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SUMMER 2014

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STEPPING IT UP

JOINING THE DOTS

MAPPING THE COMPLEX WEB OF NETWORKS THAT REGULATE GENE EXPRESSION

RIKEN Quantitative Biology Center
Researchers at the Laboratory for Cell Signaling Dynamics in Osaka are using advanced imaging techniques such as total internal reflection fluorescence microscopy to observe individual molecules in living cells.

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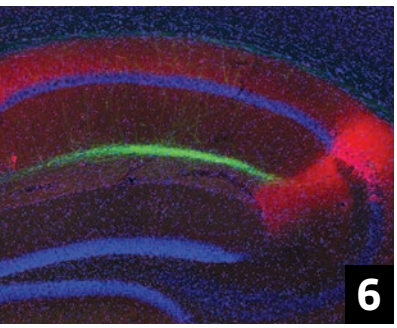

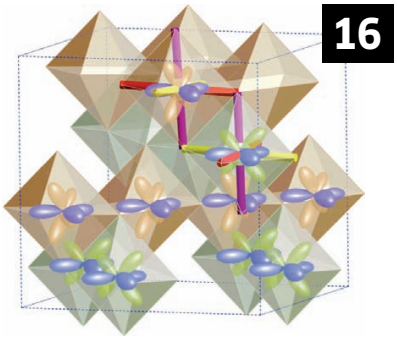

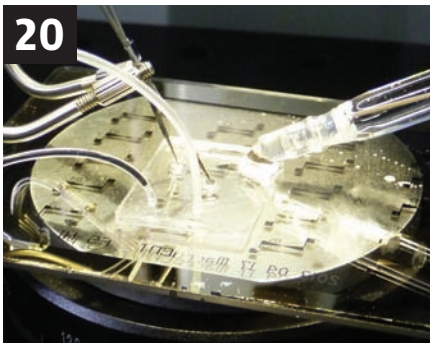

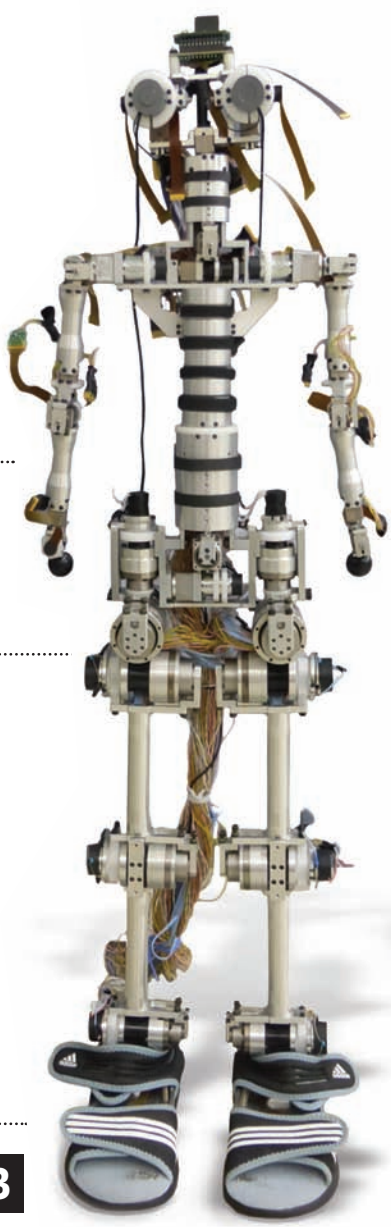

Our new look

Cover story: Researchers at the RIKEN-led FANTOM5 consortium are mapping the complex web of networks that regulate gene expression. **Page 12**

Welcome to the new-look *RIKEN RESEARCH*. Since 2006, this online and print publication has showcased some of the best of RIKEN's world-class scientific research. Through the new quarterly print format we hope to make our broad-ranging research more accessible to readers, and to better reflect the breadth and depth of our work.

While the staple of *RIKEN RESEARCH* remains the crisp and informative "Research highlights", which present recently published research for non-specialist readers, we also aim to show RIKEN from different angles. First, in our "Perspectives" articles, we present overviews of the major fields that we are engaged in as part of our efforts to address the challenges facing humanity today. And in the "Places" section, we look at a specific RIKEN location. Finally, in "People", we highlight the outstanding individuals, from Japan and around the world, who truly make RIKEN what it is.

In this issue, our "Perspectives" and "Places" articles examine the RIKEN Quantitative Biology Center (QBiC). Researchers at the center focus on measuring intracellular molecular dynamics, modeling cell behavior and designing artificial systems that recreate biological phenomena. QBiC research teams are developing a holistic understanding of biological systems to ultimately contribute to advances in drug discovery and the engineering of energy-efficient technologies.

Carrying out work in these areas requires the manipulation of enormous amounts of data, and computational power is a key requirement. The world-leading K computer located in Kobe makes RIKEN an ideal place for advancing this science. We are delighted to be able to report that RIKEN was selected to develop a new exascale computer, projected to achieve as much as 100 times the speed of the K computer, which is scheduled to go into operation in 2020. We hope that the new computer will contribute to further advances in the many computation-rich fields of research.

Maintaining a healthy gut reaction

Sidonia Fagarasan

Team Leader

Laboratory for Mucosal Immunity
RIKEN Center for Integrative Medical Sciences

How and when did you join RIKEN?
I joined RIKEN in 2001, when a new institute dedicated to the study of immunology was established at the Yokohama campus. I applied for a team leader position at the recently launched Research Center for Allergy and Immunology, which was later reorganized into the RIKEN Center for Integrative Medical Sciences. I was strongly encouraged by my mentor Tasuku Honjo at Kyoto University despite my own fears that I might not be mature enough to take on such a position of leadership. Looking back, I think that the committee members took a risk, and I am grateful to them for it.

Please describe your role at RIKEN.
My team explores the dialogue between the bacteria and immune cells in the gut. The intestine is colonized by trillions of bacteria that perform vital metabolic and protective functions and have an influence on the immune system. Our studies thus far have determined that the gut microbial landscape is also shaped by complex interactions with the host immune system. The team that I lead is studying this bidirectional flux of information between the host immune system and bacterial communities in steady-state conditions as a basis for understanding the causes of a breakdown in cooperation that leads to disease.

How did you become interested in your current field of research?
I am a trained gastroenterologist and microbiologist. During my years of practice, I encountered many patients with inflammatory bowel diseases or gastrointestinal infections and realized the need for more research on the fundamentals of host–bacterial interactions in the gut. When I later joined Honjo's immunology laboratory in Japan, it was a natural transition for me to select a field of study related to mucosal immunology, even though most immunologists at the time were oblivious to the importance of gut microbes for immune system function.

I consider RIKEN to be the best possible place to start a scientific career.

What made you decide to become a scientist?
I never intended to become a scientist because the title always sounded very pompous to me. But I was always eager to experiment—to try to find the answers to very simple questions that I considered interesting. I remember reading a book by the Hungarian biochemist Albert Szent-Györgyi on his discovery of vitamin C and being thrilled by the world that he described in those pages. I think it was his book that inspired me to go into research.

What is the best thing about working at RIKEN?
RIKEN is an institution that offers an incredible support system and allows researchers to pursue their scientific quests. I consider RIKEN to be the best possible place to start a scientific career. I believe that it is among the top scientific environments in Japan—perhaps even in the world—and hope that it will continue to be so.

Please tell us about your professional and personal goals.
My goal is to conduct high-quality basic research that could have an impact on translational medicine, thereby fulfilling my dreams as both a medical doctor and a researcher.

Connecting the circuits that control memory

Thomas John McHugh

Team Leader

Laboratory for Circuit and Behavioral Physiology
RIKEN Brain Science Institute

How and when did you join RIKEN?
As a student, I participated in the first RIKEN Brain Science Institute (BSI) Summer Program in 1999 and had visited RIKEN on several other occasions. Later, as a newly independent scientist, I was looking for a job that had the funding, colleagues and infrastructure to allow me to tackle big and interesting questions. I started my lab at RIKEN in April 2009. The BSI is unique in what it has to offer and I was delighted to be given the opportunity to join it.

Please describe your role at RIKEN.
My lab looks at how circuits in the brain interact to store, organize, recall and use memories. I have a very international team of researchers including individuals from the United States, the United Kingdom, Japan, Russia, France, Switzerland, India and Taiwan. My job is to keep them motivated, happy and well supplied so that they can pursue their science.

> *The RIKEN Brain Science Institute is unique in what it has to offer and I was delighted to be given the opportunity to join it.*

How did you become interested in your current field of research?
I have been studying memory and learning since I began graduate school twenty years ago. I was interested in taking a genetic approach to solving complex biological questions. Although I had no background in neuroscience, I fell into a collaboration between two labs at the Massachusetts Institute of Technology (MIT) in the United States, which offered me the rare opportunity to learn how to both create and analyze transgenic mice. It really was a matter of being in the right place at the right time.

What made you decide to become a scientist?
My interest in genetics stems from the fact that my eldest brother, to whom I am quite close, was born with Down's syndrome. As I started to learn more about biology, I became fascinated by the impact of his genes on his development and behavior.

What is the best thing about working at RIKEN?
A common complaint among my friends with labs in the United States or Europe is that the pressures of continual grant writing and teaching leave them with little time to do the job they were actually hired to do—leading and conducting research. At RIKEN, however, I have the time to focus on the science and am still able to run my own experiments, which is fantastic.

Please tell us about your professional and personal goals.
I want to continue to do what I enjoy: coming to the lab every day to ask interesting questions with engaging and motivated people. I would like to stay in Japan for the long term because I enjoy running my lab at RIKEN and my family is very happy living here.

How do you balance family life with your work at RIKEN?
Key to addressing this challenge is an understanding partner, staying focused and setting priorities in your life. I have two young children and my daily goal is to get home before they need to go to bed so that we can get a little playtime together.

Careers at RIKEN

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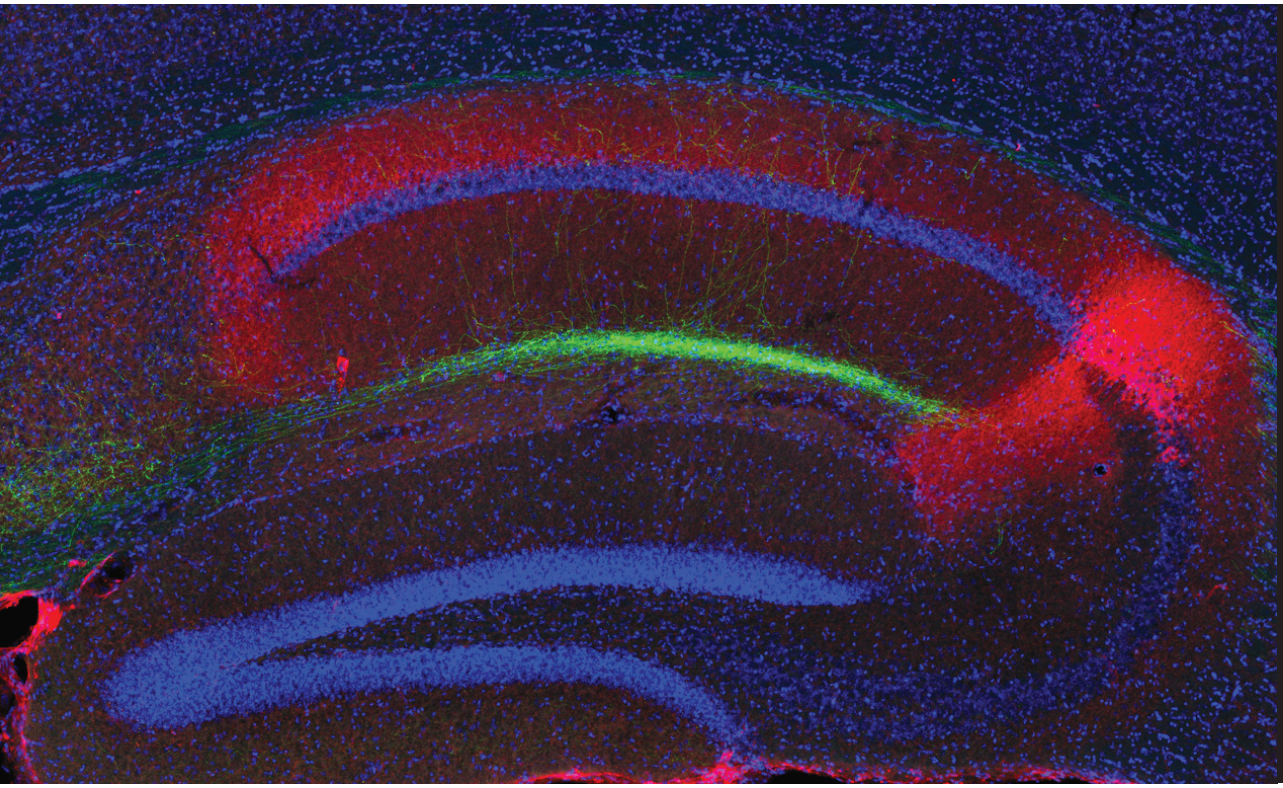


Fluorescent staining shows that island cells (green) project into the CA1 region of the hippocampus.

Island cells regulate memory

A neural network in the brain controls how associations are made between events separated in time

Memory formation requires the brain to create links between events at different points in time. These kinds of associations can be made by networks of neurons in the brain, but little is known about the networks involved. Susumu Tonegawa, Takashi Kitamura, Michele Pignatelli and colleagues from the RIKEN–MIT Center for Neural Circuit Genetics have now gained insight into how one such network is regulated[1].

Events separated in time become linked in the brain by connections between a region called the medial entorhinal cortex layer III (MECIII) and another called CA1 in the hippocampus—the brain's memory center. Tonegawa's team found that these connections are part of a neural network that is regulated by cells in the nearby entorhinal cortex layer II (ECII).

Most cells in the ECII make connections with an area of the hippocampus called the dentate gyrus. Using fluorescent staining, the researchers discovered a subset of ECII cells, called 'island cells', that make connections with CA1, the same area that connects to MECIII cells (see image).

To investigate the function of island cells, the researchers used a technique called optogenetics. This involved artificially expressing light-responsive proteins in the island cells that can activate or deactivate the cells. This allowed the team to control island cell activity using an external light source.

Tonegawa's team performed a series of experiments using mice. "We used a form of Pavlovian conditioning characterized by two steps," explains Pignatelli. "The first was a training phase in which the mouse was exposed to a tone, followed after 20 seconds by a mild electric shock. The day after, the mouse was exposed to the same tone and we measured its freezing response."

The freezing response is a sign of fear and indicates how strongly the mice associate the tone with the electric shock. Using optogenetics, the team found that they could reduce the fear response by activating island cells, or increase it by deactivating them.

The effect was opposite in MECIII cells, leading the researchers to conclude that whereas MECIII cells create the association of the tone with the electric shock, ECII cells disrupt this process.

Pignatelli says that this regulatory mechanism ensures that only useful associations are made in the brain: "In nature, you need to associate certain events with each other, but you also need to avoid the association of other sets of events. Our findings reveal for the first time the neural circuits responsible for the temporal association and dissociation of events."

Reference

1. Kitamura, T., Pignatelli, M., Suh, J., Kohara, K., Yoshiki, A., Abe, K. & Tonegawa, S. Island cells control temporal association memory. *Science* **343,** 896–901 (2014).

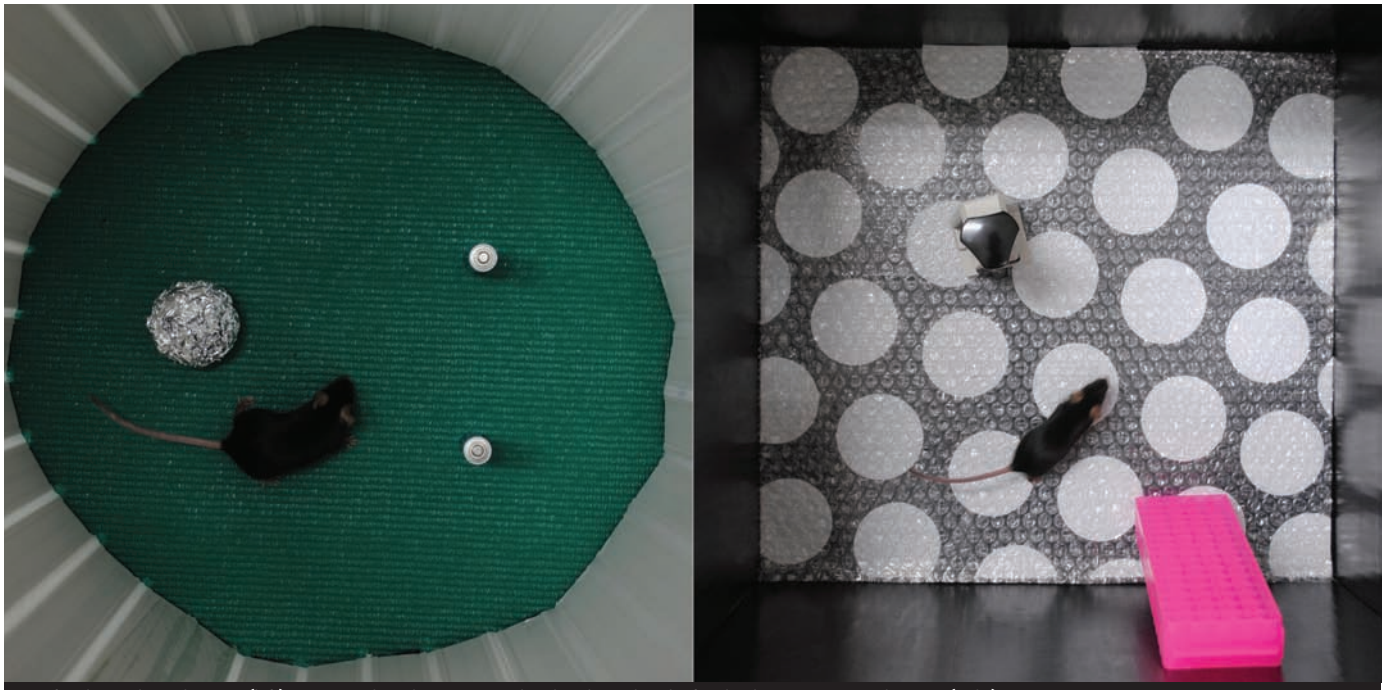
Sensing subtle differences in the environment

A region of the hippocampus in the brain helps sense differences between existing memories and new experiences

The hippocampus is an important region of the brain that encodes spatial memory. It consists of a number of subfields that have specialized functions in memory storage and retrieval, but the precise role of some of the subfields remains unclear. Thomas McHugh and colleagues from the Laboratory for Circuit and Behavioral Physiology at the RIKEN Brain Science Institute have now discovered that in mice, the CA2 subfield senses small changes in the environment that are at odds with their spatial memory[1].

McHugh and his colleagues sought to determine the role of each subfield of the hippocampus in sensing familiar and new environments through a series of mouse experiments, focusing on the often overlooked CA2 subfield. They first exposed mice to a familiar environment and then moved them back to their home cage. The researchers then either put the mice back in the first location or moved them to a new location that the mice had never experienced (see image).

The research team examined similarities and differences in the way hippocampal subfields responded to the two environments by a procedure known as catFISH—cellular compartment analysis of temporal activity by fluorescence *in situ* hybridization. This technique allows the timing of neuronal activity to be



Mice familiar with one location (left) were rested in a home cage and replaced in either the familiar location or a new location (right).

determined and permits the assessment of contextual memory by observing changes in response to environmental manipulations.

The researchers found that in most cases, there was more overlap in the response of hippocampal neurons in all subfields when the mice were replaced in the first location after their time in the home cage compared with placement in the new location. However, in mice with a mutation in the CA3 subfield that causes CA3 neuronal activity to be uncoupled from the animal's sensory environment, the change in CA2 response to a novel environment did not appear. The finding suggests that the CA3 inputs to CA2 modulate the ability of CA2 to sense novel environments.

In a final experiment, the researchers introduced more subtle changes to the environments during the second placement by taking objects from one location to the other. A distinct change in CA2 neuronal activity was found during these exposure intervals as a response to more subtle changes to the animals' environment. The CA2 subfield may therefore be the most sensitive to subtle differences between existing memories and new experiences. "In future studies, we plan to use genetic approaches to control CA2 activity in order to understand its direct effect on behavior," says McHugh.

Reference

1. Wintzer, M. E., Boehringer, R., Polygalov, D. & McHugh, T. J. The hippocampal CA2 ensemble is sensitive to contextual change. *The Journal of Neuroscience* **34,** 3056–3066 (2014).

Banding together to control movement

The motor cortex in the brain uses multiple frequency bands to coordinate movement

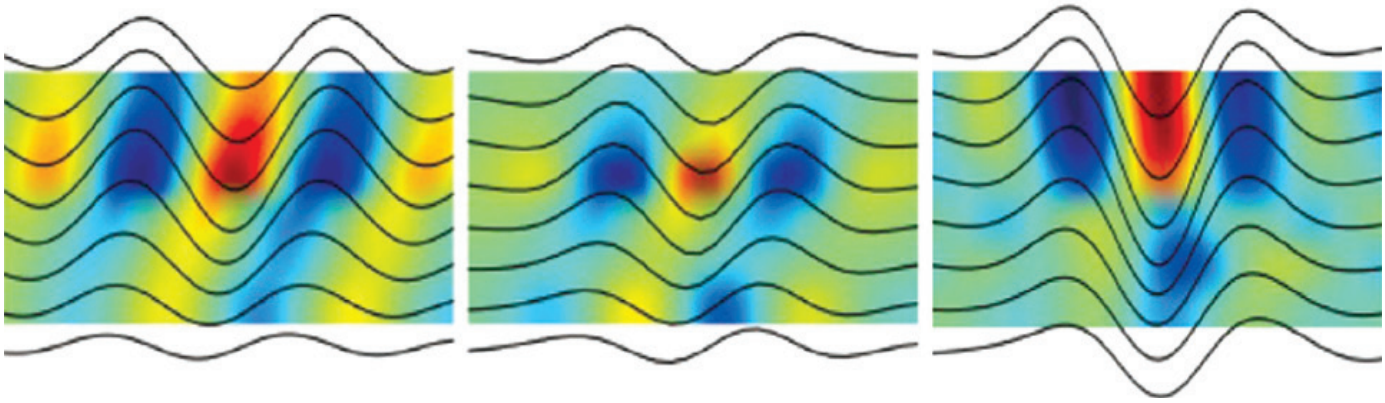
Synchrony is critical for the proper functioning of the brain. Synchronous firing of neurons within regions of the brain and synchrony between brain waves in different regions facilitate information processing, yet researchers know very little about these neural codes. Now, new research led by Tomoki Fukai of the RIKEN Brain Science Institute reveals how one region of the brain uses multiple brain-wave frequency bands to control movement[1].

Control of movement requires activation of numerous muscle groups in correct sequence, a function achieved by the motor cortex. To investigate the contribution of brain waves to this process, Fukai and his colleagues inserted multichannel electrodes into the motor cortex of rats to record brain-wave patterns as the animals learned to push, hold and then pull a lever to obtain a food reward. They also developed a machine-learning technique to extract spike sequences of individual neurons from the recorded waves.

Fukai and his colleagues found that brain waves of different frequencies appeared during distinct stages of the movements. Fast gamma waves, with frequencies of around 100 hertz, were most prominent when the rats pushed or pulled the lever, whereas slow gamma waves, with frequencies of 25–40 hertz, peaked when the rats held the lever to prepare for the next pull. Theta waves (4–10 hertz) peaked while the rats held the lever, and the initiation of the pulling movement coincided with a specific phase of these oscillations (see image).

Both frequencies of gamma waves were coupled to the theta waves such that the peaks of all three brain-wave frequencies occurred at the same time. The activity of different types of nerve cells in different layers of the motor cortex was also synchronized with specific brain-wave frequencies. Importantly, cells encoding different stages of the sequential movements fired in distinct phases of the theta waves.

The results suggest that theta waves play an important role in coordinating the



Typical slow gamma (left), fast gamma (center) and theta (right) brain-wave patterns measured during voluntary actions in rats.

neuronal activity underlying the planning and execution of voluntary movement. Theta waves are known to be important for the processing of spatial information in the hippocampus, but this is the first time that a similar code has been observed in the motor cortex.

"We are currently using machine-learning techniques to study how phase-locked spikes in different layers of the motor cortex encode motor information," says Fukai. "We are also studying whether a similar oscillatory coordination takes place in the prefrontal cortex during decision-making."

Reference

1. Igarashi, J., Isomura, Y., Arai, K., Harukuni, R. & Fukai, T. A θ–γ oscillation code for neuronal coordination during motor behavior. *The Journal of Neuroscience* **33,** 18515–18530 (2013).

Faster cell mixing leads to larger plants

The speed at which the contents of plant cells are mixed is closely linked to plant size

The contents of plant cells undergo continuous mixing by a process known as cytoplasmic streaming. Yet although this process was discovered more than 200 years ago, its exact purpose has remained unclear. Motoki Tominaga from the Live Cell Molecular Imaging Research Team at the RIKEN Center for Advanced Photonics and colleagues have now shown that the speed of cytoplasmic streaming is a key factor in determining plant size[1].

Cytoplasmic streaming is a 'stirring' mechanism thought to ensure that nutrients and other molecules are evenly distributed throughout the cell. The movement is generated by a protein called myosin XI, which has a motor domain that allows it to slide along actin filaments in the cell's skeleton.

"It has been shown that the genetic knockout of myosin XI proteins leads to growth defects in plants," explains Tominaga. "While this raises the possibility that plant cell size is related to cytoplasmic streaming, it is difficult to elucidate the link using conventional methods."

To investigate, Tominaga and his colleagues had to devise a new approach. Rather than knocking out myosin XI, they changed the speed of its movement in thale cress, *Arabidopsis thaliana*, by swapping the motor domain for either a faster version from an alga called *Chara corallina* or a slower version from humans.

Introducing the faster motor domain increased the speed of cytoplasmic streaming and led to larger than normal plants with bigger leaves and greater height and weight. Correspondingly, the slower human motor domain resulted in the opposite—slower cytoplasmic streaming and smaller than normal plants (see image). The differences in plant size were found to be due to differences in the sizes of cells rather than their number. The researchers' results indicate that faster cytoplasmic streaming allows plant cells to grow larger, making it a key factor in plant size.

Tominaga says these findings not only demonstrate the function of cytoplasmic streaming for the first time, but could also provide a new way of controlling plant size so that plants may overcome the constraints of their environments.

"Many attempts have been made to enhance plant size for crops, but if done artificially, physical stimuli such as wind and rain can

Thale cress plants with different myosin XI motor domains. Compared with the wild-type plant (left), plants with fast myosin XI are larger (middle) and plants with slow myosin XI are smaller (right).

cause the taller plants to fall," says Tominaga. "Our system may solve this problem. By expressing slower myosin XI at the shoot and faster myosin XI in other tissues like the leaf and root, we could develop plants that have high resistance to wind and rain but increased biomass."

Reference

1. Tominaga, M., Kimura, A., Yokota, E., Haraguchi, T., Shimmen, T., Yamamoto, K., Nakano, A. & Ito, K. Cytoplasmic streaming velocity as a plant size determinant. *Developmental Cell* **27,** 345–352 (2013).

Many ways to grow

Plant hormones with signaling pathways that seemingly overlap play distinct roles in controlling growth

Cytokinins are plant hormones that help to coordinate plant growth and development in response to different environmental triggers. They primarily differ from one another in their 'side-chains'—chemical modifications that are tacked on by specialized enzymes. Research from Hitoshi Sakakibara and co-workers at the RIKEN Center for Sustainable Resource Science now demonstrates clear differences in the functions of these various hormones[1].

Compared to a normal plant (left), thale cress plants lacking the capacity to produce the cytokinin tZ (right) exhibit poor shoot growth and smaller rings of leaves.

The thale cress plant, *Arabidopsis thaliana*, makes heavy use of two particular cytokinins, N^6-(Δ^2-isopentenyl)adenine (iP) and *trans*-zeatin (tZ), which act on the same three receptor proteins. This raises the question of whether they have meaningfully different effects on plant growth. "Although there is this side-chain diversity in natural cytokinins, it has been an open question whether or not the diversity is biologically relevant," explains Takatoshi Kiba, a research scientist in Sakakibara's group.

To resolve this question, the researchers generated genetically modified plants that lacked the capacity to manufacture tZ. The cytokinin tZ is produced from a precursor of iP, which undergoes processing by the cytochrome P450 monooxygenase enzyme CYP735A. Accordingly, plants lacking CYP735A showed dramatically reduced tZ levels, with a compensatory increase in iP production. The absence of CYP735A also had a striking effect on growth, resulting in stunted shoots crowned with narrower rings of leaves (see image). Spraying with tZ restored normal shoot growth to the mutant plants, whereas treatment with iP did not. Interestingly, tZ appeared to play a minimal role in root development, although the hormone can be produced in plant roots and trafficked throughout the plant.

Kiba and his colleagues subsequently generated tZ-deficient plant strains that also lacked various subsets of the three cytokinin receptors. Two of these receptors bind to both iP and tZ equally well, and the researchers anticipated that further deficits in shoot growth might be mitigated in plants retaining either of these receptors due to the elevated iP levels. Instead, the growth defects were even more pronounced than in plants lacking tZ alone, demonstrating that the cytokinins are not interchangeable.

The results suggest that a selective boost in tZ cytokinin activity could be a winning strategy for crop improvement. "Increasing the proportion of tZ by overexpressing CYP735A results in shoot-growth enhancement without reducing root growth," says Kiba. In future work, the Sakakibara lab will delve deeper into the mechanisms that allow plant cells to differentiate tZ and iP signals, as well as the systems that help to shuttle the hormones throughout the plant.

Reference

1. Kiba, T., Takei, K., Kojima, M. & Sakakibara, H. Side-chain modification of cytokinins controls shoot growth in *Arabidopsis*. *Developmental Cell* **27,** 452–461 (2013).

A disruptive genetic presence in the brain

The brains of schizophrenia sufferers display increased numbers of self-replicating genomic elements

More than 40 per cent of the human genome consists of self-replicating genetic elements known as retrotransposons. Most of these repeated elements are evolutionarily conserved in the genome and are no longer able to replicate. Some elements, however, such as a part of long interspersed nuclear element-1 (LINE1), retain the ability to insert themselves throughout the genome and disrupt gene function. Tadafumi Kato and colleagues from the RIKEN Brain Science Institute, in collaboration with Kazuya Iwamoto of the University of Tokyo and researchers from other institutes in Japan, now have findings that suggest a link between such insertions and schizophrenia[1].

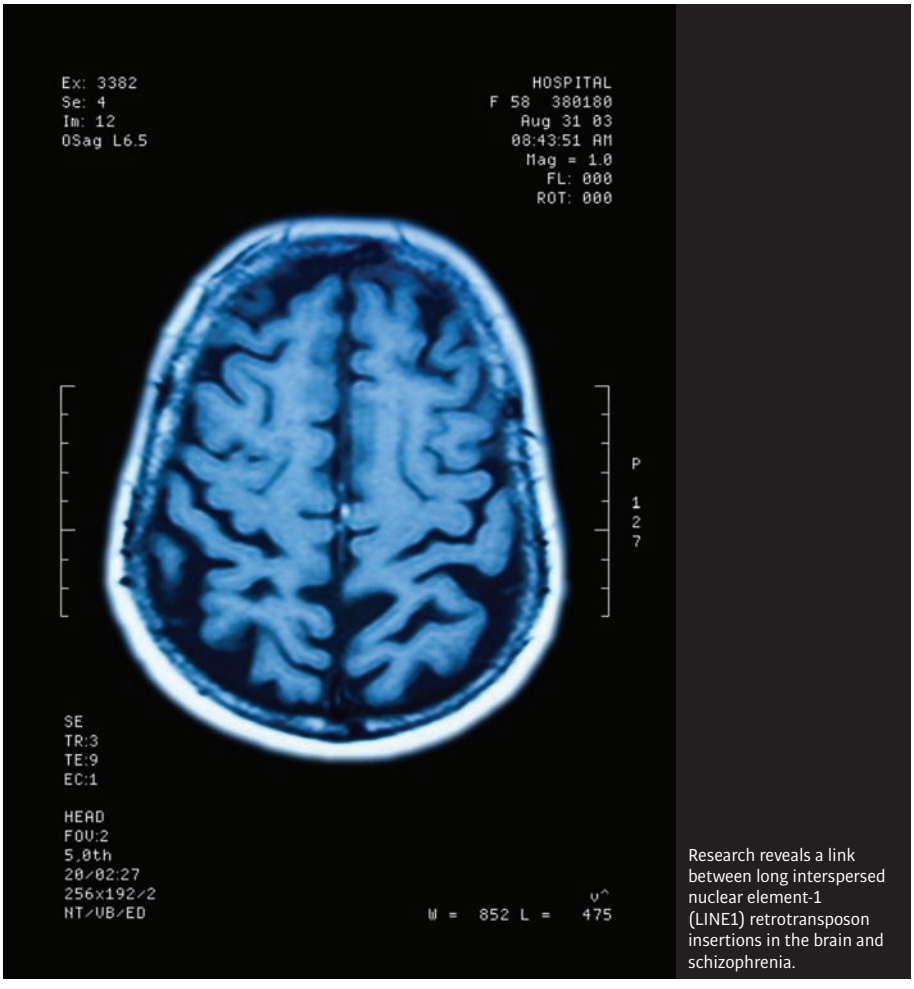
For decades, scientists have struggled to identify genetic elements that contribute to this illness. "Many weak hereditary risk factors have been identified, but their effect is small," says Kato. "Recent studies, however, have also shown a role for new mutations that are not present in parents." One such study uncovered evidence of a link between elevated LINE1 insertion in the brain and the neurodevelopmental disorder Rett syndrome, suggesting a new direction to explore. This led Kato and Iwamoto to profile the number of copies of LINE1 present in postmortem tissue samples from patients with different mental disorders.

Their analysis revealed a notable increase in LINE1 copy number in brain tissue from schizophrenic patients. As conditions during gestation and early infancy can increase the probability of developing schizophrenia, the researchers examined brain LINE1 content in animal models of this disease that simulate the impact of risk factors like prenatal infection. Both monkey and mouse models showed increased LINE1 levels relative to control animals. "The results indicate the possibility of environmental factors affecting DNA in the brain and thus modifying this disease," says Kato. Stem-cell-derived neurons with a missing genomic segment known to be linked to schizophrenia also exhibited a consistent increase in LINE1 copies, indicating that LINE1 activity is also affected by genetic determinants.

Although the frequency of LINE1 insertion into gene-coding regions was similar between patients and healthy controls, the researchers observed striking differences in their distribution. The genes affected in schizophrenics were significantly more likely to be involved in synaptic communication or other neuronal functions than those in the control group. The finding suggests that symptoms of schizophrenia may be exacerbated by genetic and environmental factors that activate LINE1 and thereby disrupt such genes, although more research will be needed to confirm these findings. "Our research has paved the way for basic research using animal models or patient-derived stem cells," Kato notes.

Reference

1. Bundo, M., Toyoshima, M., Okada, Y., Akamatsu, W., Ueda, J., Nemoto-Miyauchi, T., Sunaga, F., Toritsuka, M., Ikawa, D., Kakita, A. *et al.* Increased L1 retrotransposition in the neuronal genome in schizophrenia. *Neuron* **81,** 306–313 (2014).



Research reveals a link between long interspersed nuclear element-1 (LINE1) retrotransposon insertions in the brain and schizophrenia.

Biology

Making sense of the cellular rulebook

Atlases of functionality for specific genomic elements across a diverse range of cell types reveal insights into how different tissues establish their biological and functional identities

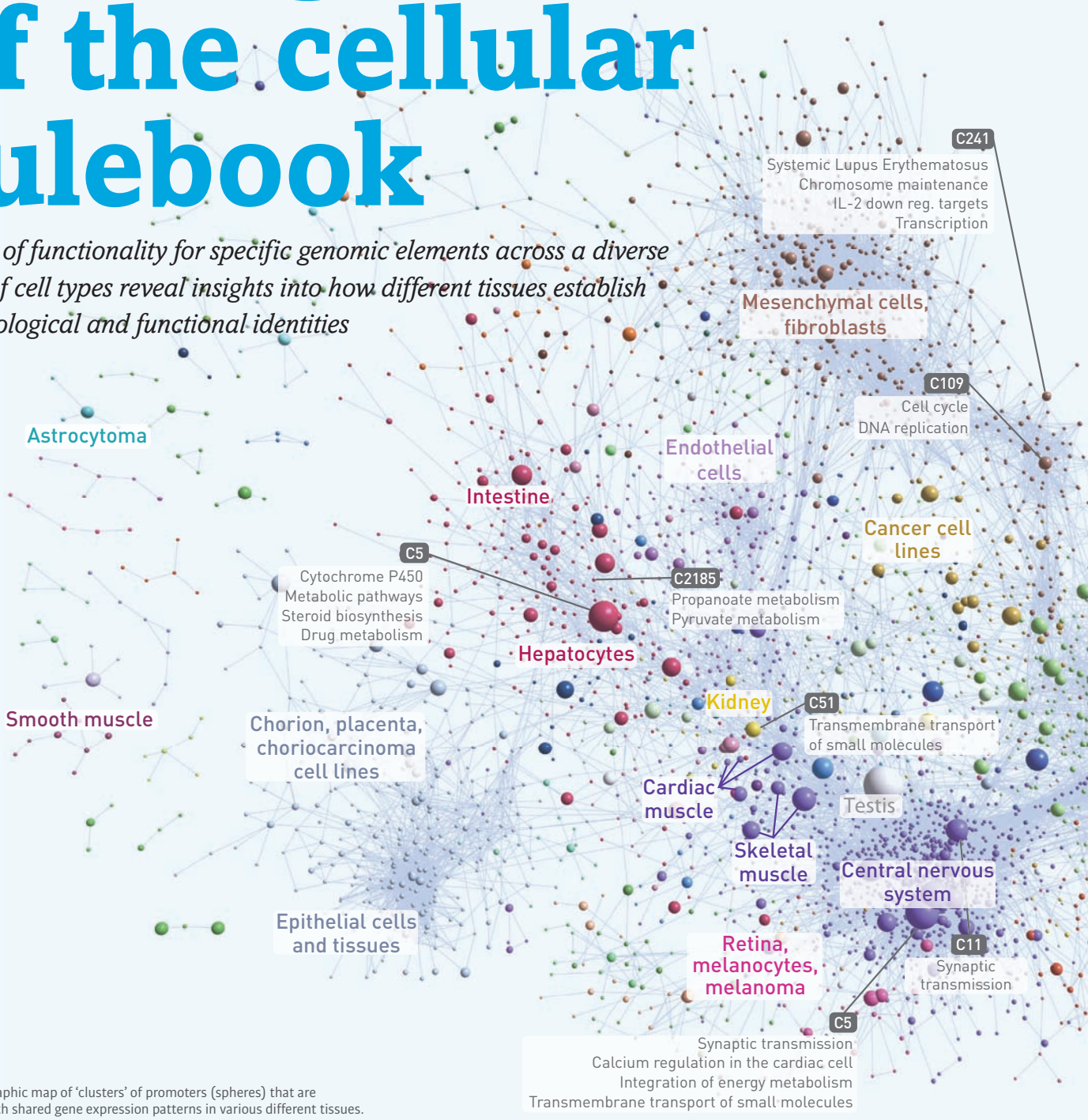
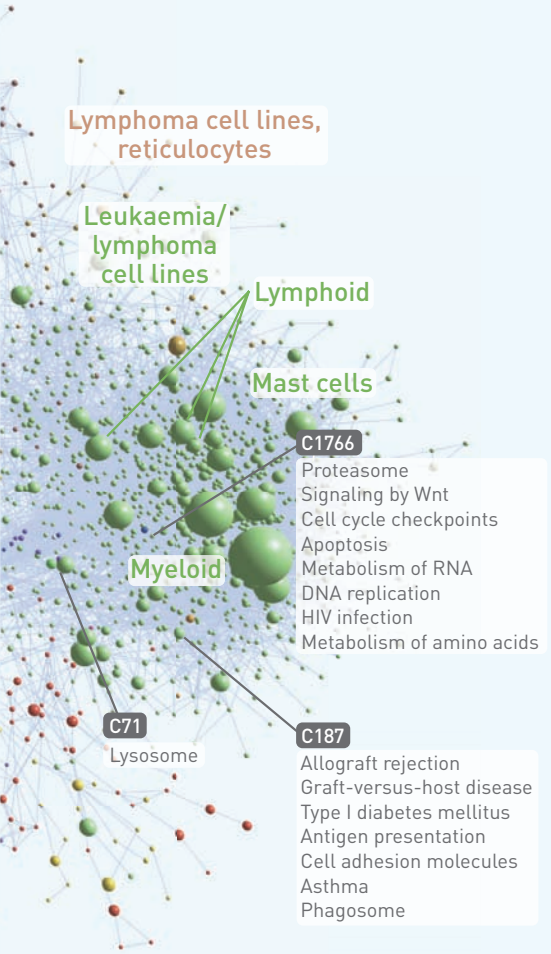


Figure 1: A graphic map of 'clusters' of promoters (spheres) that are associated with shared gene expression patterns in various different tissues.



The cell is an immensely complex biological system involving a multitude of components that work together to drive the cellular machine. Identifying how all of the components fit together in any given cell type is a challenge in itself—integrating the pieces into a functional whole across a wide variety of cell types is an undertaking on a different scale entirely. Yet this is the ambitious goal of the international FANTOM5 consortium, led by Alistair Forrest, Piero Carninci and colleagues from the RIKEN Center for Life Science Technologies and Yoshihide Hayashizaki from the RIKEN Preventive Medicine & Diagnosis Innovation Program, which has made important progress in assembling a functional blueprint for the myriad genomic elements that control gene expression across hundreds of different mammalian cell types[1,2].

Nearly 15 years ago, the first iteration of the Functional Annotation of the Mammalian Genome (FANTOM) project set out to identify every gene that undergoes active transcription to produce an RNA message. The recent fourth-generation FANTOM4 consortium combined experimental techniques and computational tools to identify the interactions between various transcription factor proteins and the promoter DNA sequences that regulate gene function. As a proof of concept, the consortium examined how regulatory pathways interact to drive maturation in a class of immune cells known as monocytes. "Through integration of binding site predictions and expression levels of RNA transcripts, we were able to predict key changes in transcription factor activities during differentiation," says Forrest.

The FANTOM5 consortium is expanding this analysis to a far greater scale, encompassing roughly 200 different cell types derived from human and mouse tissue samples. "The aim is to build transcriptional regulatory network models for the majority of mammalian cell types," says Forrest.

Seeking similarities and differences

A research effort of this magnitude—involving many thousands of samples, prepared and examined by 260 scientists in 20 countries—requires powerful analytical tools. Forrest's team designed a computational platform called ZENBU to simplify collaborative analysis of such large volumes of experimental data. Although the FANTOM5 project also examined numerous cultured cell lines, the primary focus was on cells isolated from human donor tissue, requiring standardized workflows for analyzing tiny amounts of RNA from small numbers of cells without introducing biases that might skew the data.

The researchers employed a variant of cap analysis of gene expression (CAGE), a technique developed by RIKEN scientists as a means to home in on active genes by sequencing the beginnings of RNA transcripts. Using ZENBU and other tools to map these sequences back to the genome, the researchers identified peaks

FANTOM5
FUNCTIONAL ANNOTATION OF THE MAMMALIAN GENOME

Functional Annotation of the Mammalian Genome

FANTOM is an international research consortium established in 2000 by Yoshihide Hayashizaki and his colleagues to assign functional annotations to the full-length complementary DNAs that were collected during the RIKEN Mouse Encyclopedia Project. FANTOM has since developed and expanded over time to encompass the fields of transcriptome analysis. The project is moving steadily up the layers in the system of life, progressing from an understanding of the 'elements'—the transcripts—to the 'system' of an individual life form—the transcriptional regulatory network. Representing the latest phase of the FANTOM project, FANTOM5 expands on previous efforts, with the aim of generating both a map of the majority of human promoters and comparative transcriptional regulatory network models of each cellular state.

Figure 2: A group photo of scientists from the FANTOM5 consortium at a meeting in the winter of 2011.

of activity representing likely transcription start sites (TSSs) for nearly 94% of the known human genes.

Many promoters were associated with multiple TSSs that exhibited different activity levels in different cell types. More generally, the vast majority (80%) of human TSSs showed strong tissue specificity, exhibiting activity in fewer than half of the various cell types profiled (Fig. 1). "Mammalian promoters are often complex entities consisting of tightly packed independent transcription initiation regions with different cell-type-specific preferences," says Forrest. The researchers were able to identify different combinations of transcription factors that manage this specificity at various promoters.

Promoters are generally close to the TSS, but transcription is also modulated by 'enhancer' sequences that can be relatively distant. Interestingly, many enhancer sequences give rise to short RNAs of unknown function, called enhancer RNAs (eRNAs), which made it possible for the FANTOM5 consortium to profile these genomic elements with CAGE. FANTOM5 collaborators Albin Sandelin from the University of Copenhagen in Denmark and Michael Rehli from the University Hospital Regensburg in Germany spearheaded this analysis with Forrest and Carninci. The results correlated closely with other known predictors of enhancer location. As eRNA production appears to be primarily restricted to active enhancers, the FANTOM5 group was able to identify large numbers of tissue-specific enhancers as well as a small but notable subset that acts broadly across cell types.

Part of a bigger picture

These studies represent only the first round of data from the FANTOM5 project (Fig. 2), but the clinical possibilities are already tantalizing. For example, preliminary analysis suggests that numerous genetic variations that have been linked to human disease but lie outside of known gene-coding regions may instead affect enhancers characterized by FANTOM5.

"One enhancer variant associated with diabetes led to a 50% reduction in enhancer activity, while another associated with Crohn's disease led to a 10% reduction in enhancer activity," says Forrest. Such insights could help scientists assign definite functions to the many enigmatic mutations routinely uncovered in clinical genomics research.

The next stage should yield even greater biomedical dividends. Building on the present work, which focuses on cells 'at rest', the consortium is now investigating shifts in gene activity associated with normal biological processes such as growth and development as well as external triggers such as infection. "We are looking at the series of events that happen as a cell transitions from one stable state to another at the level of promoters, enhancers and transcription factors," says Forrest. A spin-off project from FANTOM5 aims to conduct a similar analysis for cancer in an effort to identify perturbations in gene expression networks that contribute to tumor formation and growth.

More generally, the outputs of the FANTOM5 project will also be used to bolster and extend the utility of data from other large-scale research efforts, such as the detailed genomic map produced by the Encyclopedia of DNA Elements (ENCODE) consortium based in the United States. "This integration is already happening," says Forrest, "and I think that the FANTOM5 dataset will be used as a reference expression atlas for years to come."

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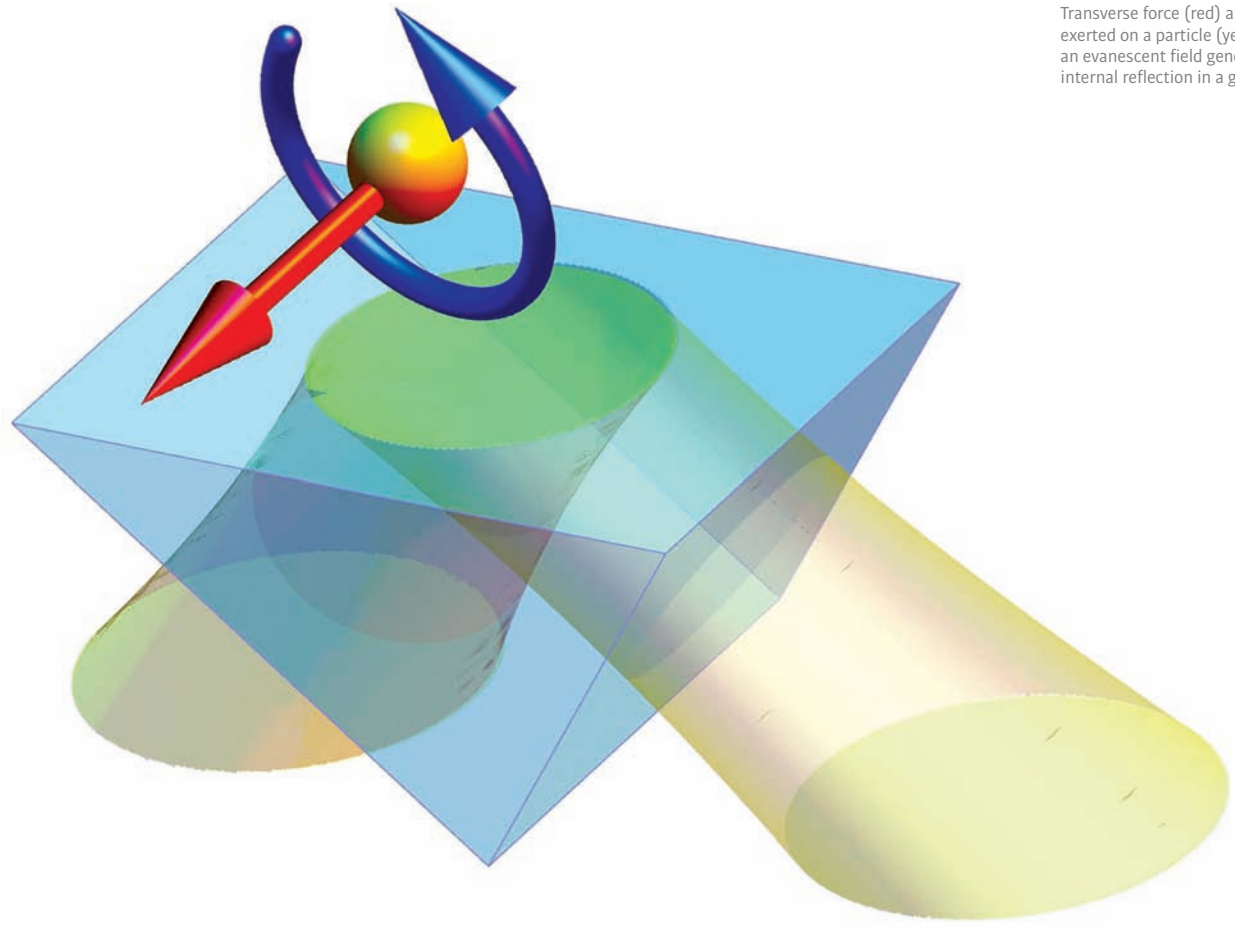

Transverse force (red) and torque (blue) exerted on a particle (yellow sphere) in an evanescent field generated by total internal reflection in a glass prism.

A new twist in the properties of light

Evanescent electromagnetic waves are found to have very different fundamental dynamical properties to those of normal light

Light has some well-established dynamical properties that have defined our understanding of electromagnetic radiation for over a century. Two of the most fundamental of these properties are that photons of light carry momentum in the direction of propagation, and a 'spin' about the propagation axis defined by the electromagnetic wave's circular polarization. These properties play critical roles in a range of everyday phenomena and experimental interactions between light and matter.

Konstantin Bliokh from the RIKEN Interdisciplinary Theoretical Science Research Group (iTHES) and Aleksandr Bekshaev and Franco Nori from the RIKEN Center for Emergent Matter Science have now made the remarkable discovery that a particular type of light known as evanescent waves possesses unexpected dynamical properties that are in sharp contrast with previous knowledge about light and photons[1].

Evanescent waves are produced, for example, when light undergoes total internal reflection at a boundary with another medium. In such situations, the main electromagnetic wave is reflected back into the originating medium and an evanescent wave is produced in the second medium. The evanescent wave decays rapidly away from the boundary but can propagate along the interface.

By investigating the dynamic characteristics of evanescent waves, Nori's team discovered that the momentum and spin of these waves have transverse components that are oriented at right angles to the plane of propagation. Equally surprising, they also found that the transverse momentum, and not the transverse spin, is determined by the wave's circular polarization—precisely the opposite to the dependence seen in normal light.

"Although these extraordinary properties seem to be in contradiction with what is known about photons," explains Bliokh, "we have shown that they reveal what is known as 'spin momentum'—an enigmatic quantity that was introduced more than 70 years ago to explain the spin of quantum particles."

The research team's analysis suggests that these extraordinary properties of evanescent waves do in fact manifest in light–matter interactions, potentially leading to effects that are impossible to achieve and observe using normal light. For example, evanescent waves exert a transverse force and a transverse torque on small particles, where the force is dependent on the circular polarization but the torque is not (see image).

"Such remarkable properties, revealed in very basic objects, offer a unique opportunity to investigate and observe fundamental physical features that were previously hidden in usual propagating light and were considered impossible," concludes Bliokh.

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A quick chill releases magnetic frustration

The delicate interplay between electronic properties and crystal structure explains how 'frustrated' magnets escape magnetic deadlock at low temperatures

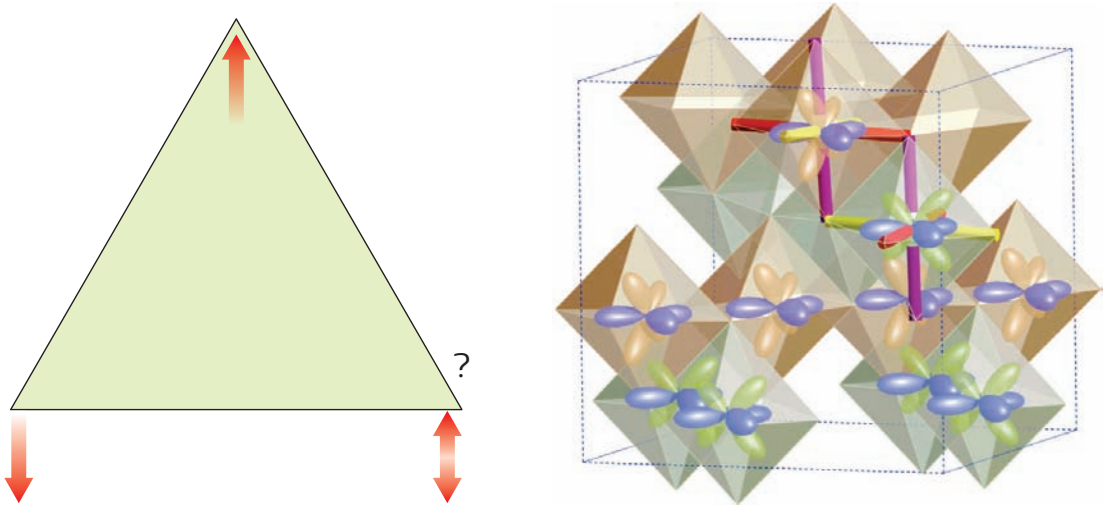
Magnetism in a material arises from how its electrons behave, which is influenced by the material's structure and the way that atoms and magnetic 'spins' of electrons are ordered within it. Frustrated magnets are a special type of magnet in which the crystal structure prevents the most energetically favorable arrangement of magnetic spins from being achieved, resulting in a magnet that is deadlocked in an unfavorable state.

Seiji Niitaka from the RIKEN Low Temperature Physics Laboratory, Hidenori Takagi from the RIKEN Magnetic Materials Laboratory and colleagues from other RIKEN centers and Japanese institutions have now discovered how small changes in crystal structure can help such magnets release their frustration[1].

The research team investigated the magnesium vanadium oxide compound MgV_2O_4. The magnetic vanadium ions in this material form a three-dimensional network consisting of a regular triangular unit with intrinsic geometrical spin frustration (see image). However, at very low temperatures, this compound shows strikingly simple magnetic ordering with significantly less frustration. The ordering of magnetic spins follows the temperature-related structural phase transition of the atoms, suggesting that the crystal structure and magnetic properties of MgV_2O_4 are linked.

Studying the material's atomic arrangement with high precision required a combination of careful sample preparation and highly precise measurement techniques, explains Niitaka. "We were able to study the crystal structure only at the RIKEN SPring-8 Center, which has x-rays of high brightness and a high-performance camera." Importantly, Niitaka, Takagi and their colleagues also succeeded for the first time in growing single crystals of MgV_2O_4 at sufficiently high quality for such experiments.

The team's x-ray investigation revealed that the atomic bonds between vanadium and oxygen atoms on the crystallographic plane are of different lengths, and that in the low-frustration state the orientation of these long and short bonds alternates between adjacent layers. This distortion is attributed to variations in



Geometric frustration in the triangular structural unit of the magnesium vanadium oxide compound MgV_2O_4 (left). Crystal structure of MgV_2O_4, showing the variation in electron states between adjacent layers (right).

the electron state between layers, which influences spin interactions. As a consequence, the deadlock of magnetic spins is lifted and the material can escape frustration.

The discovery of the role of electron orbitals in this process could be important for understanding not only frustrated magnetic materials, but also other materials characterized by strong interactions between electronic and structural properties. "This interplay between the different degrees of freedom of electrons in a material is one of the intriguing behaviors leading to rich physics in materials," says Niitaka.

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The debut of the antihydrogen beam

The first production of a stable beam of antihydrogen atoms will help researchers in the hunt for fundamental differences between matter and antimatter

The standard model of particle physics suggests that matter and antimatter are equal and opposite in every way. Yet the observable Universe is made almost entirely of matter—an asymmetry that remains one of the greatest unsolved mysteries in physics.

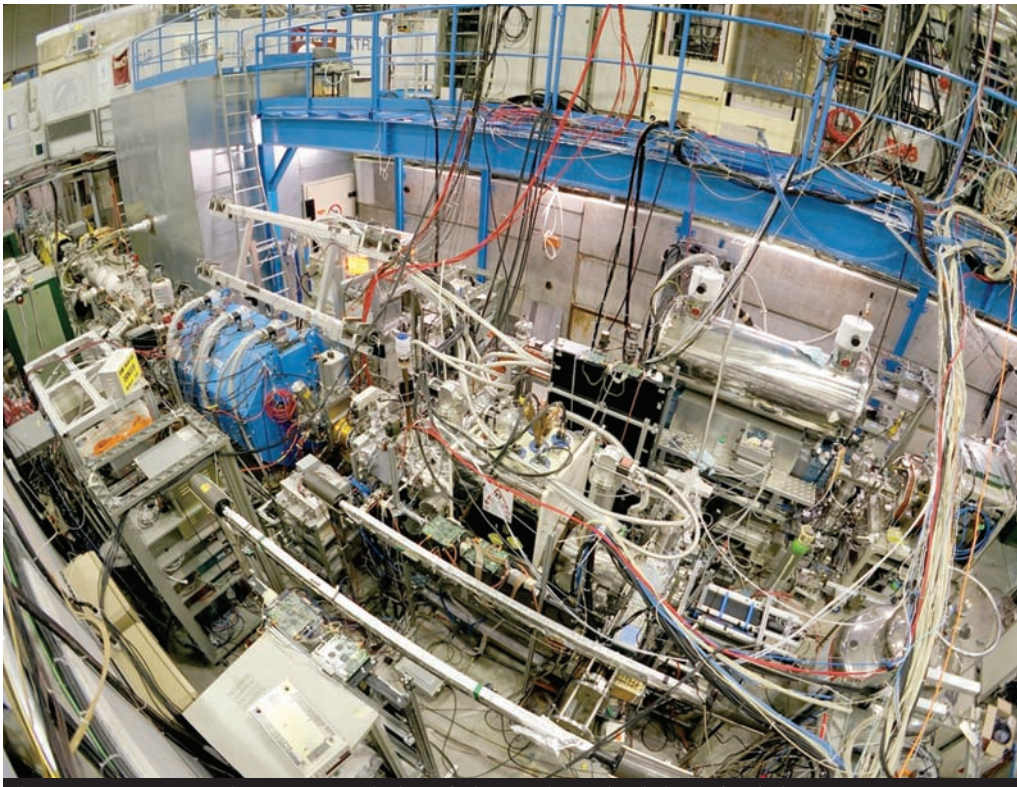
In an advance that could make it possible to search for tiny differences between matter and antimatter and help to explain their imbalance in the cosmos, Yasunori Yamazaki and colleagues from the RIKEN Atomic Physics Laboratory, in collaboration with researchers from around the world, have now succeeded in creating a stable beam of antihydrogen atoms[1].

Hydrogen contains a positively charged proton and a negatively charged electron. The electron can occupy two slightly different ground states depending on how its spin aligns with the proton's spin—a distinction known as hyperfine splitting.

Antihydrogen, on the other hand, consists of a negatively charged antiproton and a positively charged antielectron —or positron. One of the core tenets of the standard model—charge–parity–time (CPT) symmetry—dictates that antihydrogen should exhibit exactly the same hyperfine splitting as hydrogen. "If it is different," says Yamazaki, "we can immediately conclude that CPT symmetry is violated, and the standard model should be replaced by some other theory."

Measuring the hyperfine splitting of antihydrogen is extremely difficult. Since antimatter is instantly annihilated when it comes into contact with ordinary matter, researchers trap it using magnetic fields. However, magnetic fields can alter the energy of the hyperfine transition, making the measurement less precise. To avoid this, Yamazaki and his co-workers involved in the ASACUSA (Atomic Spectroscopy and Collisions Using Slow Antiprotons) experiment at the CERN (European Organization for Nuclear Research) particle physics facility instead produced a beam of antihydrogen atoms that can be detected at a distance, away from any interfering magnetic fields.

The antihydrogen beam is produced by mixing antiprotons and positrons in a special alignment of magnetic and electric fields known as a cusp trap. The antihydrogen atoms escape the trap as a beam and can be detected 2.7 meters away. Lower-energy antihydrogen atoms will be needed, however, to measure hyperfine splitting. "Our next goal



The ASACUSA experiment at the European particle physics facility CERN has produced a beam of antihydrogen.

is to produce a 'cold' beam of antihydrogen atoms in the ground state," says Yamazaki.

The test for hyperfine splitting will involve exciting the antihydrogen atoms using 1.4 gigahertz radiowaves, which can cause the spontaneous change from one spin alignment to the other. The research team is hopeful that these groundbreaking tests could be run by the end of 2014.

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Capturing a fleeting starburst

The MAXI instrument aboard the International Space Station gets a ringside seat as a white dwarf undergoes a spectacular and short-lived nova explosion

On 11 November 2011, astronomers witnessed a distant star erupt in an incredibly powerful explosion. An international research team including Mikio Morii and colleagues from the MAXI Team at the RIKEN Global Research Cluster has now reconstructed the event from a handful of telescopic snapshots, revealing for the first time the runaway fusion reaction that triggered the blast[1].

The star, known as MAXI J0158–744, is a white dwarf—a very old and dense star, exhausted of hydrogen and roughly the size of the Earth yet as heavy as our Sun. MAXI J0158–744 is partnered with a much younger, larger star, which it had been voraciously 'feeding' off by sucking hydrogen and helium to resurrect fusion reactions in a fresh layer on its surface. The renewed fusion reactions proved to be unstable and sparked a nova explosion—a massive outburst that blasted the freshly acquired material into space.

Low-energy x-rays from the blast were first detected by the MAXI instrument aboard the International Space Station (see image). Roughly four minutes later, the nova's light reached a climax. "The peak brightness of MAXI J0158–744 was 5 million times greater than that of our Sun's normal emission," says Morii. "It was the first observation of the initial fireball phase of a nova."

Some 22 minutes after the explosion, MAXI detected that the sudden ejection of matter had peaked and that the star's glowing halo was beginning to shrink. The MAXI team alerted the orbiting Swift x-ray telescope, which swung into action. Less than 11 hours after the initial observation, Swift confirmed the

The white dwarf MAXI J0158–744 (left) underwent a nova explosion fueled by material drawn from its neighboring star (right). X-rays from the powerful nova explosion (inset) were detected by the MAXI instrument.

location of the white dwarf and reported that it was emitting low-energy x-rays as it burned off the remaining hydrogen and helium.

The whole process happened more quickly than is typical of novae. Yet it was also much fainter, implying that relatively little mass was ejected in the explosion. The team's findings suggest that the white dwarf was unusually massive, giving it a higher surface gravity that put the accumulated material under even greater pressure. This meant that less fuel was needed to trigger the explosion and a briefer nova with less ejecta was produced.

The researchers are now hunting for other examples of nova ignition. "We have already started the search for similar transient x-ray emissions using MAXI data from more than four years of observations," says Morii. His team is also preparing a successor to MAXI that will seek out novae across a much wider field of view. ■

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Magnetic twins are more efficient

Pairs of nanoscale magnetic vortices could ultimately lead to more efficient memory devices

The efficient control of magnetization at nanometer scales holds much potential for the next generation of high-density data storage. One promising approach is the use of skyrmions—nanoscale vortices in the magnetic moment, or 'spin', of a material. Xiuzhen Yu and colleagues from the RIKEN Center for Emergent Matter Science and the Japanese National Institute for Materials Science have now reported the first experimental observation of bound pairs of skyrmions[1].

Skyrmions are attractive because they are very small and can be made to 'flow' and move by the application of electrical current at current densities well below those needed to manipulate domain walls in conventional ferromagnetic materials. This means they could be used to create memory devices that operate at much lower power and at higher data density than conventional magnetic systems.

It has been theoretically predicted that skyrmions could be found in bound pairs called biskyrmions. The two bound skyrmions are predicted to have oppositely oriented spin vortices and the biskyrmion to have a topological charge of two—twice that of a single skyrmion.

Using Lorentz transmission electron microscopy, Yu's team successfully observed biskyrmions in a thin layered sheet of manganese oxide containing lanthanum and strontium at very low temperature (20 kelvin) under an applied magnetic field (see image). The team was also able to make the biskyrmions move by exciting the material electrically at current densities of around 107 amperes per square meter— a thousand times smaller than that typically needed for conventional magnetic manipulations.

"We have realized such theoretically anticipated biskyrmions under a magnetic field and have also found that they can be driven with a current density three orders of magnitude lower than that needed to drive ferromagnetic domain walls," says Yu. The result is expected to lead to the development of novel low-power, high-density magnetic memories.

The researchers found that the physical size of the biskyrmions depends on the thickness of the magnetic material and the strength of the applied external magnetic field. Importantly, the biskyrmions appear to be relatively stable, although they disappear at temperatures higher than 50 kelvin and under magnetic fields stronger than 0.4 tesla.

In future work, Yu says that the difference in the behavior between skyrmions and biskyrmions needs to be explored in more detail. "The doubled topological charge in the biskyrmion may cause a difference in the dynamics and also modify the skyrmion transport properties compared to the case of the single skyrmion." ■

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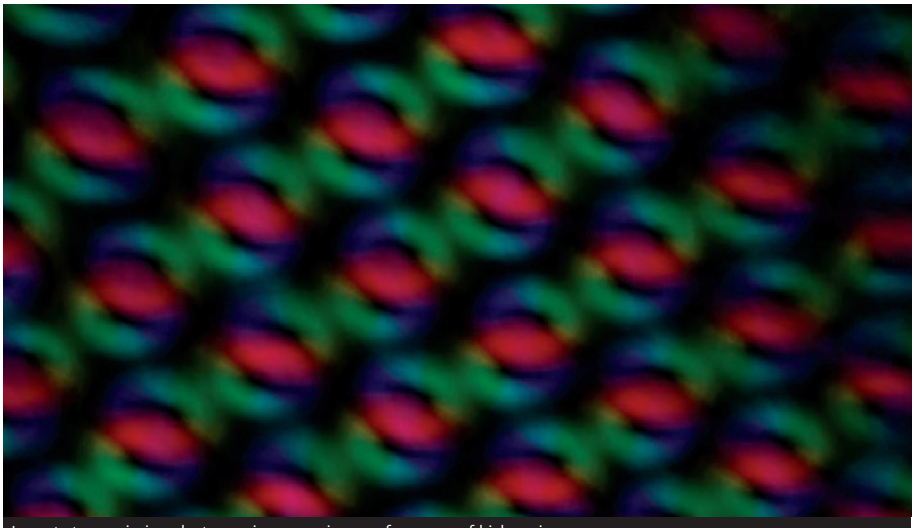

Lorentz transmission electron microscopy image of an array of biskyrmions.

RIKEN Quantitative Biology Center

Mimicking living systems

The Quantitative Biology Center is measuring, modeling and designing living systems to determine the fundamental rules that govern their behavior

Contact information
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Life can be viewed through a paradoxical lens as a large number of nonliving elements cooperating in a network. The rules that govern these networks are perhaps science's greatest mysteries. Advances in high-resolution imaging, in which Japan is a global leader, have significantly improved our understanding of biological systems. The RIKEN Quantitative Biology Center (QBiC) was established in 2011 to enhance existing technologies and apply them to the most fundamental living unit—the cell—to identify the principles that regulate molecular and cellular networks.

As one of RIKEN's newest centers, QBiC was envisioned by director and renowned biophysicist Toshio Yanagida to have both an interdisciplinary research and education remit. Mathematicians, biologists and engineers at QBiC work together in 20 laboratories spread across Osaka, Kobe and Yokohama, with easy access to some of RIKEN's award-winning facilities such as the K computer and SACLA (SPring-8 Angstrom Compact free-electron Laser).

A calculated look

QBiC focuses on three core areas of research: measuring intracellular molecular dynamics, modeling cell behavior and designing artificial systems that recreate biological phenomena. Through close collaboration, QBiC's Cell Dynamics, Computational Dynamics and Cell Design research teams are developing a holistic understanding of the cell—knowledge that will ultimately contribute to advances in drug discovery and the engineering of energy-efficient technologies, among others.

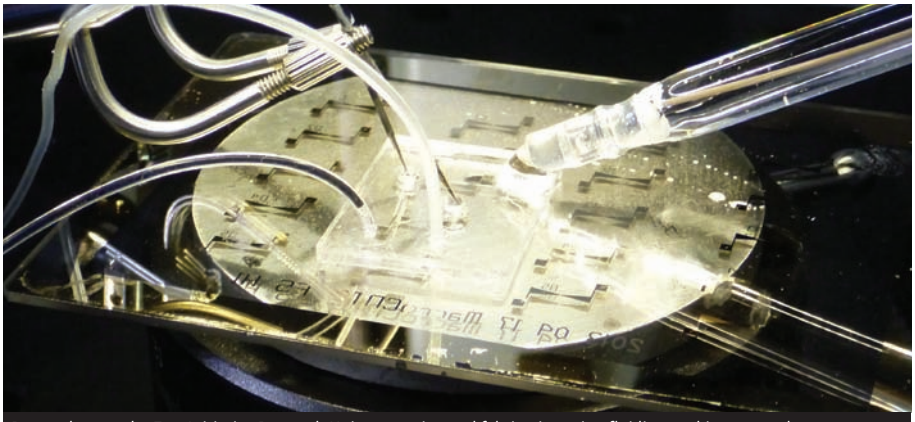
Laying the groundwork for the collaborative efforts are QBiC's experimentalists at the Cell Dynamics Research Core, who are developing new imaging and detection techniques to quantitatively measure cellular processes such as transcription and translation as they happen. These include improvements to the spatial and temporal resolution of microscopy for real-time observation of molecules in the cell, synthesis of quantum dot fluorescent probes that emit infrared light for visualizing cellular dynamics *in vivo*, and the application of mass spectroscopy and nuclear magnetic resonance techniques for analyzing the living cell as a whole.

New measurement tools and the information they generate are helping theorists at QBiC to develop realistic algorithms and models of biological systems. Among other projects, researchers in the Computational Dynamics Research Core are using supercomputers to simulate molecular dynamics at millisecond timescales, as well as to predict the interplay between macromolecules in the cell and the interaction between cells in a tissue or organ.

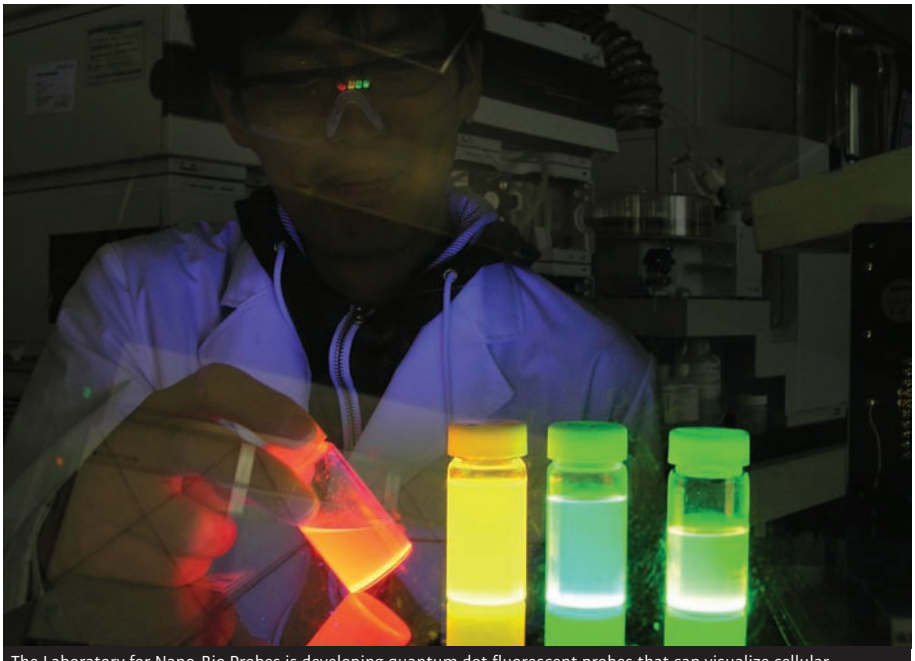
Engineering biology

With the proliferation of new instruments, measurements and models, synthetic biologists at QBiC are designing artificial systems that mimic their *in vivo* counterparts from biological components. Current research being performed at the Cell Design Research Core employs multiple strategies such as novel DNA synthesis, genome engineering using artificial restriction enzymes and rapid generation of genetically modified cells, including embryonic stem cells, to ultimately mass produce chimeric mouse models. Discoveries that emerge from these efforts will expand into new branches of the biological sciences, such as protein synthesis, membrane division, spatial and temporal distributions in a cell, and the logic that controls gene networks.

Biological systems seemingly play by a set of impenetrable rules. However, by taking a deeper look at how their various elements co-exist in dynamic environments, QBiC scientists are on course to explaining the invisible order that determines life.



Researchers at the Frey Initiative Research Unit are testing and fabricating microfluidic nanobiosensors that can measure the properties of biological systems.



The Laboratory for Nano-Bio Probes is developing quantum dot fluorescent probes that can visualize cellular dynamics in real time.

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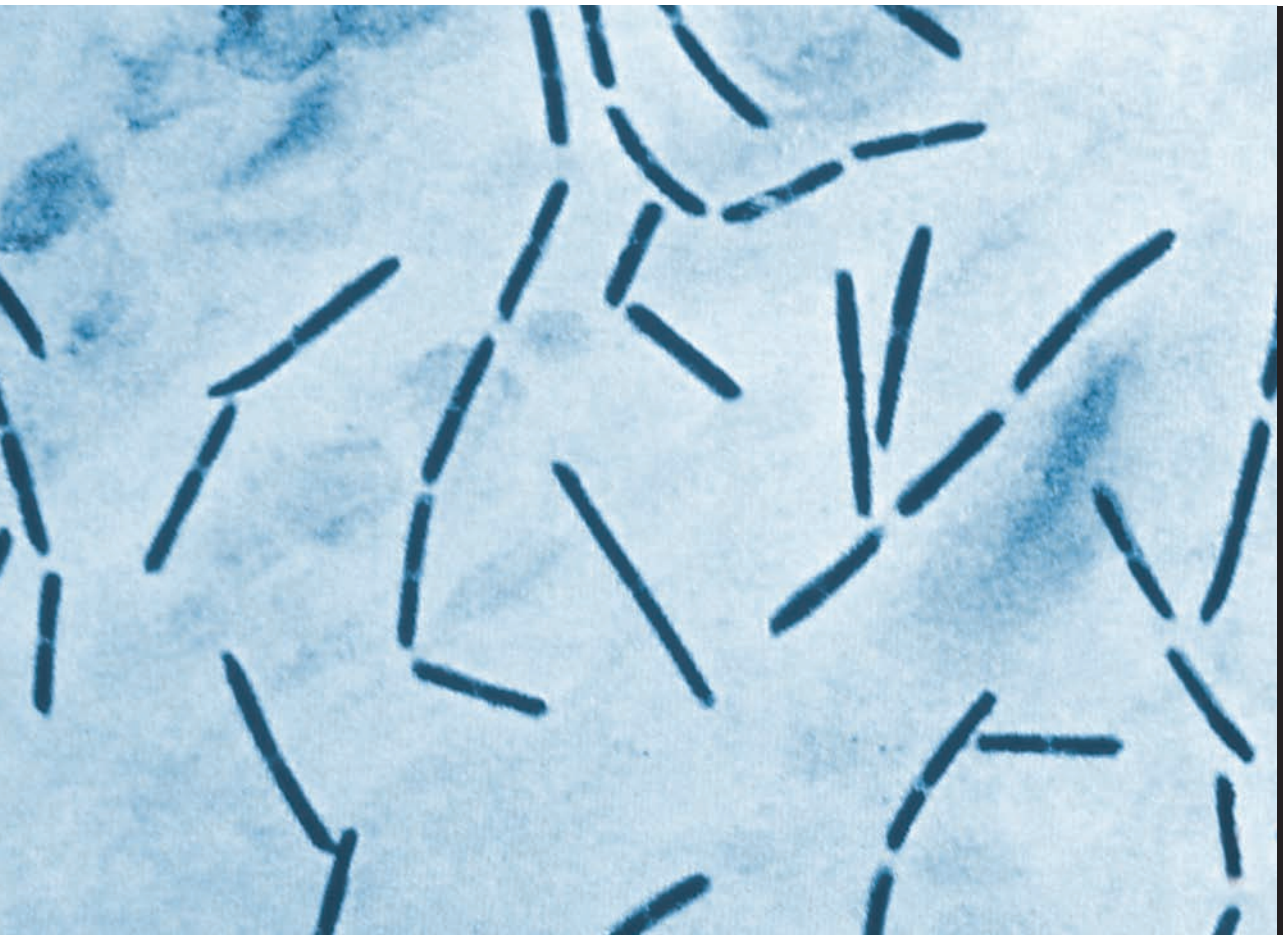

Commensal bacteria belonging to the Clostridiales order help to control inflammation in the mammalian gut.

Friends in low places preserve gut health

Bacteria living in the mammalian intestine help to digest dietary fiber, generating metabolites that also control gut inflammation

The bacterial communities that live in our intestines should not be considered freeloaders—they contribute substantially to our well-being in a number of ways, including assisting in the breakdown of otherwise indigestible dietary fiber. Hiroshi Ohno from the RIKEN Center for Integrative Medical Sciences and colleagues have now discovered a mechanism by which this digestive assistance also helps to prevent gut inflammation[1].

Bacteria belonging to the order Clostridiales are known to metabolize indigestible dietary fiber to produce metabolites, such as short-chain fatty acids (SCFAs). Ohno's team found that mice lacking gut commensal bacteria including Clostridiales exhibit bloating within the cecum —the start of the large intestine—after consuming a high-fiber diet. However, this problem could be repaired by transplanting a Clostridiales-enriched gut bacterial community (see image).

In other recent work, Ohno and his colleagues demonstrated that these same bacteria stimulate regulatory T (T_{reg}) cells, which specifically prevent the immune system from overreacting. Ohno suspected that this and his most-recent observation might be connected. "This led us to hypothesize that bacterial metabolism of dietary fiber may be the cause of T_{reg} induction," he says.

After searching for metabolic products that were elevated following consumption of a high-fiber diet, the researchers focused on one SCFA, butyrate, as a likely candidate. Butyrate proved capable of converting immature 'naive' T cells into T_{reg} cells in culture, and a maize starch diet that had been chemically enriched with butyrate invoked a similarly potent T_{reg} response within the mouse colon. The same butyrate-enriched maize starch diet failed to elicit T_{reg} proliferation

Image courtesy of the Centers for Disease Control and Prevention

in mice lacking commensal microbes, suggesting that in addition to butyrate, bacterial components are required as an antigen to be recognized by naive T cells.

Butyrate modulates the activity of enzymes that introduce chemical modifications known as 'epigenetic marks' to chromosomes, which can dramatically affect gene expression, and Ohno and his colleagues identified alterations that selectively activate T_{reg}-specific genes. "Bacterial butyrate affected the epigenetic status of naive T cells to propel their differentiation into T_{reg} cells within the colonic tissue," he says. This resulted in a strong protective effect, and the increased numbers of T_{reg} cells that developed following consumption of a butyrate-enriched diet ameliorated inflammation in a mouse model of colitis.

These findings may thus reveal a critical component of the pathology of human inflammatory bowel diseases as well as a potential means for treatment. Ohno and his colleagues now hope to explore whether the same mechanism is also relevant in the inflammatory response to food allergies.

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Growing brains in the lab

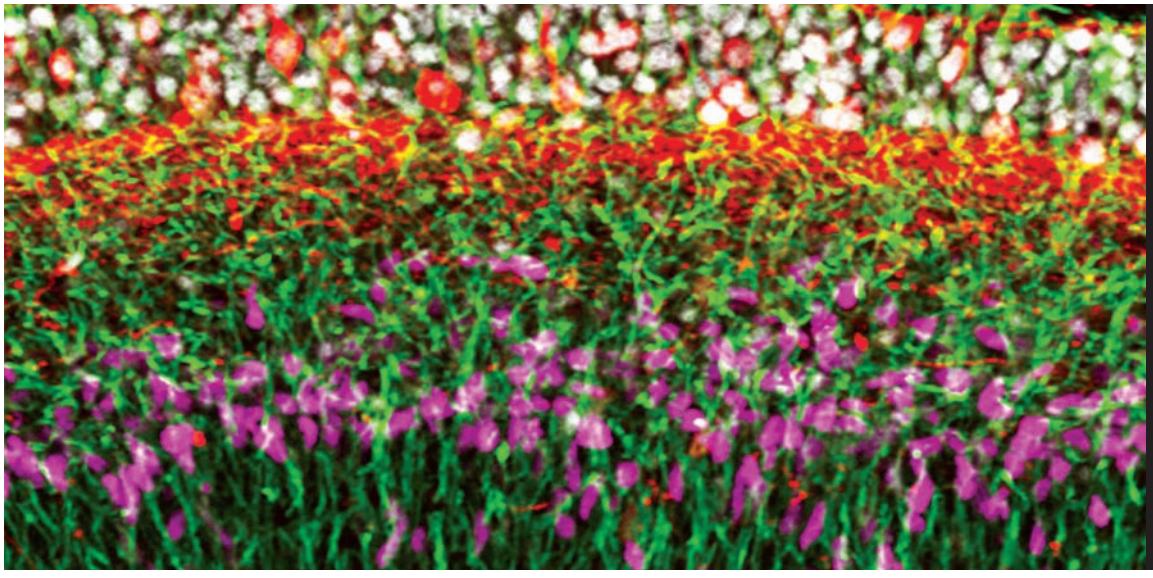
Human embryonic stem cells can be induced to spontaneously form developing brain tissue

During development, the nervous system forms as a flat sheet called the neuroepithelium on the outer layer of the embryo. This sheet eventually folds in on itself to form a neural tube that gives rise to the brain and spinal cord—a process that involves the proliferation and migration of immature nerve cells to form the brain at one end and the spinal cord at the other. Yoshiki Sasai, Taisuke Kadoshima and colleagues from the RIKEN Center for Developmental Biology have now shown that human embryonic stem (ES) cells can spontaneously organize into the cerebral cortical tissue that forms at the front, or 'brain' end, of the developing neural tube[1].

Sasai and his colleagues previously developed a novel cell culture technique that involves growing ES cells in suspension, and have shown that these cells can self-organize into complex three-dimensional structures. They have already used this method to grow pieces of cerebral cortex and embryonic eyes from mouse ES cells. And more recently, they have shown that human ES cells can also organize into embryonic eyes containing retinal tissue and light-sensitive cells.

In their most recent work, Sasai's team treated human ES cells grown using their cell culture system with signaling molecules that induce the formation of nervous tissue from the outer embryonic layer. They found that the cells spontaneously organize into neuroepithelial tissue that then folds up to give a multilayered cortex (see image).

During human embryonic development, the neural tube thickens at both ends. In particular, the front end thickens dramatically as waves of cells migrate outward to form the layered cerebral cortex and other parts of the brain. An important finding of the team's is that the front end of the neural tube appears to thicken due to the growth of radial glial fibers, which span the thickness of the tube



Human embryonic stem cells spontaneously organize into neuroepithelial tissue containing multiple zones after growing for 70 days in culture.

and guide migrating cells, rather than due to the accumulation of immature cells within the tube, as previously thought.

The findings also highlight critical differences between the development of the neural tube in mice and humans. While in humans, the inner surface of the neural tube and the intermediate neuroepithelial zone underneath it contain distinct populations of neural progenitors resembling radial glia, the progenitor population in the latter is not present in the developing mouse cortex.

"Efficient generation of cortical tissues could provide a valuable resource of functional neurons and tissues for medical applications," says Kadoshima. "By combining this method with disease-specific human induced pluripotent stem cells, it will also be possible to reproduce complex human disorders."

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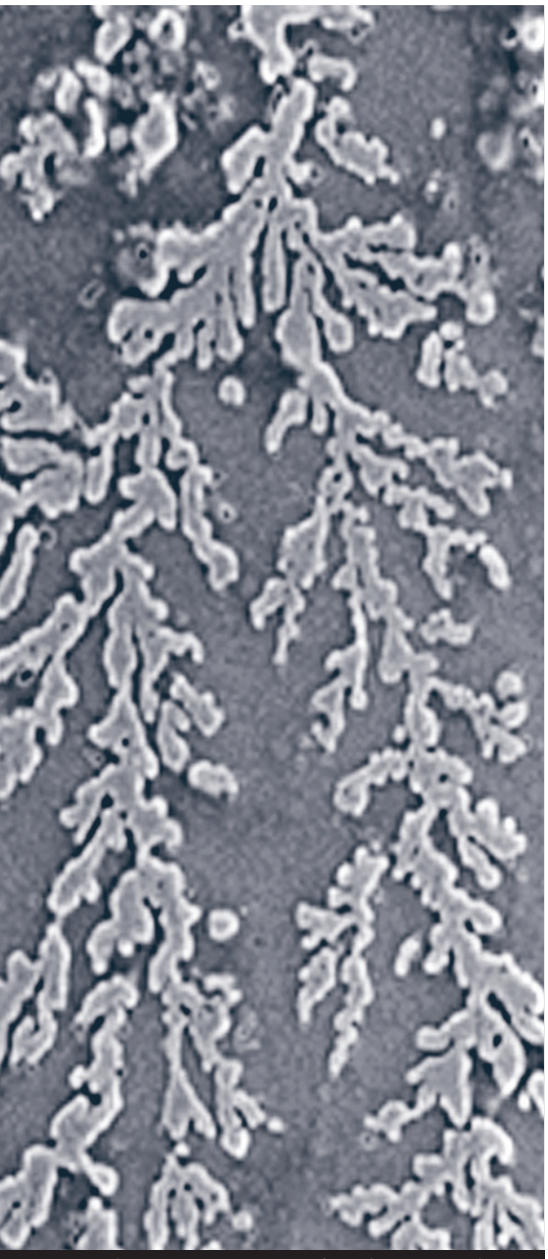

Electron microscopy image of measles virus particles firmly attached to the surface of a slide with a photoreactive polymer.

Pinning down viruses with a light touch

A light-activated polymer allows scientists to construct precise arrays of virus particles as a foundation for faster and more efficient diagnostic assays

Antibodies in the bloodstream hold the history of a patient's past infections. Clinicians probe this history by immobilizing protein particles or proteins of clinical interest onto the plastic surface of an assay plate and looking for antibodies that 'stick' to those targets after incubation with a serum sample. Yoshihiro Ito and colleagues from the RIKEN Center for Emergent Matter Science have now devised a superior assay that allows researchers and clinicians to obtain such diagnostic information more efficiently[1].

For some time now, scientists have been detecting DNA using 'microarrays'—glass slides featuring immense numbers of precisely positioned spots, each containing a different target sequence of interest. Unfortunately, translating this approach for protein analysis has been problematic. "DNA is made of only four bases and the range of chemical functional groups is very limited," says Ito. "But proteins are composed of 20 amino acids with many chemical functional groups, which makes it difficult to immobilize different proteins on the same surface using a single method."

Ito and his colleagues found some success with photo-immobilization, in which proteins are mixed with polymers that essentially turn into 'glue' when bombarded with ultraviolet (UV) light. However, as existing polymers are poorly suited for whole virus particles, the team embarked on a search for a superior photo-immobilization medium. The search pointed to photoreactive polyethylene glycol (PEG), which offers numerous advantages. Notably, PEG forms a layer on the slide surface that resists binding by nontarget molecules, thereby yielding more accurate assay results. PEG also responds to lower levels of irradiation, minimizing the risk of UV damage to bound proteins or viruses.

After confirming that the photoreactive PEG layer can successfully affix virus particles (see image), the researchers developed an automated workflow that enabled them to analyze antibody binding from tiny volumes of patient serum on a microarray in just 20 minutes. By comparison, a standard plate-based assay requires far more serum and takes a few hours to complete. The tests also

showed essentially equivalent performances in analyzing patient antibodies for five different viruses, with a false-negative rate lower than 5 per cent in most cases.

The system is now undergoing further development with the RIKEN venture company Consonal Biotechnologies, and Ito sees great potential for rapidly testing a larger number of samples against a greater number of targets. "Our microarray system achieves short detection times with small amounts of blood," he says. "We hope to contribute to society by commercializing this useful system."

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Serotonin boost explains an anesthetic's antidepressant effect

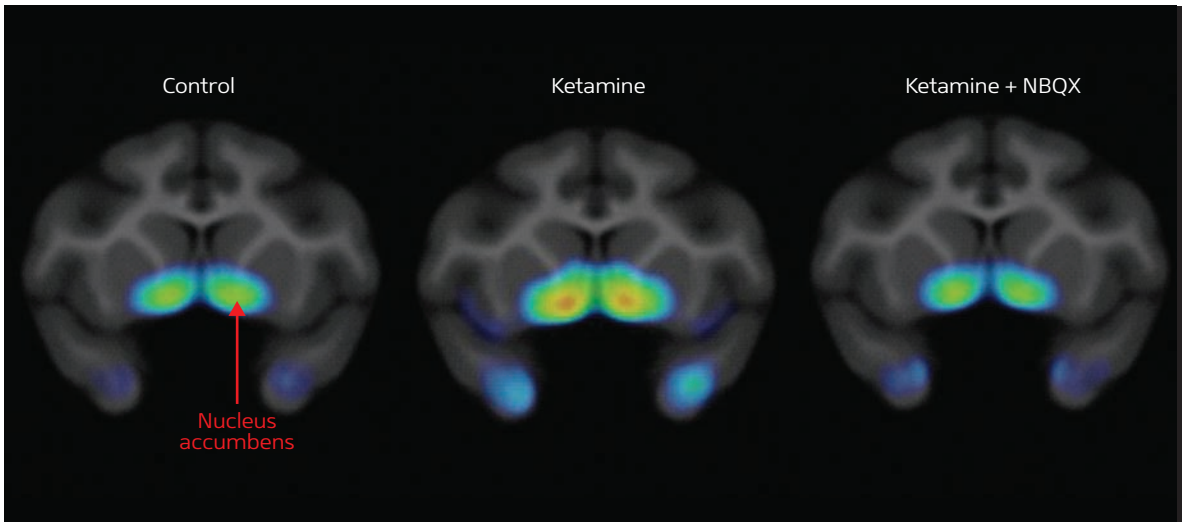
Molecular imaging reveals that the anesthetic drug ketamine helps to reverse depression by altering serotonin signaling in the brains of monkeys

The anesthetic drug ketamine has recently been shown to have a rapid antidepressant effect in psychiatric patients who do not respond to other medications. However, physicians still do not fully understand what the drug does in the brain. Using molecular imaging techniques, a research team led by Hirotaka Onoe and colleagues from the RIKEN Center for Life Science Technologies has now discovered that ketamine increases the activity of serotonin in brain areas involved in regulating motivation and mood[1].

Many antidepressants target the re-uptake of serotonin, a neurotransmitter molecule with a range of important functions that has been linked to feelings of happiness. To investigate the possible effect of ketamine on serotonin signaling, Onoe's team, in collaboration with researchers from Karolinska Institutet in Sweden, used a molecular imaging technique called positron emission tomography (PET) to study the brains of four rhesus macaque monkeys before and after ketamine administration. PET allows researchers to map the location of radioactively labeled 'tracer' molecules in the brain in three dimensions. Onoe's team used two tracers linked to serotonin signaling—one bound to the serotonin 1B receptor (5-HT1B) and another bound to the serotonin transporter (SERT).

The researchers found that ketamine significantly affected serotonin neurotransmission in two specific regions of the brain: the nucleus accumbens and the ventral pallidum, both of which play pivotal roles in the brain's reward circuit. Ketamine significantly increased the binding of the tracer chemical to the 5-HT1B receptor, indicating an upregulation of this key serotonin mediator. At the same time, the drug significantly reduced binding to SERT, a transporter that terminates the action of serotonin in the neural synapse.

"Ketamine might modulate brain serotonergic neurotransmission by regulating



A positron emission tomography (PET) scan of a monkey brain showing that ketamine treatment activates serotonin 1B (5-HT1B) receptors in the nucleus accumbens. This activation is reversed by the addition of NBQX, a drug known to block ketamine's antidepressant effect.

its receptor and transporter expression on the synaptic membrane," says Onoe. "Since the nucleus accumbens and the ventral pallidum are known to be critical parts of the neural circuit associated with motivation, ketamine might directly affect motivation in depressive patients."

The researchers also investigated the effects of combining ketamine with a drug called NBQX, which had previously been reported to attenuate the effects of ketamine in rodents. They found that addition of NBQX interfered with the action of ketamine on 5-HT1B receptors (see image) but had no impact on SERT levels. The findings suggest that much of ketamine's antidepressant action appears to be due to an increase in activity of postsynaptic 5-HT1B receptors in the nucleus accumbens and ventral pallidum—and not as a result of the drug's effect on SERT.

Reference

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A boost for photosynthetically derived bioplastics

Enhanced photosynthesis is crucial for improving bioplastic yields from cyanobacteria

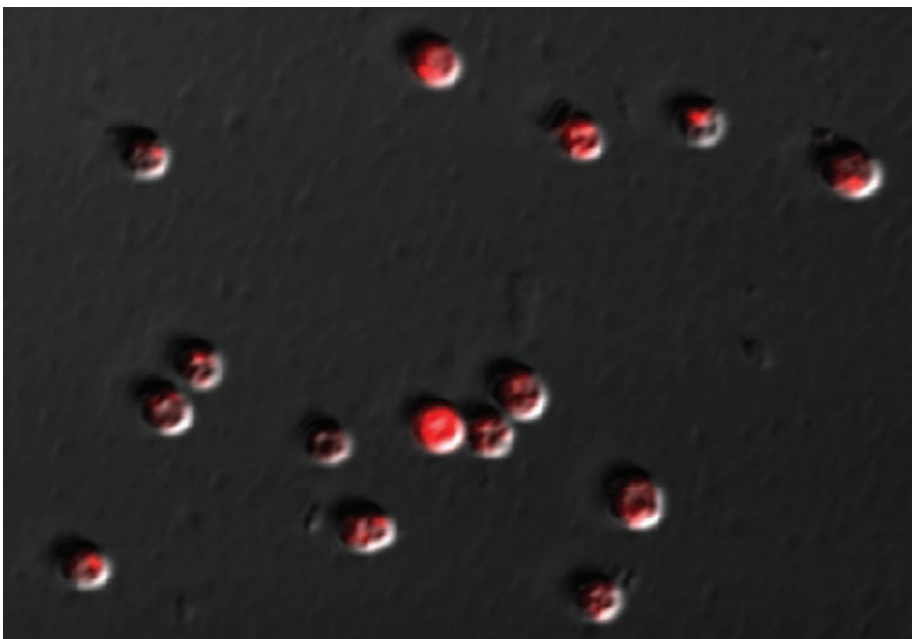
Traditional petrochemical plastics are made from nonrenewable fossil fuels and cannot be readily assimilated back into the environment at the end of their useful life. Thus, there is considerable interest in replacing petrochemical plastics with biodegradable alternatives that are derived from more sustainable resources. Unfortunately, efforts to produce plastics by more sustainable means have yet to yield economically viable alternatives.

Minami Matsui, Nyok-Sean Lau and colleagues from the Synthetic Genomics Research Team at the RIKEN Center for Sustainable Resource Science have now led research that has significantly advanced the potential of bacterial fermentation-based plastics[1].

Polyhydroxyalkanoates (PHAs) are linear polyesters produced by bacterial fermentation of sugars or lipids. However, cultivation of the heterotrophic bacteria used so far to produce PHAs requires expensive organic substrates as a carbon source. An alternative approach is to use photosynthetic organisms, such as cyanobacteria, which extract carbon from atmospheric carbon dioxide and have minimal nutrient requirements. Yet this approach has also been stymied by low conversion rates and an inadequate understanding of the factors that promote PHA accumulation.

In collaboration with Kumar Sudesh from Universiti Sains Malaysia, the research team created a genetically modified variant of the cyanobacterium *Synechocystis* sp. strain 6803 that produced PHA levels as high as 14 per cent of the cellular biomass—a record for cells grown without an external carbon source (see image). In the modified strain, the first enzyme involved in PHA production is replaced with another enzyme, acetoacetyl-CoA synthase from *Streptomyces* sp. CL190.

The team then compared the gene expression in their PHA overproducer with its unmodified counterpart. "The PHA-overproducing line actually expressed lower levels of most of the other genes directly involved in PHA synthesis," explains Matsui. Instead, the most striking set of genes that were expressed at higher levels in the modified line were in fact those encoding a wide range of components of the photosynthetic apparatus.



A microscope image of a genetically modified variant of the cyanobacterium *Synechocystis* sp. strain 6803. Red dye indicates the likely accumulations of polyhydroxyalkanoates.

The findings suggest that increased expression of genes involved in various aspects of photosynthesis reflects an enhancement of the cyanobacterial capacity for carbon fixation to accommodate the increased diversion of carbon to PHA formation.

"Enhancing carbon fixation in cyanobacteria by manipulating the proteins involved in the process seems like the most obvious next frontier for the sustainable production of bioplastics, biofuels and other chemicals," says Matsui. "Cyanobacteria are ideally suited to showing that this is possible. And if we can achieve this with cyanobacteria, it is likely that the same or a similar approach might work in higher plants." ■

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Additional factors improve stem cell generation

Proteins found in unfertilized egg cells enhance the efficiency and speed of induced pluripotent stem cell production

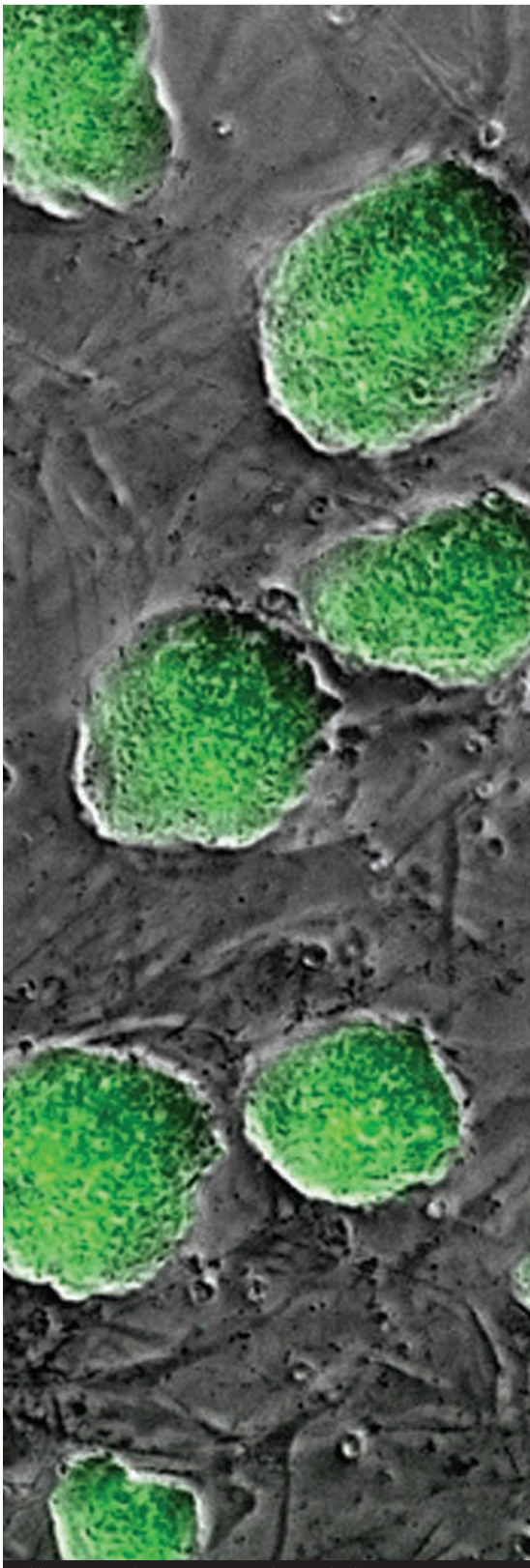
Induced pluripotent stem (iPS) cells are created by 'reprogramming' specialized adult cells into embryonic stem-like cells. Although a remarkable process, the procedure remains slow and inefficient, involving induced expression of four specific protein factors found at high levels in embryonic stem cells. By adding two further proteins found in unfertilized egg cells, or oocytes, to this standard recipe of reprogramming factors, a collaborative team of researchers led by Shunsuke Ishii and colleagues from the Molecular Genetics Laboratory at the RIKEN Tsukuba Institute have now discovered a way to generate iPS cells with increased speed and efficiency[1].

There are many ways to coax terminally differentiated adult cells into a pluripotent state. One of the most commonly used methods is based on expression of the four 'Yamanaka factors'. These proteins —Oct3/4, Sox2, Klf4 and c-Myc—are found in embryonic stem cells but not in oocytes.

Through cloning studies using a technique known as somatic cell nuclear transfer, scientists have shown that oocytes retain the natural ability to reprogram adult cells. Ishii and his colleagues hypothesized that the proteins found in oocytes might therefore assist in the generation of iPS cells.

The researchers focused on two proteins in particular, TH2A and TH2B. Both are variants of histone proteins that affect how tightly DNA is wrapped into chromatin —the complex of DNA and proteins that makes up the contents of the cell's nucleus. By studying mice engineered to lack these proteins, Ishii and his colleagues showed that TH2A and TH2B are highly expressed in oocytes and, upon fertilization, help to activate the paternal genome of incoming sperm. These observations highlighted TH2A and TH2B as strong candidates for oocyte-specific reprogramming factors that might induce pluripotency through a different mechanism to that of the Yamanaka factors.

Working with mouse embryonic fibroblast cells, the researchers added TH2A and TH2B to the Yamanaka factors and obtained more than 20 times the number of iPS cells in less than half the normal time compared to using the Yamanaka factors alone. The researchers also showed that TH2A and TH2B could function as direct substitutes for two of the Yamanaka factors, allowing for an alternative four-part recipe.



Adding TH2A and TH2B proteins to the standard reprogramming recipe promotes the production of induced pluripotent stem cells (green).

"There are two ways to use these findings," explains Ishii. "Firstly, use of TH2A/TH2B may help to increase the quality and differentiation capacity of iPS cells. Secondly, use of TH2A/TH2B may help to generate iPS cells from cell types, such as certain somatic cells, that have low efficiency for iPS cell generation."

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The secret ingredient that strengthens silk biomaterials

The addition of pectin molecules significantly improves the mechanical properties of silk-based hydrogels as tissue engineering substrates

The human body has limited ability to self-repair damage to cartilage or bone. Implantable 'bioscaffold' materials that can be seeded with cells can potentially be used to regenerate these critical tissues. One such biomaterial under consideration is silk hydrogel—a nontoxic, natural substance produced by combining silk proteins with water to form an aqueous gel. Keiji Numata from the RIKEN Center for Sustainable Resource Science and colleagues have now devised a way to reinforce silk-based bioscaffolds through the addition of pectin-based fibers[1].

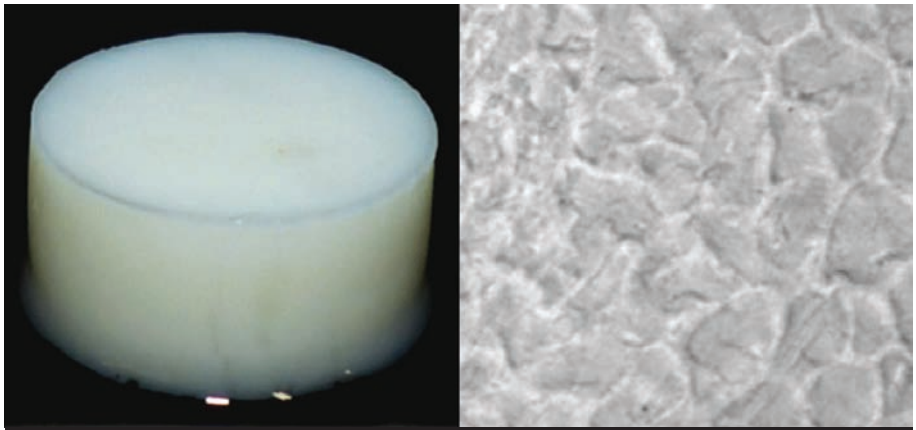
Due to their high bound-water content, silk hydrogels have been shown to accelerate the growth of human mesenchymal stem cell (hMSC) adhesion proteins. hMSCs can differentiate into bone and cartilage cells and are therefore ideal precursors for tissue regeneration strategies. Unfortunately, silk hydrogels often have insufficient mechanical strength for practical applications.

To boost the elasticity and mechanical strength of silk hydrogels, Numata and his colleagues turned to pectin, a polysaccharide found in plant cells that is widely used as a food thickening agent. The team suspected that silk's arginine and lysine amino acids could interact with pectin to form a strong molecular network (see image). "Silk is a hydrophobic polymer, while pectin polymers are hydrophilic," says Numata. "The key challenge was to make a stable polymer blend from these two materials."

The researchers obtained an aqueous silk solution by extracting proteins from silkworm cocoons and filtering them through dialysis membranes. They then mixed the silk solution with pectin, sealed the mixture in a container fitted with a dialysis membrane, and immersed the container in methanol. The methanol regulated the displacement of water through the dialysis membrane, producing a semi-solid silk–pectin hydrogel.

Studying the silk–pectin hydrogel under a microscope revealed the formation of a heterogeneous network of silk fibers and random coils of silk proteins associated with the pectin. The hydrogels also displayed greatly improved strength as well as enhanced water-holding capacity, which resulted in the release of water during mechanical compression—an action that mimics natural cartilage behavior. Cell culture experiments showed that hMSCs had exceptional viability on the silk–pectin substrate, and the bioscaffold itself was demonstrated to degrade on exposure to enzymes, encouraging the integration of differentiating cells into surrounding host tissue.

Numata notes that whereas most hydrogels are prepared from purely hydrophilic polymers, the extraordinary strength exhibited by this example of a hydrophobic/hydrophilic mixture suggests that this unconventional strategy could open a new avenue for hydrogel synthesis.



The addition of pectin to silk proteins produces a soft, tissue-like hydrogel (left) with a complex microstructure (right) that results in exceptional strength.

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Quantitative biology

Seeing the big picture in cells

Toshio Yanagida is director of the RIKEN Quantitative Biology Center
Recently honored as a Person of Cultural Merit by the Japanese government, Yanagida is a leading scientist in the area of dynamic biological systems. His research focuses on the development of new technologies, such as single-molecule imaging, for the study of biological molecular motors.

Biology requires both bottom-up studies of molecular and cellular interactions and top-down approaches that examine larger molecular and cellular networks. By identifying the common characteristics of these dynamics, researchers in quantitative biology are working toward models of whole cells and even entire tissues. These models—informed by data from advanced imaging techniques—act as virtual laboratories for exploring how biological systems respond to environmental changes and disease.

Laboratories at the RIKEN Quantitative Biology Center are designing microscopy systems to measure the behavior of single molecules in living cells at unprecedented spatial and temporal resolution.

The future of medicine, agriculture and biotechnology relies on scientists continually learning more about molecules and cells and their interactions. By necessity, many biological studies focus on individual components of these systems, such as pinpointing the roles of specific genes in the progression of diseases. However, the biological systems that determine life comprise many components that operate in complex and dynamic networks and are influenced by external environmental factors.

The challenge remains to develop new measuring, imaging and analytical techniques that reveal these networks and their workings. Incorporating this information into models that describe cells and tissues in their entirety will give researchers the capability to design artificial systems that predict the body's response to drugs or show how to make cells behave in useful ways. These are the ambitious aims of scientists working in the expanding field of quantitative biology.

> *The future of whole-cell models will require researchers to exploit the full capabilities of supercomputers.*

Although a seemingly overwhelming task, certain common themes have emerged, allowing generalized rules to be adopted within whole-system models. One dominant theory at the RIKEN Quantitative Biology Center (QBiC) is that dynamic biological networks are scale-invariant, which means that such rules apply from the molecular, subnanometer level up to the whole-tissue level.

Helpful heterogeneity

Within any dynamic biological network, there will be cells or molecules that are otherwise identical but react to external stimuli in very different ways. A given population of cells will contain a subset of individuals that thrive in the current environment and another subset of individuals that do not. Should the environment change, then the new conditions might favor the latter subset. This population characteristic is known as heterogeneity.

One famous demonstration of heterogeneity is the peppered moth, which exists with both white and black body colorings. As a consequence, different individuals are camouflaged against different tree barks. In a normal environment with white trees, the white moth prospers, but in the presence of heavy industry, the tree bark becomes darkened by pollution and the black-bodied moth is favored.

Similarly, at the subcellular level, there is heterogeneity in the states that molecules can adopt, allowing them to serve different functions. For example, signaling proteins within a cell respond to external stimuli by binding to nearby so-called effector molecules. Advanced single-molecule

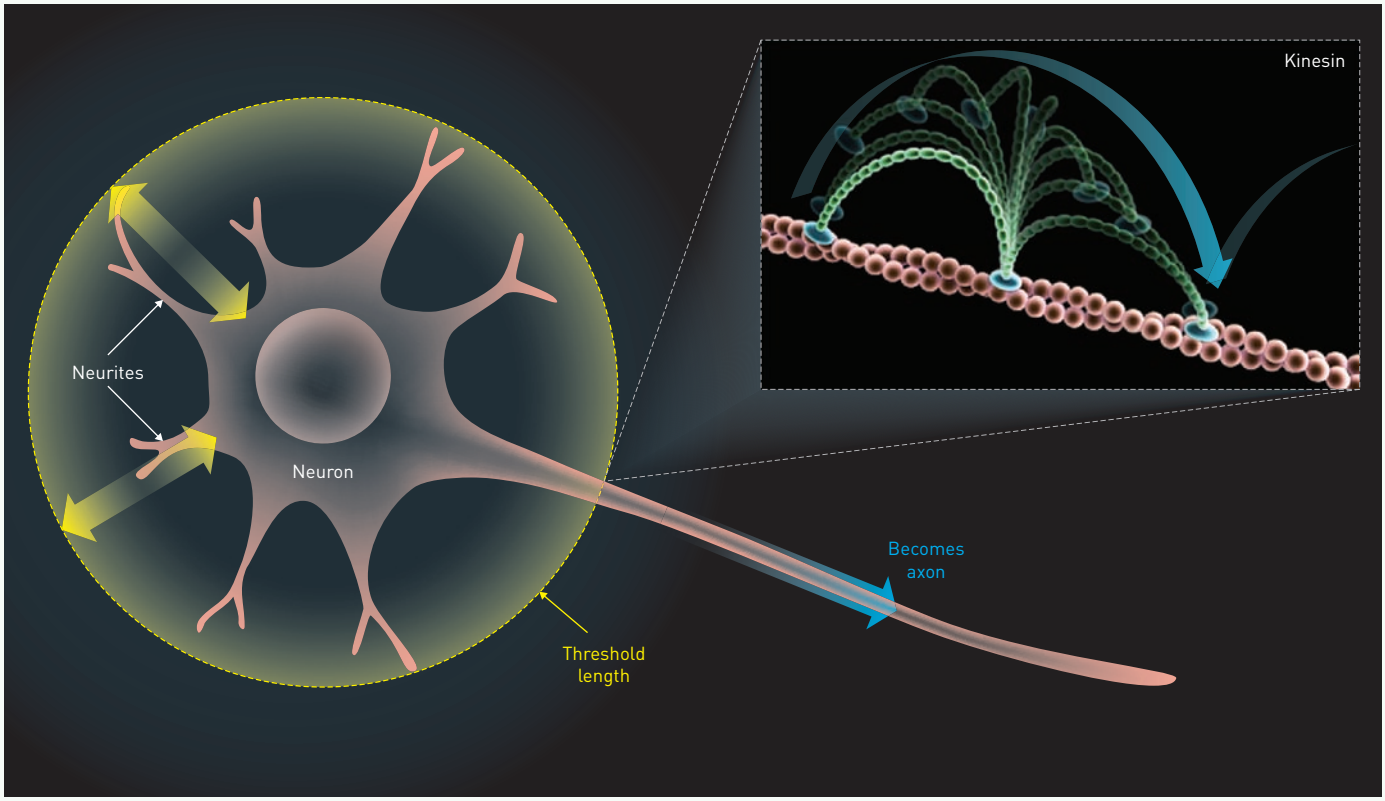

Figure 1: Observing Brownian motion with single-molecule imaging
Neurons start with multiple, identical neurites that are growing and shrinking (yellow arrows). Eventually, one neurite will pass the threshold length and not retract, continuing to grow into an axon (blue arrows). The rest become dendrites. Material needed to grow the axon is transported by kinesin, which, like other motor molecules including myosin, moves in a hand-over-hand fashion driven by Brownian motion (inset).

microscopy and spectroscopy studies by QBiC and others have verified that signaling molecules exist in several different structural states, which bind to different effectors in order to play different regulatory roles[1].

The random nature of nature

Typically, heterogeneity emerges spontaneously through random noise. Hence, any realistic models must include relationships based on random, or stochastic, principles.

Stochastic processes are particularly important in the nanoscopic world. At these scales, movement is largely governed by Brownian motion—the random movement of molecules caused by collisions with surrounding particles. The cargo transporter molecule myosin, for example, has a two-headed structure that moves in a hand-over-hand fashion to climb along cytoskeletal filaments of actin. While such a movement might appear deterministic, single-molecule imaging has shown that when one head of the myosin molecule is free, it moves back and forth along the actin filament under Brownian motion. The head waves around until it chances upon the optimum position further up the filament, where it makes a strong bond and thus pulls its cargo to the targeted location in the cell. These findings by QBiC researchers could have unexpected spin-off applications, such as informing the development of nanosized motors for miniature devices.

Seeing how cells break out

Heterogeneity and stochastic processes can also help explain how primitive cells change shape to become more specialized. All cells are symmetrical to begin with before they receive signals that initiate reorganization of their structure. This is well illustrated by neurons in the brain. Mature neurons have many protrusions, comprising one long axon and multiple shorter dendrites. Initially, these structures are identical, all undergoing a series of dynamic extensions and retractions. Eventually, one protrusion does not retract and continues to grow into an axon; all others become dendrites[2] (Fig. 1). Single-molecule imaging studies have shown that the axon grows via stochastic processes through the accumulation of growth proteins.

This type of symmetry breaking can also be seen in whole-cell movement, or chemotaxis. This is the process by which a cell moves either toward or away from an area rich in chemicals, such as food or toxins. When signaling molecules within a cell pick up an external chemical signal, certain subcellular structures move from their original, random positions to assemble at a specific location, causing the cell to move. Research at QBiC has shown that cells can achieve the high sensitivity required for chemotaxis because their internal molecular components are in a constant state of flux.

Researchers at the RIKEN Quantitative Biology Center make up a vital part of the global effort to understand complex biological systems.

Bringing the whole cell together

By taking an integrated approach to analyzing the structural, spatial and temporal properties of cells, researchers at QBiC make up a vital part of the global effort to understand complex biological systems. Imaging techniques are vitally important for this work: through innovations in microscopy, molecular probes and other measurement systems, researchers hope to obtain simultaneous nanometer and millisecond resolutions for the three-dimensional imaging of molecules inside living cells.

Computational biologists have the daunting task of incorporating this information into models. Modeling protein interactions requires a timescale of milliseconds and a resolution of femtoseconds (one quadrillionth of a second). For this reason, the future of whole-cell models will require researchers to exploit the full capabilities of supercomputers and develop algorithms based on the unifying principles described above.

Two computational tools lead this research at QBiC: MDGRAPE-4, a supercomputer designed to perform molecular dynamics simulations; and E-Cell, a software platform for modeling complex, heterogeneous and dynamic systems inside the cell. MDGRAPE-4 simulates the behavior of atoms and predicts how this will determine molecular behavior whereas E-Cell predicts how molecules operate at larger scales. Such comprehensive descriptions of activity at different scales will provide not only a better understanding of heterogeneous populations but also the potential to control them.

This control comes in the form of the third component of research at QBiC: synthetic design. RIKEN scientists are using data from imaging and computational work at QBiC to synthesize proteins without the need for a cell and to create lab-on-a-chip devices that can efficiently sort cells. These technologies are helping other researchers, for example, in creating strains of laboratory mice with specific traits that are useful for studying the effects of diseases and developing drugs.

At its core, QBiC is creating synthetic systems that act like biological ones. This work is possible thanks to a symbiotic relationship among researchers, from experimentalists to computational theorists to synthetic biologists. By continuing to uncover the operating principles that determine a cell's state, researchers at QBiC are innovating technologies with the aim of controlling cell behavior.

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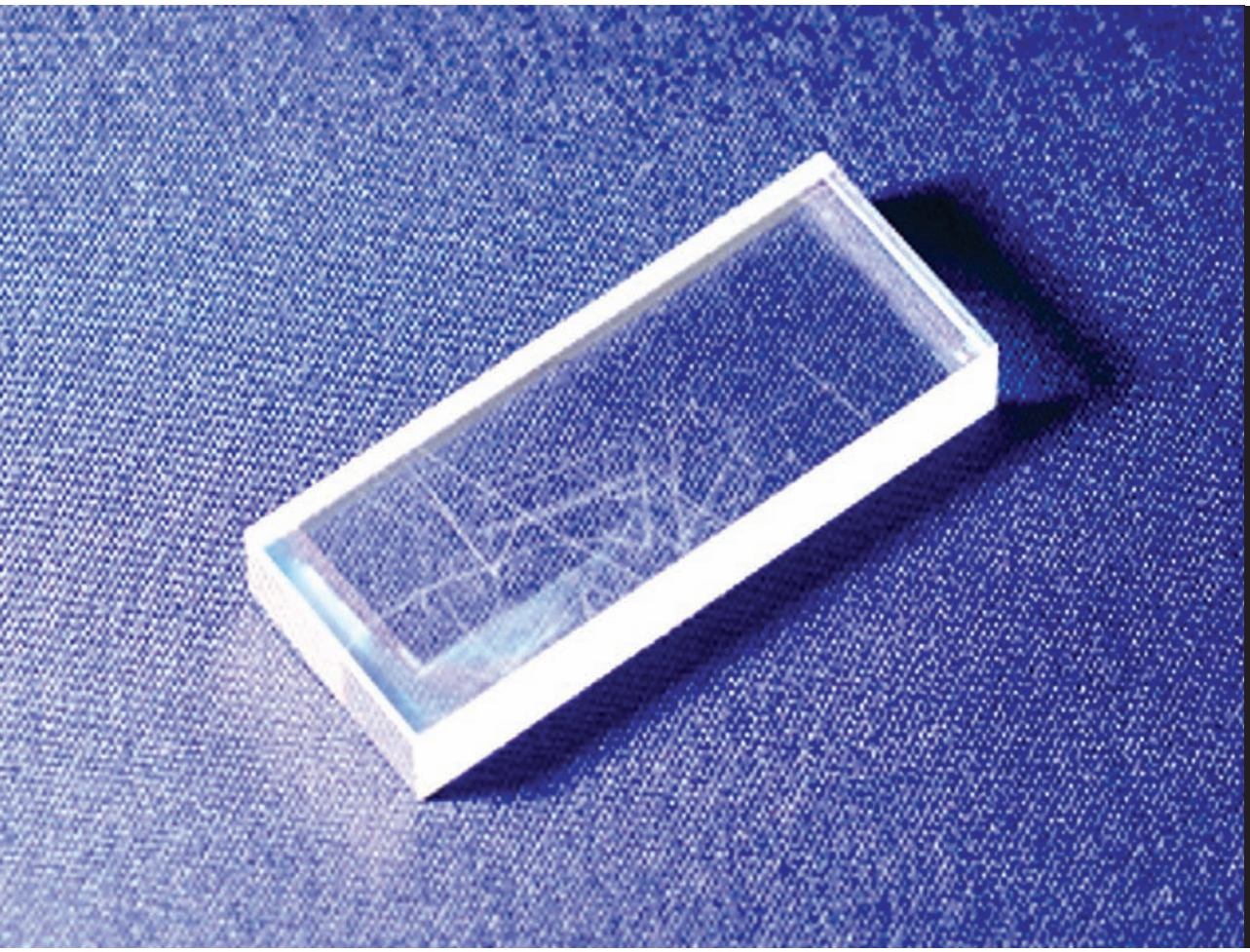

A crystal of lithium niobate with an alternating stacking structure that gives the material nonlinear optical properties.

A step up for terahertz detection

Nonlinear optical materials convert terahertz radiation into infrared light for simplified detection

Terahertz radiation, part of the frequency spectrum of light between microwaves and infrared, can pass through many materials and is potentially useful for applications such as airport security scanning. Commercial use of the technology, however, has been held back by the difficulty in detecting terahertz signals. Kouji Nawata and colleagues from the Tera-Photonics Research Team at the RIKEN Center for Advanced Photonics have now developed a system that can upconvert terahertz radiation to higher-frequency infrared light for more efficient detection[1].

"Conventional terahertz detectors are thermal sensors that convert terahertz energy into heat," explains Nawata. "This can cause the sensitivity of these detectors to become worse in hot environments."

Optical detection after frequency conversion represents an attractive solution. Nawata's team achieved this goal by taking advantage of the unusual properties of nonlinear optical materials, which have an optical response that is dependent on light intensity. These materials are useful because they provide a way of making two beams of light interact indirectly, where a high-intensity beam of light sets the optical properties of the crystal and thus influences the propagation of a second, lower-intensity pulse. An example of such a nonlinear optical process is difference frequency generation (DFG), which creates a third beam of light with a frequency that is roughly equal to the difference of the two incident beams.

Using their nonlinear optical material in a DFG configuration, the researchers were able to take a 1.6-terahertz pulse and combine it with a high-intensity laser beam to generate a near-infrared signal. They demonstrated

that the intensity of the DFG infrared light was proportional to the incoming terahertz radiation, proving the scheme to be a useful way of measuring the strength of the incoming terahertz pulse. The approach was also sensitive, detecting pulses of terahertz light with a miniscule 25 femtojoules of energy.

The real advantage of this technique, however, is its flexibility. Nawata and his colleagues engineered their nonlinear material (see image) by stacking layers of lithium niobate such that the atomic crystal orientation was alternated between layers. They were able to optimize detection for a specific frequency of terahertz light by changing the periodicity of this stacking or by altering the angle between the light propagation and the stacking directions.

"One potential application that we would like to develop is terahertz wireless communication," says Nawata. "This technology could enable speeds a thousand times faster than present gigahertz-class communication, and the concept could easily be combined with fiber optics technologies."

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Helping robots learn to walk

A two-legged robot can learn to walk on different surfaces thanks to computer code that mimics the natural adaptability of humans

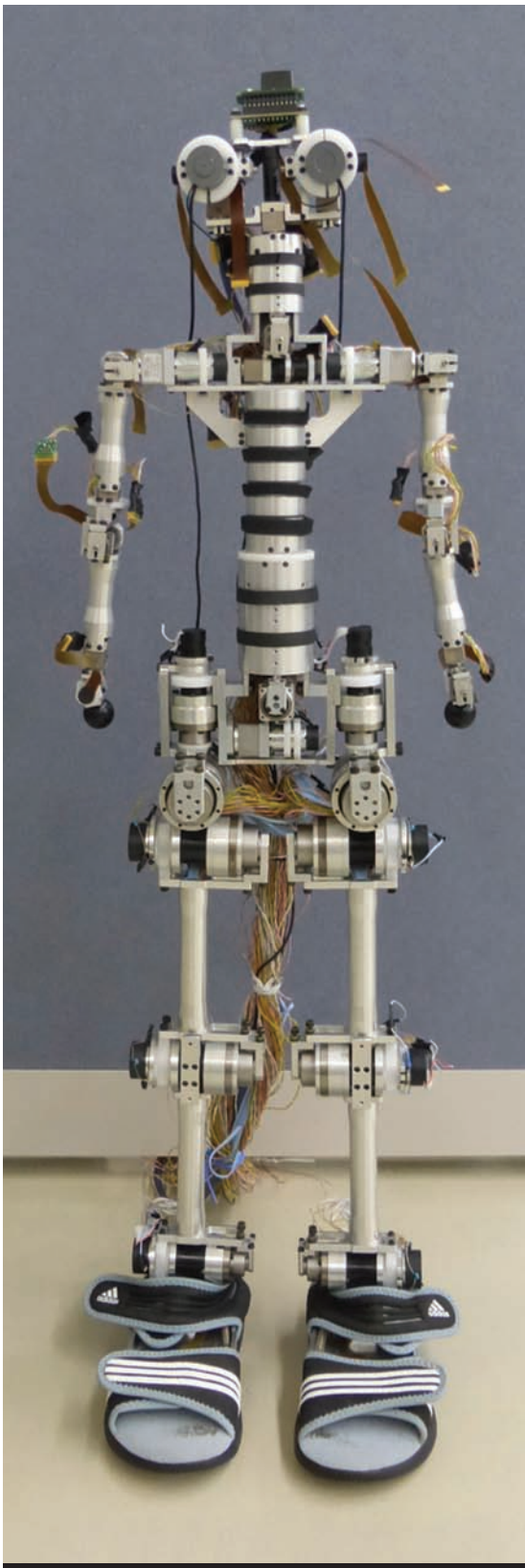
Fully autonomous robots could transform the way we live, but so far such machines remain beyond the reach of our most advanced technologies. Existing robots are generally limited to performing simple, well-structured tasks in controlled environments. To overcome unpredictable natural obstacles, robots must be able to learn from their environment and adapt their behavior in the same way as humans.

Shingo Shimoda and colleagues from the RIKEN BSI–TOYOTA Collaboration Center have now greatly improved the walking skill of a two-legged robot by programming the robot to adapt its posture in response to cues from the environment[1].

Having previously worked at the Japan Aerospace Exploration Agency (JAXA), Shimoda has long been interested in how robots can obtain the skills needed to explore natural environments on Earth and other planets. "I wondered why small animals like squirrels can move around and find food in a natural environment but robots cannot, even with great computing power," he says. "The control principles of biological systems offer important clues that we can use to implement the same adaptability in robots."

For a robot to be adaptable, it must have regulatory systems of integrated components that work together in response to the environment, just like proteins or neurons in the human body. Shimoda's team developed a 'tacit learning' scheme that enables the regulatory system in a robot to tune primitive, reflexive actions into more sophisticated, useful behavior. A computer model simulates the natural process based on interacting code structures called variable threshold neurons, achieving control of the 36 movable joints in the team's walking robot (see image).

For every movement of the robot, the researchers specified certain joints that were strictly controlled to obtain an objective, for example the swinging hip of the leg that was stepping forward. The computer model then adjusted other joints, such as those of the supporting leg, to an optimum configuration that kept the robot standing and minimized its energy consumption.



The gait of the walking robot is greatly improved by a computer model that mimics the tacit learning of animals.

In their first trial on a smooth laboratory floor, the robot fell down, but after around 10 minutes the motion was sufficiently tuned to keep the robot walking at over 7 centimeters per second. The robot was also able to adapt its walking gait to walk on natural turf and up slopes.

Shimoda believes that this same tacit-learning scheme could be applied to help robots interact with humans. "One important application would be prosthetic arms that can automatically move in response to the motions of the remaining joints," he says.

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A broader view of materials

Advanced broadband materials expand the utility of technologies for the optical characterization of materials at terahertz frequencies

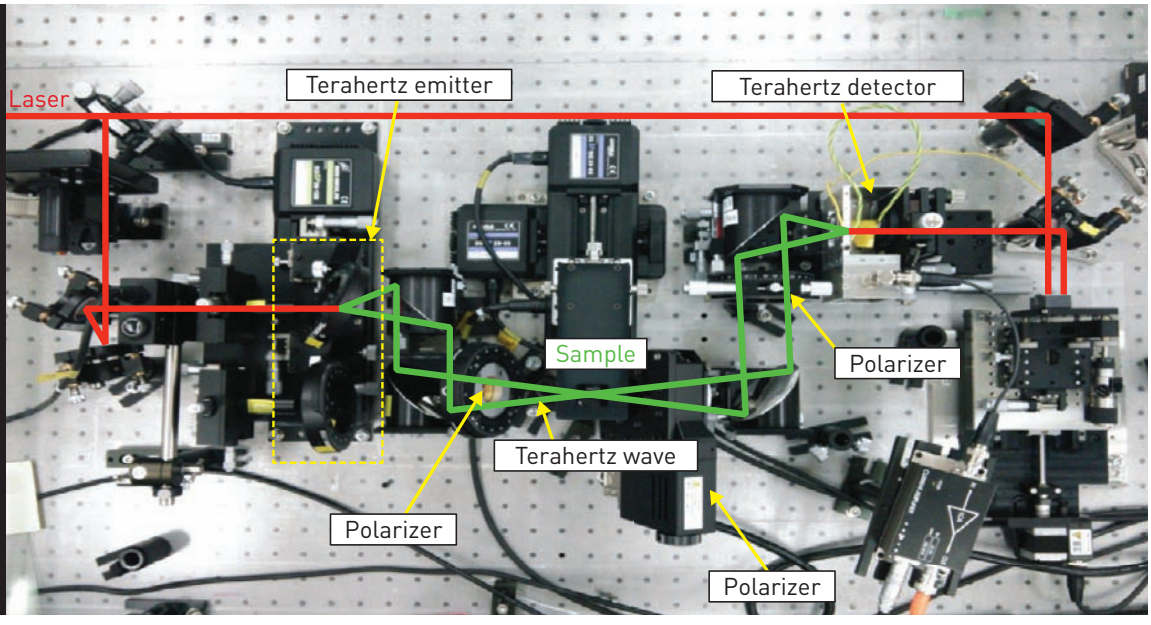
The noncontact measurement of material properties using light is used in a wide variety of applications, from airport security scanners to medical x-ray imaging and various analytical techniques. Some of the most interesting information is contained in what is known as the terahertz region of the frequency spectrum, but developing broadband spectroscopic techniques for the terahertz regime has proved difficult. Masatsugu Yamashita, Chiko Otani and colleagues from the RIKEN Center for Advanced Photonics and Canon Inc. have now developed a spectroscopy system that operates across an unprecedented range of terahertz and infrared frequencies[1].

Terahertz light, while potentially very useful for probing material properties, is difficult to handle using optical instruments because it is easily absorbed by many materials. Time-domain terahertz spectroscopy is already widely used to measure the optical properties of materials. Measurement of the polarization of light reflected from a sample in comparison to a reference mirror, or ellipsometry, provides even more information. Yamashita and his colleagues combined the two methods for use over a wide range of terahertz frequencies (see image).

Precision was a crucial aspect of the system, explains Yamashita. "Conventional terahertz reflection time-domain spectroscopy suffers from positioning error between the sample and the reference mirror, which prevents precise measurement of phase information."

Achieving the necessary micrometer precision across a broad frequency range required the use of as few light emitters and detectors as possible, and the use of broadband emitter and detector materials. The researchers used crystals of gallium phosphide or gallium selenide as light emitters, covering an unprecedented frequency range of 0.5–30 terahertz. For detection, Yamashita's team used a film of gallium arsenide, prepared at low temperature to extend its broadband characteristics. The thin film of gallium arsenide was transferred to an optically neutral substrate to avoid unwanted terahertz wave absorption.

The researchers demonstrated the excellent performance of the system across almost the entire operating frequency range. Although some unwanted light absorption in the detector and other optical components remains, which



The 'ultra-broadband' terahertz time-domain spectroscopic ellipsometry system.

prevents the system's use at certain frequencies, Yamashita is confident that these issues can be overcome by using thinner detector films or different optical components.

Once refined, the 'ultra-broadband' terahertz time-domain spectroscopic ellipsometry system could have some significant industrial applications. "An important application of the system," says Yamashita, "is the contactless and nondestructive characterization of carrier transport properties in semiconductors and the conducting polymers used in optoelectronic devices such as mobile-phone displays. This would be indispensable for improving device performance." ■

Reference

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Taking x-ray lasers to the next level

Higher-order nonlinear optical processes can now be observed using the SACLA x-ray free-electron laser

X-rays are used extensively for medical imaging and airport security, as well as for examining the atomic-scale crystallographic structure of materials. Until recently, the high-energy x-rays needed to probe materials at the atomic level could only be produced by very large ring accelerators known as synchrotrons. The development of a new type of x-ray source, called x-ray free-electron lasers (XFELs), has since made it possible to produce intense, ultrashort pulses of x-ray light that have dramatically expanded the utility of x-ray-based material characterization techniques.

Opening the door to an even broader range of experiments on ultrafast timescales, Kenji Tamasaku and colleagues at the SPring-8 Angstrom Compact Free Electron Laser (SACLA) facility (see image), part of the RIKEN SPring-8 Center, have now achieved a long-anticipated goal of inducing higher-order nonlinear optical processes using their advanced x-ray source[1].

Nonlinear optical effects are typically only observed at very high energies in materials with specific nonlinear optical properties. An example of such effects is second harmonic generation, or frequency doubling, by which two photons are absorbed by the material to create a single photon of twice the frequency. More complex third-order or even higher-order effects involving more photons are also possible, but require far higher laser power than the second-order effects such as frequency doubling.

"X-ray nonlinear optics remained almost untouched experimentally since the first theoretical work was reported in 1969," says Tamasaku. "There have been some reports on second-order nonlinear processes, but none on third-order effects." The SACLA facility is one of the few places in the world where such an observation has become possible.

In their experiments, the researchers focused the x-ray laser pulses onto a crystal made from pure germanium. High-precision mirrors

The SPring-8 Angstrom Compact Free Electron Laser (SACLA) facility in Harima.

developed over the past decade with partners from Osaka University made it possible to achieve the high light intensities needed to induce a third-order nonlinear process known as two-photon absorption, in which two x-ray photons are absorbed simultaneously and a photon of higher-energy light is emitted. The two-photon absorption process was verified by analyzing the emitted light using a special x-ray spectrometer.

The successful observation of two-photon absorption opens up the possibility of observing a broad range of related phenomena, comments Tamasaku. "Our first demonstration of this third-order nonlinear effect successfully removes this experimental barrier, so that experimental and theoretical physicists can now design experiments using x-ray two-photon absorption, and theorists can consider the possible applications for such processes."

Reference

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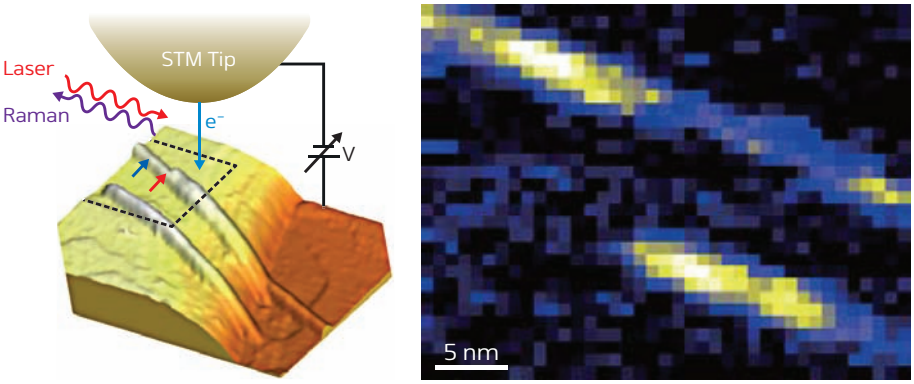
Carbon nanotubes go under the microscope

Ultrahigh-resolution optical imaging of individual carbon nanotubes is now possible under user-friendly conditions

Carbon nanotubes are expected to be used in a myriad of applications ranging from military protective clothing to hydrogen storage. Due to their nanometer dimensions, however, the structure and surface chemistry of individual carbon nanotubes cannot be easily studied using conventional techniques. Norihiko Hayazawa and colleagues from the Near Field NanoPhotonics Research Team at the RIKEN Center for Advanced Photonics have now developed a high-resolution microscopy technique that can resolve individual carbon nanotubes under ambient conditions[1].



A schematic of the scanning tunneling microscope-based tip-enhanced Raman spectroscopy (STM-TERS) procedure (left) and resulting image (right).

Raman spectroscopy is widely used to probe the characteristics of materials with high precision. It involves exciting the material surface with a laser and then measuring the change in laser energy after it is scattered from the surface. Tip-enhanced Raman spectroscopy (TERS) is used to achieve close to molecular resolution by passing a metallic tip across the material surface to enhance the Raman signals of nearby molecules. TERS using an atomic force microscope (AFM) tip is capable of simultaneously assessing the structure and surface chemistry of materials at a resolution of around 10–20 nanometers —far below the diffraction limit of conventional optical microscopes.

Replacing the AFM tip with a scanning tunneling microscope (STM) tip has recently been shown to improve the resolution of the technique dramatically. The position of the metallic STM tip can be controlled more precisely than that of an AFM, making it possible to scan a material with a gap between the tip and surface of less than 1 nanometer. Strong coupling between electronic resonances called 'plasmons' of the tip and material surface across this narrow gap in STM-TERS further improves the resolution of the technique (see image).

"With our STM-TERS system, we have achieved resolution of 1.7 nanometers, meaning that carbon nanotubes can be visualized at the dimensions of their diameter," explains Hayazawa. "This makes it possible for the first time to extract the local property of the carbon nanotubes optically without averaging."

Whereas previous nanoscale STM-based techniques and STM-TERS methods have required cryogenic temperatures and ultrahigh vacuums, the STM-TERS technique developed by Hayazawa's team can be used with a compact chamber at ambient pressure and temperature. This significantly broadens the range of materials that can be probed. "DNA sequencing, protein dynamics on biological membranes, and organic solar cells all require ambient conditions," explains Hayazawa.

As well as using the technique to probe other materials at ultrahigh resolution, the researchers hope to be able to reveal previously undiscovered physical properties of carbon nanotubes.

Reference

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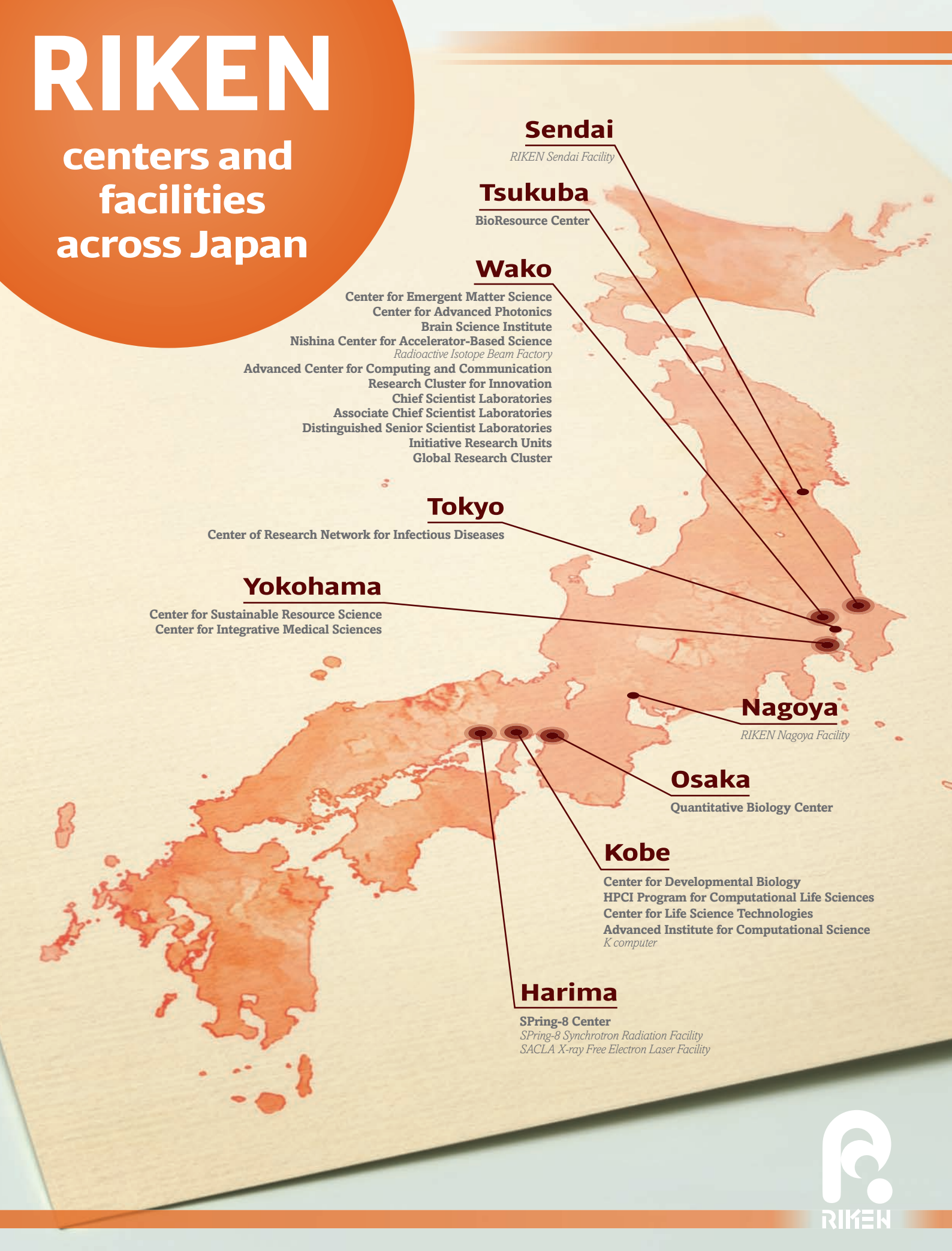

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