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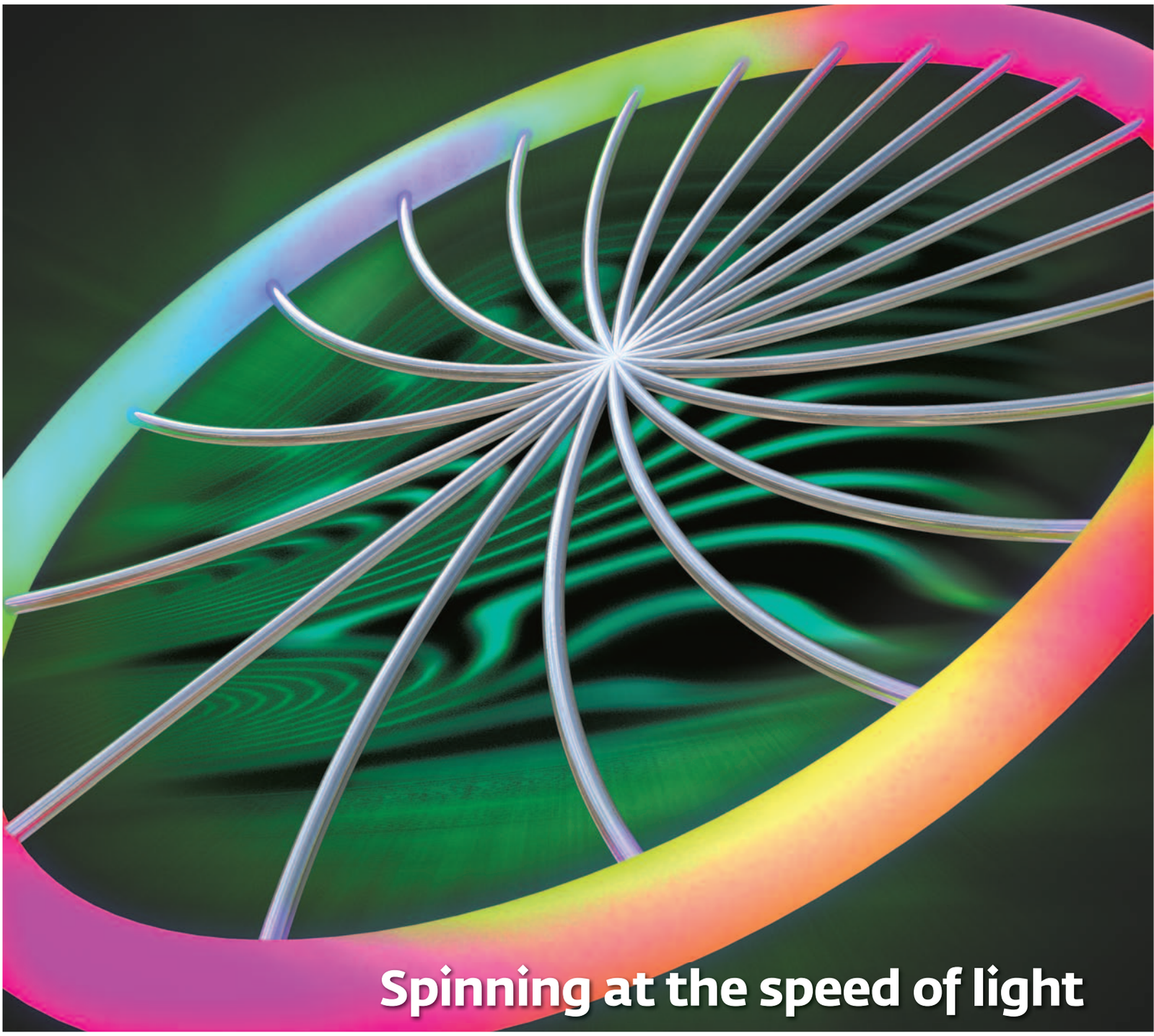

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RIKEN

Biology

Pinpointing the origins of arthritis

A large genome-wide association study helps researchers in Japan unravel connections between rheumatoid arthritis and ethnicity

A large, RIKEN-led, international team of genome researchers has found nine new associations of gene regions or loci with the autoimmune disease rheumatoid arthritis. The loci, found in ethnically Japanese populations, had been undetected in previous European studies. Three of the loci are also associated with other autoimmune conditions in the Japanese, one with systemic lupus and two with Graves' disease.

The researchers, led by Kazuhiko Yamamoto of the RIKEN Center for Genomic Medicine in Yokohama, Japan, believe they have now identified almost all the important genes involved in rheumatoid arthritis in people of Asian heritage. The work, published recently in *Nature Genetics*[1], demonstrates that there are loci associated with rheumatoid arthritis both specific to Asian populations and shared with people of European heritage.

"In time, we think the genes we have identified will be good candidates as drug targets," says senior research scientist Yuta Kochi, one of the corresponding authors of the paper. "And we hope that eventually we will be able to predict outcomes such as the severity of the disease or likely drug response given an individual's genotype."

Rheumatoid arthritis is a chronic and painful inflammatory condition that can afflict many tissues and organs, but mainly attacks flexible joints causing cells to generate excess fluid and swell (Fig. 1). Although it can affect people of any age, rheumatoid arthritis is normally considered a condition of ageing typically beginning to affect sufferers at between 40 and 50 years old.

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Figure 1: Rheumatoid arthritis in the joints is a debilitating condition, particularly for the elderly.

Making sense of large data sets

The researchers used genome-wide association (GWA) studies to determine the location of genes with links to the development of the condition. Such studies use microarray technology to compare patterns of the gene variants or alleles in a population of people with a particular condition—in this case rheumatoid arthritis—with those of people who do not have the disease. When a specific allele is found to be significantly more frequent in people with a disease, then it is said to be 'associated' with that disease. These associated alleles are then considered to mark a region of the human genome that influences the risk of developing that disease.

In the case of rheumatoid arthritis, previous GWA studies in European populations by other researchers had unearthed more than 35 genes associated with susceptibility to the condition. The RIKEN-led team, however, believed that studies in Asian populations might reveal further ethnically specific genetic associations (Fig. 2), as had been the case for other medical conditions reported in the literature.

GWA studies can discriminate susceptibility even more finely if several populations from different sources are combined in a meta-analysis that provides

Figure 2: The Japanese population has specific genetic associations with autoimmune diseases such as rheumatoid arthritis.

larger numbers and greater variability. Thus, Yamamoto and his colleagues initially combined the data from the Biobank Japan Project, Kyoto University and the Institute of Rheumatology Rheumatoid Arthritis for populations of ethnically Japanese people.

In total, across about 2 million gene variants, they analyzed the DNA of 4,074 individuals suffering from rheumatoid arthritis and compared these data against 16,891 people who did not have the disease. They found seven regions that showed significant association with rheumatoid arthritis. Four were previously known from the European studies and three were new discoveries.

Yamamoto and colleagues then conducted another study: they compared two independent groups of 5,277 Japanese rheumatoid arthritis cases against 21,684 controls and added them to the first study in a combined meta-analysis. This resulted in the discovery of another six previously unknown regions of genetic association.

Widening the net

From previous studies, other researchers had reported that for some regions, susceptibility to rheumatoid arthritis was associated with increased risk to other autoimmune diseases. Yamamoto and colleagues therefore undertook GWA studies of populations of Japanese people suffering from systemic lupus erythematosis or from Graves' disease. They observed significant association of one rheumatoid arthritis locus with lupus and two others with Graves' disease.

Finally the team conducted a multi-ancestry comparative analysis between Japanese and European groups. They found significant associations with rheumatoid arthritis at 22 loci in the Japanese population and 36 in the European. Of these, 14 of the loci were shared.

"This analysis provided evidence of significant overlap in the genetic risks of rheumatoid arthritis between Japanese and Europeans," the researchers note. Even so the spectrum of genes associated with rheumatoid arthritis in Asian populations is substantially different from those identified in European populations.

"We think our findings could have several applications, although these will take time to develop," Kochi says.

"First, we suggest that the genes identified by GWA studies will provide useful candidates to test as drug targets. Second, because different alleles may influence the functioning of an individual, they may also influence the course of the disease in an individual. So, knowing the genotype may allow us to predict the outcomes of a disease in individuals, such as severity or response to drugs," he explains.

"In order to pursue these ideas, we will undertake functional studies of the genes to investigate how they cause rheumatoid arthritis. In addition, in collaboration with researchers in hospitals, we will undertake clinical studies to investigate the association between the different alleles and clinical outcomes." ■

1. Okada, Y., Terao, C., Ikari, K., Kochi, Y., Ohmura, K., Suzuki, A., Kawaguchi, T., Stahl, E.A., Kurreeman, F.A.S., Nishida, N., *et al*. Meta-analysis identifies nine new loci associated with rheumatoid arthritis in the Japanese population. *Nature Genetics* **44**, 511–516 (2012).

ABOUT THE RESEARCHER

Kazuhiko Yamamoto graduated from The University of Tokyo School of Medicine in 1977. After finishing his training and residency in internal medicine and rheumatology, he conducted basic research in the laboratory of Professor T. Tada in Tokyo and Professor G. Haemmerling in Heidelberg. He then started his own research projects and also served as clinical rheumatologist at The University of Tokyo Hospital. He then moved to St. Marianna University School of Medicine as an associate professor and to the Medical Institute of Bioregulation, Kyushu University, as a professor. He is now a professor and chairman of the Department of Allergy and Rheumatology, The University of Tokyo Graduate School of Medicine. He also serves as a team leader of the Laboratory of Autoimmune Diseases, Center for Genomic Medicine, RIKEN. Yamamoto's research interests include genetic analyses of rheumatoid arthritis and other autoimmune diseases, T-cell receptor gene transfer for controlling autoimmune disorders and characterization of newly identified regulatory T cells.

Rotations at the speed of light

An effect occurring for rotating objects at the speed of light has surprising relevance to everyday applications

It is tempting to believe that effects arising from Einstein's theory of relativity, where objects move at speeds close to the speed of light, arise mainly at very large length scales, for example the movement of planets and stars. However, as Konstantin Bliokh and Franco Nori from the RIKEN Advanced Science Institute have demonstrated, this is not necessarily so. The researchers have shown that a combination of relativistic motions and rotation effects can lead to a rather general phenomenon that occurs for a range of objects, from black holes to small beams of light or electrons[1].

When an object is moving close to the speed of light, relativistic effects occur. For example, to an external observer an object moving very fast appears squeezed in the direction of the object's motion (Fig. 1). This so-called Lorentz contraction arises from the timing in which light from the fast-moving object arrives at the observer. Bliokh and Nori have now shown that if such an object also rotates at the same time, for example a flywheel, then the rotating motion is also affected. The spokes in the wheel appear distorted in a way that makes them seem denser in one direction than the other. This is a general effect. For electron beams for example, it would appear as if the electrons accumulate mainly on one side. Hence, this effect has been named a relativistic Hall effect, as it is an analog of the usual Hall effect, where moving electrons in a magnetic field accumulate on one side of a material.

The effect on the spokes of a wheel bears a striking similarity to a problem in conventional photography known as the rolling-shutter effect. There, the way a CMOS-based image sensor, in for example a mobile phone camera, is continuously read out from one side to the other causes distortions that look very similar to the spokes of a flywheel. "The rolling shutter effect emulates the relativistic deformations as it introduces a mathematically very similar time delay to an object as the relativistic effects," explains Bliokh.

Such analogies to photo and video cameras might also point to wider possibilities of observing the relativistic Hall effect in real-world systems that are mathematically identical to relativistic motions. But it is applicable to truly relativistic systems as well. "The relativistic Hall effect can play a role in astrophysical systems involving rotating black holes or vortex-like beams of light," says Nori. ■

1. Bliokh, K.Y. & Nori, F. Relativistic Hall effect. *Physical Review Letters* **108**, 120403 (2012).

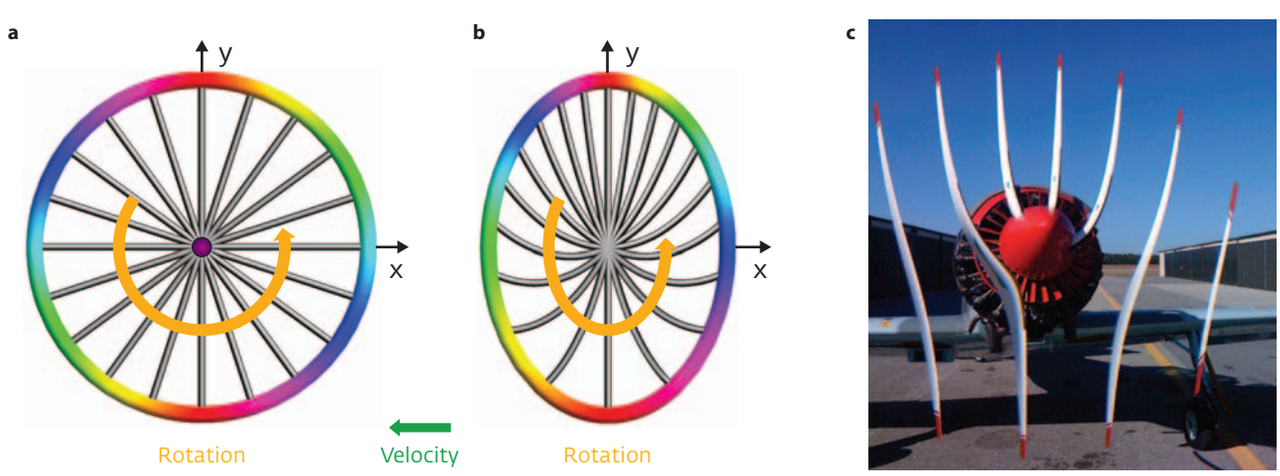

Figure 1: Relativistic flywheel (a and b): When a round flywheel (a) moves horizontally, close to the speed of light, it appears distorted to an observer (b). The rolling-shutter effect (c): CMOS camera chips are continuously read out from left to right of the image. This can lead to distortions of fast-moving objects that are strikingly similar to the relativistic Hall effect.

Magnetic vortices with electric sense

Observation of magnetic vortices in an insulating material suggests a route to energy-efficient electronic devices

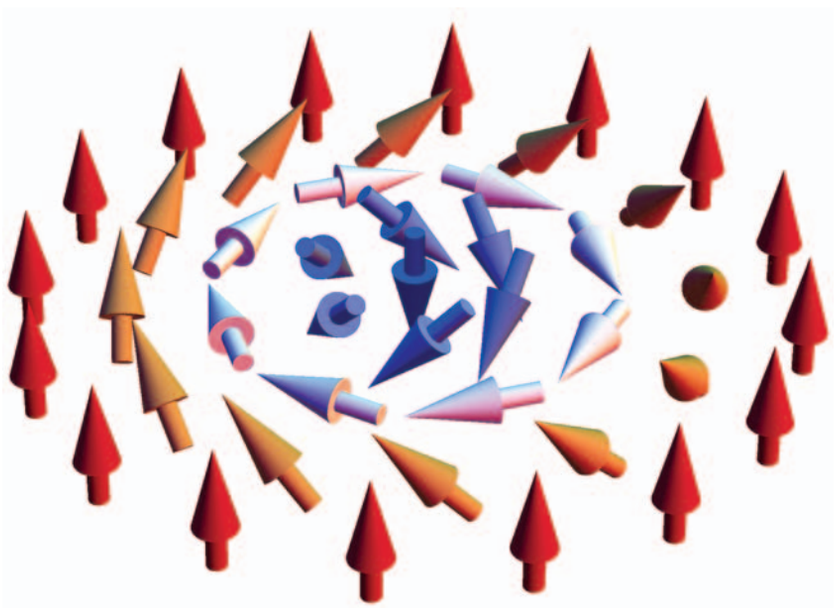

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Figure 1: An artistic representation of a magnetic vortex. In magnetic materials, spins—represented here as arrows—can adopt arrangements such as the skyrmion structure shown above that are robust against external perturbations.

In the field of magnetic materials, a rapidly expanding area of study concerns stable nanometer-scale spin arrangements. Spins are the fundamental magnetic entities in solids, and patterns made of several spins could be useful components in applications such as memory devices. Applied physicist Shinichiro Seki and his team in Japan, including members from the RIKEN Advanced Science Institute, Wako, now report the observation of one such spin pattern, called a 'skyrmion', in a material where it can be manipulated with electrical fields[1]. This finding could help to make future devices based on spin structures more energy-efficient.

Skyrmions are magnetic structures in which spins adopt a vortex pattern (Fig. 1). They are a famed example of spin textures that are, by virtue of their specific spatial arrangements, robust against external perturbations. This robustness is important for device applications. Also, skyrmions are only tens of nanometers in diameter, making them promising candidates for use in compact electronic components.

In the past few years, scientists have observed skyrmions in a range of metals, which are conducting materials. Now, Seki and his colleagues have detected skyrmions in an insulating material, Cu_2OSeO_3. In addition to being non-conductive, Cu_2OSeO_3 has another important property: the spins—that is, the elementary magnetic units—are ordered in well-defined patterns as are their electric counterparts. "Materials with both magnetic and electric order are called multiferroics," explains Seki. "For Cu_2OSeO_3, emergence of electric ordering has now been reported for the first time."

The coupling of magnetic and electric order in this material has important ramifications. "Our analysis suggests that each skyrmion can carry some form of electric order, which implies that the dynamics of skyrmions is controllable through external electric fields," says Seki. This kind of skyrmion manipulation is not possible in conducting materials, where electric fields cause electrons to flow, which in turn leads to detrimental heating effects.

Progress in this field is fast. In recent weeks, further evidence has been reported that skyrmions induce electric order, and additional experiments and analysis by Seki and colleagues[2], supported by theoretical work by collaborators in China and the United States[3], helped in identifying the microscopic origin of magnetoelectric coupling in Cu_2OSeO_3.

In light of these findings, Seki expects that other insulating materials may host skyrmions or related spin arrangements. "Recent theories predict that skyrmions may appear in a broad class of magnetic materials," he says. "We therefore strongly expect further discoveries of various types of spin structures with unique magnetoelectric properties." ■

1. Seki, S., Yu, X.Z., Ishiwata, S. & Tokura, Y. Observation of skyrmions in a multiferroic material. *Science* **336**, 198–201 (2012).
2. Seki, S., Ishiwata, S. & Tokura, Y. Magnetoelectric nature of skyrmions in a chiral magnetic insulator Cu_2OSeO_3. Preprint at http://arxiv.org/abs/1206.4404 (2012).
3. Yang, J.H., Li, Z.L., Lu, X.Z., Whangbo, M.-H., Wei, S.-H., Gong, X.G. & Xiang, H.J. Strong Dzyaloshinskii–Moriya interaction and origin of ferroelectricity in Cu_2OSeO_3. Preprint at http://arxiv.org/abs/1206.4792 (2012).

Nanoscale constrictions open up

State-of-the-art transport measurements reveal that certain geometries of bottleneck junctions have unexpected effects on particle flow

If people bump into one another too often at a building's entrance, the owner could opt to build a larger doorway. But physicists from the RIKEN Advanced Science Institute in Wako have shown that this intuitive solution may not always work—especially at the nanoscale. The team of David Rees, Hiroo Totsuji, and Kimitoshi Kono has discovered that when crystalline electrons pass through a narrow 'nanoconstriction', particle movement sometimes decreases as the bottleneck gets wider[1].

Normally, researchers have trouble understanding how electron systems move through constrictions because of the multiple classical and quantum forces acting on these particles. The RIKEN team recently solved this problem by studying surface-state electrons on liquid helium cooled to nearly absolute zero[2]. At these temperatures electrons are trapped in a two-dimensional layer on the helium surface, where the only interactions present are classical charge repulsions between electrons, creating an ideal environment to study the fundamentals of correlated particle transport.

The researchers constructed a special point-contact device on a silicon wafer to observe the dynamic electron transport (Fig. 1). A series of tiny grooves on the wafer first draws the low-temperature liquid helium into two opposing 'reservoir' regions through capillary action. Then, a voltage-controlled 'gate' controls the current of surface electrons between the reservoirs by gradually opening a narrow bottleneck junction.

Rees and colleagues reduced the temperature of the device below 1 K to freeze the surface electrons into a triangular lattice known as a Wigner crystal. The team then applied a potential between the reservoirs to slowly push the electron crystal through the bottleneck. Surprisingly, the movement of these crystalline electrons was hardly straightforward: at periodic intervals, current suddenly dipped when the gate opened wider.

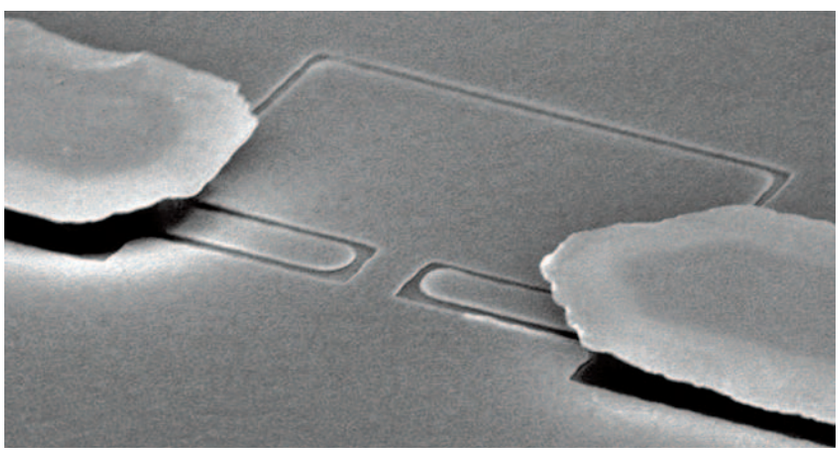

Figure 1: A scanning electron microscope image of a microchannel device that squeezes electron crystals on the surface of liquid helium through a narrow bottleneck (center).

Molecular dynamics simulations revealed that interactions between the Wigner crystal lattice and the nanoconstriction's geometry caused the unusual current oscillations. If the nanoconstriction had a different width than the spacing between electrons in the Wigner system, the electron crystal weakened and flowed unimpeded between the reservoirs. But when the confinement potential matched the lattice spacing, the crystal stayed strong, preventing particles from moving through the bottleneck.

Rees notes that these oscillations between weak and strong crystal regimes may have implications for other systems involving constrictions. "For systems such as colloids in a thin channel, grains passing through a silo, or even pedestrians walking through doorways, our results show that the geometry of such bottlenecks can be crucial in determining the rate of particle flow," he says. ■

1 Rees, D.G., Totsuji, H. & Kono, K. Commensurability-dependent transport of a Wigner crystal in a nanoconstriction. *Physical Review Letters* **108,** 176801 (2012).

2. Rees, D.G., Kuroda, I., Marrache-Kikuchi, C.A., Höfer, M., Leiderer, P. & Kono, K. Point-contact transport properties of strongly correlated electrons on liquid helium. *Physical Review Letters* **106,** 026803 (2011).

Stabilizing heavy bonds

Success in preparing and isolating a heavy ketone paves the way for synthesizing novel catalysts

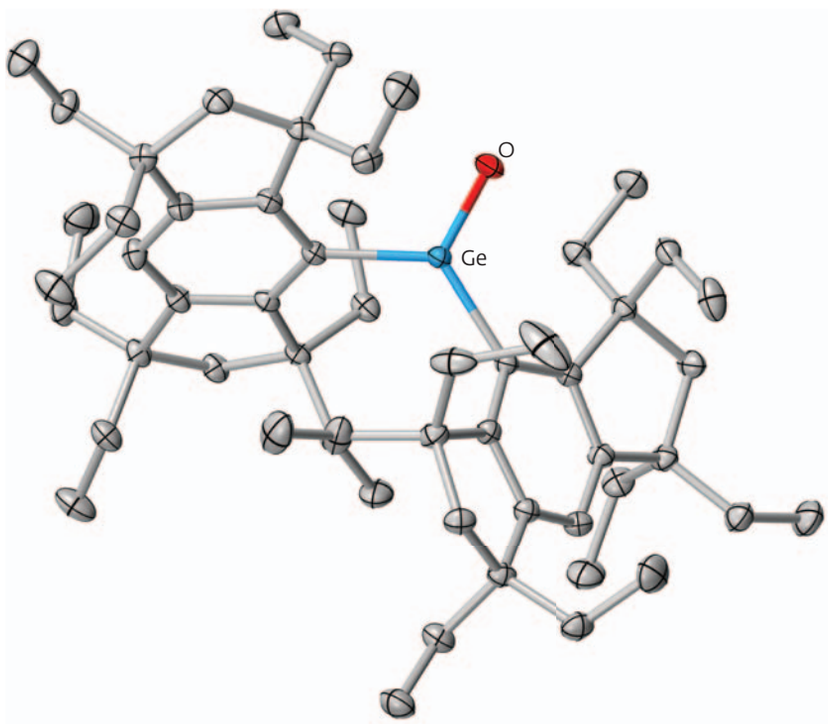

Figure 1: An x-ray molecular structure of germanone protected with bulky Eind groups.

The carbon–oxygen (C=O) double bond is an important chemical motif, particularly in compounds called ketones. Chemists expect that substituting the carbon for a heavier atom would produce 'heavy ketones', which are attractive targets to further understanding of chemical bonding and for synthesizing novel molecules. Now, Kohei Tamao at the RIKEN Advanced Science Institute in Wako, Japan, and colleagues have—for the first time—isolated a heavy ketone, where the carbon is replaced by germanium[1].

The researchers were motivated by the importance of ketones in both nature and industry: carbon dioxide (CO_2) contains two C=O double bonds, while acetone, or nail polish remover, has only one. Ketones react reliably with numerous chemicals and are important precursors in the synthesis of pharmaceuticals. Tamao and colleagues therefore investigated replacing the carbon in ketones with heavier atoms—in this case germanium—a first step in the quest for unusual catalytic systems.

Molecules are made of atoms joined together by chemical bonds. The properties of each atom affect the strength and reactivity of the bond. Electronegative atoms, such as oxygen, pull electrons towards themselves and become negatively charged, which make the bonds highly reactive.

"We expected the germanium–oxygen double bonds (Ge=O) to be extraordinarily polarized because of the difference in electronegativity between the two atoms," Tsukasa Matsuo, co-author, explains. Previous attempts to make molecules with a Ge=O resulted in spontaneous combination to form oligomers—or short polymers. The researchers realized that by using bulky protecting groups, they could stabilize the heavy ketones and prevent them reacting together. These large bulky groups or atoms prevent polymerization by simply getting in the way of other chemicals that otherwise react with them.

Liangchun Li, another co-author, isolated a germanium ketone, or germanone, as colorless crystals by selecting a bulky ligand known as Eind (Fig. 1). With the heavy ketone in hand, the team then compared and contrasted them to normal ketones and investigated both their physical and reactive properties.

The researchers' experiments showed that germanones are more highly reactive than regular ketones. For instance, they react readily with carbon dioxide and water at room temperature whereas regular ketones do not. Computer simulation models have confirmed this reactivity: they indicate that the Ge=O bond is almost completely charge separated.

The next step for the researchers is to create a silicon–oxygen double bond. "Thanks to this chemistry, we have a chance to develop a new catalytic system involving germanones, such as oxygen atom transfer reactions," Matsuo notes.■

1. Li, L., Fukawa, T., Matsuo, T., Hashizume, D., Fueno, H., Tanaka, K. & Tamao, K. A stable germanone as the first isolated heavy ketone with a terminal oxygen atom. *Nature Chemistry* **4**, 361–365 (2012).

Stem cells thrive on superficial relationships

Chemically preserved cell surfaces provide a healthy and supportive environment for the cultivation of stem cells

Stem cells are renowned for their capacity to develop into a wide range of mature cell types but they cannot maintain this flexibility on their own. In the body, neighboring cells help maintain this 'pluripotent' state. But to grow these cells in culture, scientists have had to devise a variety of specialized techniques.

This is especially true for embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), which are ESC-like cells derived from adult tissue. To preserve their pluripotency, these cells have typically been grown atop a supporting layer of 'feeder cells'. Now, a strategy developed by a team led by Yoshihiro Ito at the RIKEN Advanced Science Institute, Wako, promises to make ESC and iPSC cultivation considerably easier[1].

Feeder cells provide valuable growth factors for stem cells but also make culture complicated and create opportunities for contamination—an especially serious concern for clinical applications. Early attempts to isolate the key features of feeder cells have fallen short. "It was difficult to culture stem cells on growth-factor immobilized substrates," says Ito. "Feeder cells provide a complex microenvironment that cannot simply be replaced with one or several growth factors."

As an alternative, the researchers subjected feeder cell layers to chemical 'fixation' treatments that killed the cells while physically preserving them and maintaining their external structure largely intact. This resulted in a robust cell culture surface that retained virtually all of the features with which stem cells would typically interact. Mouse iPSCs maintained their pluripotent state even after extensive cultivation on feeder cells that had previously been fixed with either formaldehyde (FA) or glutaraldehyde (GA). GA fixation is a harsher treatment, but Ito and colleagues noted that GA fixed cells also provided a superior substrate, and this GA-fixed layer was robust enough to be washed and reused.

The researchers were pleasantly surprised to find that mouse iPSCs grown in this manner were virtually indistinguishable from those cultured by traditional methods (Fig. 1). "Feeder cells were believed to secrete proteins or other compounds that maintain the growth of undifferentiated stem cells," says Ito. "But fixed cells lose this secretion capability, which shows that providing the right contact microenvironment is more important for iPSCs." Given how rugged the fixed cell layers are, he anticipates that this approach could offer a commercially viable cell culture tool once it has been tested and optimized for cultivation of human iPSCs. ■

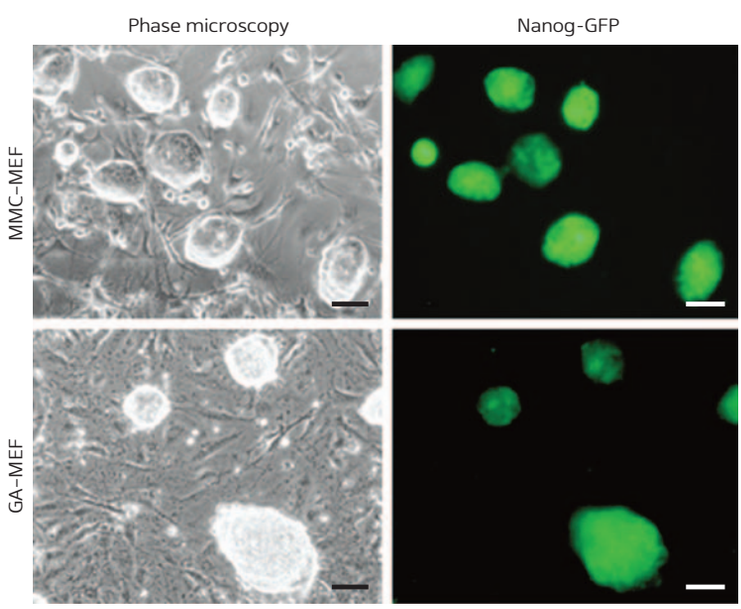

Figure 1: iPSCs cultivated atop a 'feeder' layer of mouse embryonic fibroblasts (top left) maintain expression of a fluorescent pluripotency marker (top right; green). However, these cells also thrive (bottom left) and maintain their pluripotency (bottom right) when grown on a glutaraldehyde-fixed feeder cell layer.

1. Yue, X.-S., Fujishiro, M., Nishioka, C., Arai, T., Takahashi, E., Gong, J.-S., Akaike, T. & Ito, Y. Feeder cells support the culture of induced pluripotent stem cells even after chemical fixation. *PLoS ONE* **7,** e32707 (2012).

The statistics of neuronal synchrony

A new statistical method provides a way to analyze synchronized neural activity in animals

Researchers from the RIKEN Brain Science Institute have developed a new method of statistical analysis that can estimate the extent to which the activity of multiple neurons is group-wise synchronized[1].

The synchronized electrical activity of multiple neurons gives rise to coordinated network activity. This cooperative activity is highly dynamic and widely thought to be critical for organization behavior and cognitive processes.

Current methods for the statistical analysis of synchronized activity can analyze pairs of cells or detect the existence of correlations between multiple neurons. However, there is no way of accurately determining specific groups of neurons that interact with each other, and how this activity changes with time.

Working in collaboration with researchers from Germany and the U.S. Hideaki Shimazaki and colleagues developed a statistical tool that extracts information about the interactions of neuronal activity recorded from the brains of animals as they perform actions. To do so, the team adapted and extended an algorithm typically used in GPS tracking software, allowing them to measure interacting groups of neurons and how these interactions change with time.

The researchers tested their method on computer simulations of sequences of neuronal impulses produced by two and three neurons. These initial tests suggested that the higher-level analyses performed by the new method would enable discovery of the network activity that cannot be revealed by interactions between pairs of cells.

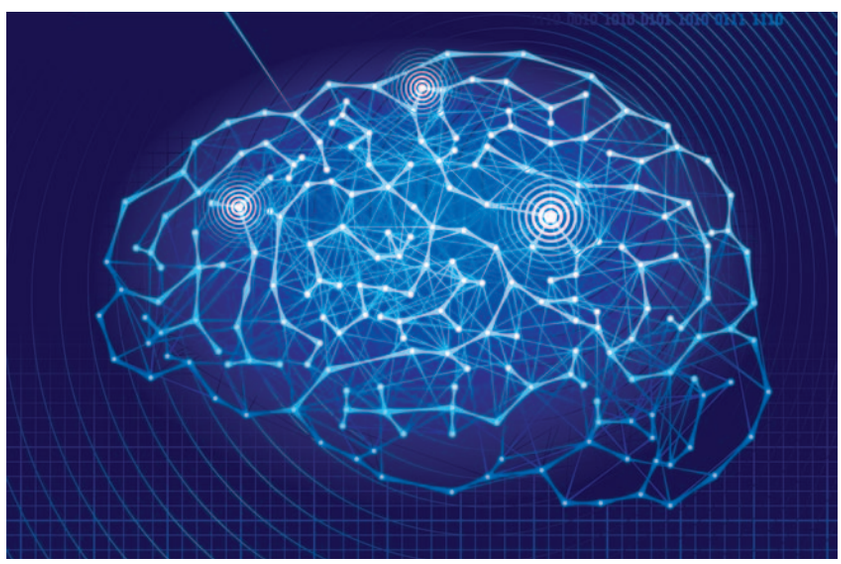

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Figure 1: Scientists can now analyze synchronized activity of multiple neurons from behaving animals with a new statistical method.

To confirm this, Shimazaki and his colleagues applied their method to a set of data obtained by simultaneous recordings of multiple neurons in the primary motor cortex in the monkey. These data were recorded in an earlier study, which demonstrated that the synchronized activity of two neurons increases when a monkey is preparing for motor action. However, this earlier study did not determine whether the cells were part of a larger group that coordinate their activity[2].

The new method enabled Shimazaki and his colleagues to analyze the activity of three neurons simultaneously. Their analyses revealed that synchronicity of the three neurons increases during the preparatory period, confirming that the neurons examined in the earlier study do indeed belong to a larger group of cells that act together.

"Currently the method is limited to analysis of a few neurons," says Shimazaki. "We would like to extend that number to hundreds or more. This would considerably increase the probability of observing assemblies of neurons involved in planning and controlling behavior." ■

1. Shimazaki, H., Amari, S., Brown, E.N. & Grün, S. State-space analysis of time-varying higher-order spike correlation for multiple neural spike train data. *PLoS Computational Biology* **8**, e1002385 (2012).
2. Riehle, A., Grün, S., Diesmann, M. & Aertsen, A. Spike synchronization and rate modulation differentially involved in motor cortical function. *Science* **278**, 1950–1953 (1997).

A new starring role for astrocytes

Identification of a novel membrane barrier in astrocytes may illuminate how neurological signaling is disrupted in patients with Alzheimer's and epilepsy

Astrocytes, previously thought of as helper cells for neurons, have recently been shown to send signals themselves. The signals are chemical not electrical and astrocytes send them to neurons, vascular cells and other astrocytes to improve the efficiency of synaptic signaling. A team led by Katsuhiko Mikoshiba and Hiroko Bannai at the RIKEN Brain Science Institute, Wako, have described the mechanism that allows astrocytes to signal each cell in their network individually[1].

Named for their star-like shape, astrocytes have a central 'soma' and many ray-like arms connecting to the cells they regulate. Healthy astrocytes send separate Ca^{2+} signals through each ray, called a 'process'. Signaling was known to be regulated by a receptor in the cellular membrane called the metabotropic glutamate receptor (mGluR5), but it was unclear how the signals were confined to individual processes. Understanding this specificity may be therapeutically important because in brains affected with Alzheimer's disease or epilepsy astrocytes send global signals, more like a megaphone broadcast than the telephone calls made by healthy astrocytes (Fig. 1).

To understand how astrocyte signaling is regulated, the researchers tagged individual mGluR5 receptors with quantum dots—semiconductor nano-crystals that emit light when excited—then observed how the receptors migrated through the fluid membrane. Video footage revealed that receptors did not pass from the process to the soma. In normal astrocytes, the mGluR5-selective diffusion barrier could, by compartmentalization of Ca^{2+} signaling, allow each process to regulate its contacting partners independently.

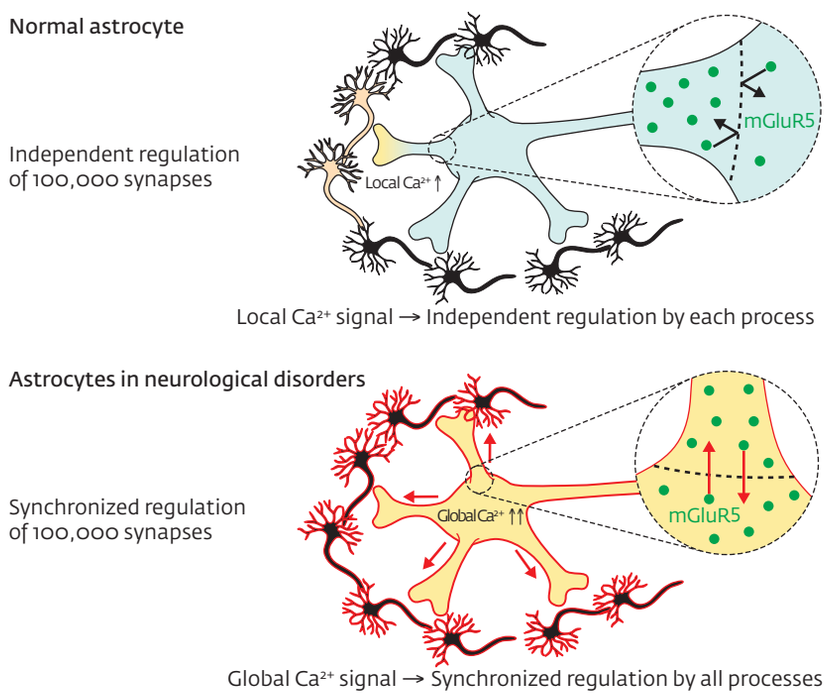

Figure 1: Healthy astrocytes send separate signals through each process, while astrocytes in neurological disorders send synchronous signals to their entire network.

To investigate the character of the barrier, Mikoshiba's team attempted to undermine it. Over-expression of mGluR5 overwhelmed the barrier, which they infer is made of proteins that interact with the cytosolic portion of mGluR5. Each barrier protein pairs with a single mGluR5 molecule and prevents it from crossing to the soma. However, the number of barrier proteins is finite and an overabundance of mGluR5 leaves some receptors free to cross into the soma, thus enabling propagation of global signals through every process in the astrocyte.

Experimental models of Alzheimer's disease and epilepsy have shown increased concentrations of mGlu5 in astrocytes. The researchers believe that understanding the molecular nature of the diffusion barrier will provide new targets for the treatment of these conditions. Once they reveal the molecular nature of the barrier, the team hopes to produce a transgenic mouse lacking the astrocytic barrier protein. "We are very curious to know the effect of global astrocytic Ca^{2+} signaling on the neuronal network and neuro-vascular coupling," says Mikoshiba. ■

1. Arizono, M., Bannai, H., Nakamura, K., Niwa, F., Enomoto, M., Matsu-ura, T., Miyamoto, A., Sherwood, M.W., Nakamura, T. & Mikoshiba, K. Receptor-selective diffusion barrier enhances sensitivity of astrocytic processes to metabotropic glutamate receptor stimulation. *Science Signaling* **5**, ra27 (2012).

Lessons from herbicide tolerance

Discovery of an uptake mechanism for key cellular components in *Arabidopsis* plants gives insights into potential cancer treatments and GM crops

Polyamines are widespread and important organic compounds involved in multiple cellular processes in living organisms. Their levels are highly regulated through a combination of processes including synthesis, breakdown and transport. However, the mechanisms of polyamine transport are still largely unknown.

Since the widely used herbicide paraquat (methyl viologen) follows the same transport pathways as polyamines, Miki Fujita and colleagues at the RIKEN Plant Science Center in Tsukuba, Japan, analyzed natural variability in paraquat susceptibility in the model plant *Arabidopsis thaliana*. They found that the gene *resistance to methyl viologen 1* (*RMV1*) is involved in the transport of both paraquat and polyamines in *Arabidopsis*[1].

Paraquat's herbicidal activity results from causing oxidative stress in plant tissues. However, Fujita and her team found that most paraquat-tolerant varieties of *Arabidopsis* remained susceptible to other oxidative stress inducers, implying tolerance was due to reduced uptake rather than limitation of paraquat's oxidative effects. By crossing different paraquat-resistant varieties, they showed that paraquat-tolerance was linked to a single locus, while cloning and complementation techniques revealed its genomic location. Using transgenic plants in which they had placed the fluorescent jellyfish protein, green fluorescent protein (GFP), under the same genetic control as *RMV1*, the researchers revealed that its expression is localized to the cell membrane (Fig. 1), indicating a likely role in uptake or transport of molecules into cells.

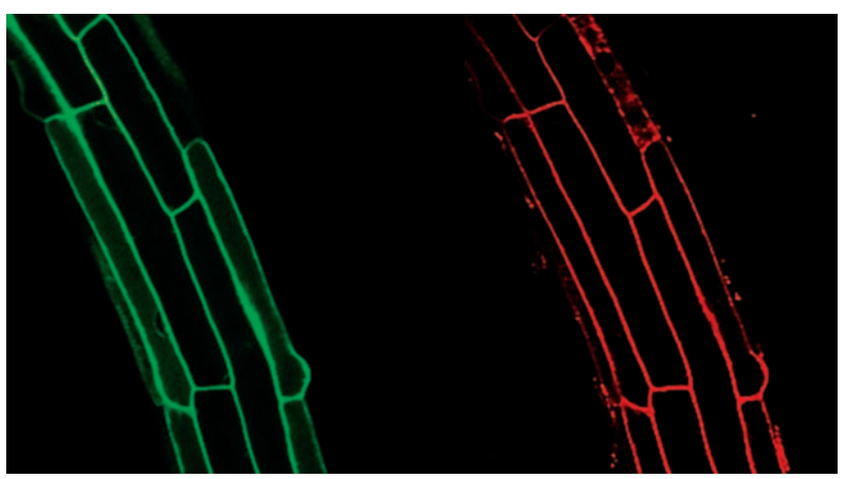

Figure 1: Fluorescent labeling reveals expression of the *RMV1* gene, localized to cell membranes of *Arabidopsis* root cells. The left panel shows the GFP-labeling, the right panel is under a red filter showing plasma membrane stained with 'FM 4-64'.

Fujita and colleagues then verified that *RMV1* mediates paraquat uptake by studying *Arabidopsis* plants in which *RMV1* is inactivated by mutation: these plants showed higher paraquat tolerance, indicating reduced paraquat uptake or transport. Many of these mutants also showed increased tolerance to certain polyamines, indicating that the same locus is involved in polyamine transport. However this effect was relatively slight, suggesting to Fujita and her team that other polyamine transporter genes might exist in the *Arabidopsis* genome.

Fujita is now aiming to identify other polyamine transporters and investigate their biological significance in terms of plant growth and stress responses. The identification of such transporters may facilitate developments in fields as diverse as cancer treatments—reduced polyamine uptake is linked to the efficacy of certain anticancer drugs—and GM crops. "It is really urgent to create the next generation of herbicide-resistant crops," Fujita says. Some hurdles remain, however: paraquat is banned in many countries due to its toxicity, but these findings are a step towards creating more environmentally friendly herbicide-resistant crops. ■

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Unsung heroes of antibody production

The discovery of a cell-specific regulatory mechanism helps overturn a long-standing model of immune system function

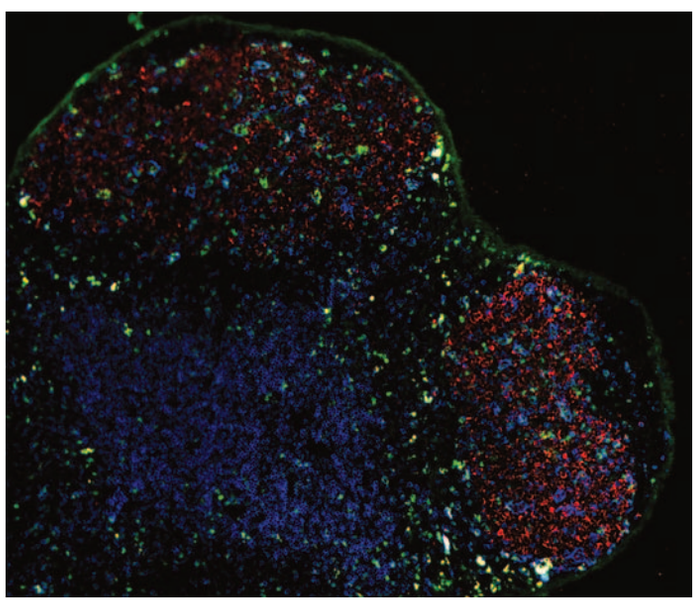

Figure 1: IL-4-secreting Tfh cells (green) reside alongside the B cells of the germinal center (red), and enable them to produce a full spectrum of antibody subtypes.

B cells are the body's antibody factories, standing by to churn out molecules that selectively target foreign threats as a component of the humoral immune response. However, this process also requires T cells to secrete a protein known as interleukin-4 (IL-4), which promotes a mechanism called 'class switching' that enables production of functionally specialized antibody subtypes.

It remains unclear exactly which T cells generate the IL-4 signal. Although many scientists favor the T_h2 subclass of helper T cells, there are strong arguments against this model as well. "This subset of cells cannot get into the follicles where these B cells are located," explains Masato Kubo of the RIKEN Research Center for Allergy and Immunology, Yokohama. "Nevertheless, most textbooks say that T_h2 cells control the antibody response."

Kubo and colleagues have now provided strong evidence that a recently discovered class of follicular T (Tfh) cells generates the IL-4 signal that kicks B cells into high gear[1]. In a previous study, his group identified several stretches of DNA within the gene encoding IL-4 that might regulate its activity[2]. One candidate sequence, CNS2, had little effect on T_h2 production of IL-4, but mice lacking this genomic region nevertheless showed severe defects in class-switching. "We started thinking other T cell subsets might be responsible for IL-4-mediated humoral immune responses," he says.

By placing a fluorescent gene under the control of CNS2, the researchers showed that this regulatory region is specifically active in Tfh cells. As their name suggests, Tfh cells reside within follicles, in close proximity to the germinal centers where B cells mature. They also actively secrete IL-4 (Fig. 1), albeit by an apparently distinct mechanism from T_h2 cells. Kubo's team found that mutant mice lacking CNS2 can produce mature Tfh cells, but these cells expressed greatly reduced levels of IL-4. By comparison, T_h2 cells were only minimally affected. Nevertheless, these genetically modified mice showed striking deficits in their production of several antibody subclasses, including immunoglobulin E (IgE) and G1 (IgG1).

These findings proved surprising at multiple levels. "IL-4 is a critical cytokine for controlling IgG1 and IgE antibody responses, but its expression in T_h2 and Tfh cells is independently regulated by distinct elements," says Kubo. "Furthermore, Tfh and not T_h2 cells are the T cell subset responsible for T_h2-type humoral immune responses." He and his colleagues are working to identify the factors that act on CNS2, which could ultimately offer useful drug targets for controlling autoimmune disease by restraining antibody production. ■

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2. Tanaka, S., Motomura, Y., Suzuki, Y., Yagi, R., Inoue, H., Miyatake, S. & Kubo, M. The enhancer HS2 critically regulates GATA-3-mediated Il4 transcription in T_h2 cells. *Nature Immunology* **12,** 77–85 (2011).

Understanding proteins in packed places

Numerical simulations reveal how protein function is affected by its naturally congested environment

The average cell of an organism is a packed environment, filled in particular with proteins—the workhorse molecules of the cell that are involved in almost every biochemical process taking place within it. A method to understand how this crowded environment affects the way that proteins function has been developed by Ryuhei Harada, at the RIKEN Advanced Institute for Computational Science in Kobe, and two RIKEN colleagues[1].

Although cells are well known to be crowded environments, studying protein function experimentally under crowded conditions is difficult, so researchers typically study proteins in dilute solutions instead. To get a more realistic picture of protein function as it actually takes place in nature, Harada and his colleagues use computer modeling.

"We carry out atom-level molecular dynamics simulations under crowded environments as a first step for understanding the cell," Harada says. The researchers started by studying how the properties of the water molecules that surround the protein change when only a thin film of water separate one protein from its neighbor (Fig. 1). These properties can in turn profoundly influence the protein's behavior.

"We were very surprised by the size of the effect that we calculated on water's properties due to crowding," Harada says. To measure the impact of these altered properties on protein function, the researchers modeled two small, simple proteins, each a sub-unit of a natural protein. They showed that crowding lowered the diffusion rates of the proteins through the cellular environment, which slowed protein dynamics.

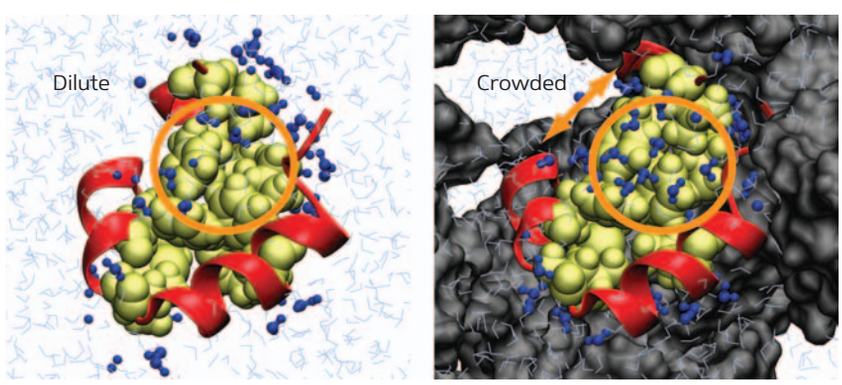

Figure 1: Under crowded conditions (right), more water molecules (blue) are found in the hydrophobic region (circled) of the villin protein (green and red), compared to dilute conditions (left). Neighboring proteins are colored black.

Harada and colleagues also noted other effects. "Probably the most important finding from our study is the significant decrease in dielectric response of water in highly crowded environments," Harada says. Water's dielectric response relates to the way that it stores electrical charge. "The reduction of dielectric response directly impacts protein conformations and stabilities under crowded conditions."

In particular, the reduced dielectric constant enhances the interactions between positively and negatively charged points in the protein structure that help to hold together its three-dimensional 'secondary' structure. The team found that the relationship is a linear one: the greater the crowding, the stronger the effect.

Harada and his colleagues are now investigating whether the observations that they made studying simple proteins will hold true for larger, more complex ones. They are also expanding their calculations to consider multiple proteins simultaneously. "We also want to develop general methodologies to simulate cellular environments based on this research," Harada says. ■

1. Harada, R., Sugita, Y. & Feig, M. Protein crowding affects hydration structure and dynamics. *Journal of the American Chemical Society* **134,** 4842–4849 (2012).

HIROYASU ISHIDA

Manager
Innovation Promotion Section
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Crafting a career at RIKEN

How and when did you join RIKEN?

I joined RIKEN in April 1997 and have been working here for 15 years. I decided to join RIKEN after learning about its status as a world-class research institute for basic research. I also connected with the idea of using scientific and technological achievements to make our societies more prosperous.

Please tell us about your work at RIKEN.

Since joining RIKEN, I have worked in several positions at the Wako Institute, Tsukuba Institute and the Ministry of Education, Culture, Sports, Science & Technology (MEXT).

I was first assigned to the General Affairs Division at the Wako Institute, where I was in charge of general administrative tasks. Then I moved to the BioResource Center at the Tsukuba Institute, where I oversaw administrative support for supplying biological materials to researchers in Japan and around the world.

While at MEXT, my responsibilities were related to acquiring funding for research on accelerator science, and support and liaison with MEXT committee offices and accelerator science researchers at various institutions.

For seven years I was based at the Technology Transfer Office, where I handled collaborative research agreements and patent licensing agreements between RIKEN and private companies in Japan and overseas.

Now as manager of the Innovation Promotion Section, I focus on how to create a better working environment for our staff and how to keep their motivation high. It is a continual learning experience for me, but I am building on the knowledge and experience I have gained at RIKEN up until now.

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

My time at the Technology Transfer Office has left the biggest impression on me. My work there specifically involved drafting both collaborative research and patent licensing agreements that were entered into with private companies, checking the contractual details, and negotiating with the parties until they were satisfied and signed the agreements.

When moving forward with a collaborative research agreement, it is important to first know what the researchers want to accomplish, and then to have a basic grasp of the research contents. When negotiating with a company, it is important to clearly understand their requirements for entering the collaboration. Knowledge of the law, intellectual property rights and business customs is also necessary.

I started with very little knowledge in these areas, but the coordinators I worked with gave me tremendous support. Originally from private companies, they were very knowledgeable about company management and the latest trends in technology. From working with them, I learned how to express my viewpoints and requests, and how to create win-win relationships with everyone involved.

These experiences have been very useful even in the new work I am doing now.

What is the best thing about working at RIKEN?

RIKEN's strengths are the diversity of staff working here with various skills, knowledge and backgrounds. RIKEN also fosters a very close relationship between researchers and administration. Both sides discuss how to achieve what researchers want to do, which creates a work environment conducive to producing top-quality output.

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President of the Slovak Republic visits RIKEN

On 28 June 2012, the President of the Slovak Republic Ivan Gašparovič and his wife Silvia Gašparovičová visited RIKEN headquarters in Wako, Saitama Prefecture, Japan, leading a distinguished delegation of 26 guests. The Slovak delegation included the Deputy Prime Minister and the Minister of Foreign Affairs, the Minister of Finance, the President of the Slovak Academy of Sciences (SAS), along with the directors of several institutes under the SAS, and officials from Cormenius University and the University of Economics in Bratislava. They were accompanied by Drahomir Stos, Ambassador of the Slovak Republic in Japan, and Takamatsu Akira, Ambassador of Japan to the Slovak Republic.

The President of the Slovak Republic Ivan Gašparovič (front row, center left) and distinguished guests of the Slovak delegation met with RIKEN President Ryoji Noyori (front row, center) and RIKEN officials during a five-day visit to Japan.

After an official welcome by RIKEN President Ryoji Noyori, the delegates had an opportunity to exchange views on the future of deepening cooperation and research exchanges between Japan and the Slovak Republic. A presentation on some of the latest developments in brain science and robotics was given by a Slovak engineer, Peter Jurica of the Laboratory for Advanced Brain Signal Processing at the RIKEN Brain Science Institute, one of RIKEN's approximately 600 foreign researchers, staff, and students.

During their official five-day visit to Japan from 26 to 30 June 2012, President Gašparovič and his wife were widely praised for visiting several towns and cities in the disaster-hit Tohoku region, including Sendai and Ishinomaki city, where they attended a wreath-laying ceremony at Kadonowaki Elementary School. Their visit also included a meeting with Japanese Prime Minster Yoshihiko Noda, and the Slovak presidential couple were received by the Emperor and Empress of Japan at the Imperial Palace in Tokyo.

Japan and Slovakia will be celebrating the twentieth anniversary of the establishment of diplomatic relations between the two nations in 2013, and various activities are being planned to deepen bilateral cooperation in areas such as trade, culture, education and tourism, as well as academic exchanges and developing future cooperation in the fields of science and technology. ■

The RIKEN ASI Science Dojo draws on the past to look to the future

As part of RIKEN's science education and training activities the RIKEN Advanced Science Institute (ASI) carried out a series of weekly public lectures dubbed the "ASI Science Dojo" between May and July 2012. During the "dojo" – which takes its name from the Japanese word for a martial arts training hall – Principal Investigators in fields covered by the activities of the ASI introduced the background and fundamentals of their research to a mixed audience of scientists and the general public. As well as helping to fulfill RIKEN's public science remit, the sessions provide a valuable opportunity for scientists with different specialisms to gain a better understanding of each others' research fields and also to contribute to the ongoing education of young researchers.

The first ASI Science Dojo of 2012 consisted of nine lectures on topics in the biosciences, culminating with a special lecture by Mitsuhiro Yanagida, a world-renowned molecular biologist and a professor of the Okinawa Institute of Science and Technology. In his seminar Yanagida drew on his extensive experience to discuss the past. The history of Japanese biological research which dates back to the 1860s in particular is not well known amongst younger researchers, contended Yanagida, as the country's many achievements in the field were fragmentary and diverse and have therefore not formed a large and coherent narrative. "Life sciences research in Japan has a rich history of achievement," said Yanagida. "However we have not done enough to spread our success story around the world. I think it is important for the future of Japan that we get the 'Story of Japan' out to an international audience."

Further sessions of the ASI Science Dojo program are planned for the near future and will focus on the topic of materials science research. All lectures in the series will be open to the public and will be offered free of charge. ■

Eminent molecular biologist, Mitsuhiro Yanagida, gave a special lecture at the first ASI Science Dojo of 2012.

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

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

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