Drosophila* Pax6 homolog Eyeless in insulin expression. *Proceedings of the National Academy of Sciences USA* **109**, 2406–2411 (2012).

ABOUT THE RESEARCHER

Takashi Nishimura was born in Hyogo, Japan, in 1976. He graduated from the Faculty of Fisheries Science, Hokkaido University, in 1999, and obtained his PhD in 2004 from the Nagoya University Graduate School of Medicine for research on neuronal polarization. After two years as a postdoctoral fellow at the same university, he joined the Juergen Knoblich lab as a postdoctoral fellow at the Institute of Molecular Biotechnology (IMBA), Austria, from 2006 to 2009, where he researched the molecular mechanism of asymmetric protein localization during neuroblast division in *Drosophila*. He then returned to Japan as a Team Leader at RIKEN Center for Developmental Biology in 2009, where he started his own research in growth regulation. His research focuses on the molecular basis for growth control and developmental timing at the cellular and tissue/organ levels using *Drosophila* as a model system.

TATSUYUKI AOSHIMA

Chief
Research Ethics Section
Safety Division

Working together with RIKEN researchers

How did you join RIKEN?

I was looking for a job related to scientific research. RIKEN was an attractive choice for me because it is a renowned international research hub in Japan, and its papers are often cited. I was offered a job in the Safety Division at the RIKEN Kobe Institute, and I believe I made the right decision to work here as I am really enjoying taking part in a variety of scientific studies in my day-to-day work.

Please tell us about your work at RIKEN.

The Safety Division at RIKEN plays a central role in creating new rules for scientific research in Japan. In fact, the government has adopted some safety guidelines developed by RIKEN. In collaboration with researchers, I investigate the correct application procedures required for research projects and ensure that researchers' studies adhere to established laws and safety guidelines. Previously I was engaged in research projects dealing with human embryonic stem cells in Kobe, but now I am in charge of application procedures to obtain approval for the clinical translation of human induced pluripotent stem (iPS) cells in Wako. I have always worked in the Safety Division, but the application procedures vary depending on the research I am involved in.

What have been the highlights of your time at RIKEN so far?

When researchers come up with new ideas for research projects, they need to consult with the Safety Division first to ensure that the projects are going to be legitimate. They appreciate all my efforts when the application is approved successfully, which is very rewarding. When the research I have been involved in achieves significant results, especially when they are reported widely in the media, I am very proud of RIKEN's achievement and I am glad to have played a part in the study's success. It makes me feel like I am a researcher on the team and it is extremely challenging and fulfilling to be involved in such cutting-edge research.

What is the best thing about working at RIKEN?

At RIKEN, managers and non-managers work closely with one another. We are encouraged to freely make suggestions to improve procedures and to highlight both positive and negative points in our feedback to senior staff. We also work closely with the researchers. Sometimes I need to request them to stringently follow laws and guidelines, and it can be challenging when we encounter problems getting approvals. Needless to say, we all share in the delight of achieving research results after an application is approved thanks to our combined painstaking efforts. This is the moment that makes me feel like I am a member of the research team.

What would you say to other people considering joining RIKEN?

People may think that a scientific background would be helpful in understanding the details of research. Even if you work in an area that is different from what you have studied at university, you can still make a contribution to research projects at RIKEN. A scientific background is not essential; what is most important is to have a clear focus in your work, and an ability to work effectively in a busy, fast-paced environment.

CONTACT INFORMATION

For details about working at RIKEN, please contact the RIKEN Global Relations Office:
Tel: +81-(0)48-462-1225
E-mail: gro-pr@riken.jp

Prime Minister Abe visits RIKEN institutes

Prime Minister Shinzo Abe (center) tours the K computer facility with RIKEN President Ryoji Noyori (left) and RIKEN AICS Director Kimihiko Hirao (right).

On 11 January 2013, the RIKEN Advanced Institute for Computational Science (AICS) and the RIKEN Kobe Institute welcomed a visit by Japanese Prime Minister Shinzo Abe. Joining the prime minister on the tour were Deputy Chief Cabinet Secretary Hiroshige Seko, State Minister of Economy, Trade and Industry Kazuyoshi Akaba, and Cabinet Office Senior Vice Minister Yasutoshi Nishimura.

At AICS, the prime minister was greeted with a presentation by RIKEN President Ryoji Noyori, who delivered an overview of the institutes' research mission and achievements. AICS Director Kimihiko Hirao also gave a presentation on the K computer, explaining its function and importance in industrial applications. The prime minister was invited to the visitor hall where he was given a bird's eye view of the extensive facility where the K computer is housed. Commending the impressive set-up, Prime Minister Abe, President Noyori and Director Hirao shared their views on the importance of acquiring the right talents for developing future technologies.

Following the informative tour of AICS, the prime minister and his entourage continued the day at the RIKEN Kobe Institute. Director Masatoshi Takeichi introduced some of the projects currently undertaken by the RIKEN Center for Developmental Biology (CDB). "Our basic research in the area of developmental biology is providing a foundation for research in regenerative medicine and drug discovery," explained Takeichi, whom at the same time emphasized the importance of a well-balanced system of support for both basic and applied research. In giving the prime minister an insight into the possibilities of tomorrow's technology, Director Shinya Yamanaka of Kyoto University's Center for iPS Cell Research and Application (CiRA) discussed the studies conducted at the CiRA and the potential development of therapeutic drugs from induced pluripotent stem (iPS) cells.

Upon completion of the presentations, Group Director Yoshiki Sasai gave the distinguished guests an overview of the CDB's cutting-edge research at the center's demonstration laboratory. One researcher who had the opportunity to present her work to the prime minister was Masayo Takahashi, a project leader spearheading clinical research in which iPS cells will be used to develop a new treatment for age-related macular disease.

The prime minister was intrigued by the visualization of optic cups formed from embryonic stem cells and retinal pigment epithelial cells generated from iPS cells, and later enquired about measures being taken for the implementation of the research.

Upon conclusion of the tour, Prime Minister Abe expressed his admiration for the dedication of the researchers at both the AICS and Kobe Institute for their continual creation of innovative medical technologies. ■

Memorandum of Understanding on research collaboration signed between RIKEN and the Institute of Chemistry, Chinese Academy of Sciences

On 30 January 2013, a ceremony was held at the RIKEN Wako campus for the signing of a Memorandum of Understanding (MoU) between RIKEN and the Institute of Chemistry, Chinese Academy of Sciences (ICCAS). Two distinguished visitors from ICCAS, Li-jun Wan and Deqing Zhang, who are the former and current directors of the institute, traveled to Japan for the ceremony.

Starting off the proceedings, the two ICCAS leaders met with RIKEN President Ryoji Noyori to discuss the current state of scientific research in China and Japan. This

The signing ceremony for the MoU. From left to right: RIKEN ASI Director Kohei Tamao, RIKEN Executive Director Maki Kawai, RIKEN President Ryoji Noyori, former ICCAS Director of the Institute for Chemistry, Li-jun Wan, and current director Deqing Zhang.

was followed by the signing ceremony, which was attended by RIKEN's President Ryoji Noyori, Executive Director Maki Kawai, Executive Director Kenji Oeda, and RIKEN Advanced Science Institute Director Kohei Tamao.

Addressing the ceremony, Kawai reflected on the history of collaboration between the two institutions and called for further cooperation involving exchanges of personnel and joint research toward the realization of a sustainable society. Rounding up the momentous day, Wan expressed his appreciation of the collaborative effort that spanned three decades, and highlighted the importance of the strengthening of scientific ties between the two countries.

The first MoU between the two institutions was inaugurated in 2007. This new agreement will further enhance collaboration, bringing the vision of establishing a joint center one step closer to fruition. ■

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For further information on the research presented in this publication or to arrange an interview with a researcher, please contact
RIKEN Global Relations Office
2-1, Hirosawa, Wako, Saitama, 351-0198, Japan
TEL: +81 48 462 1225
FAX: +81 48 463 3687
E-Mail: rikenresearch@riken.jp

www.rikenresearch.riken.jp

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