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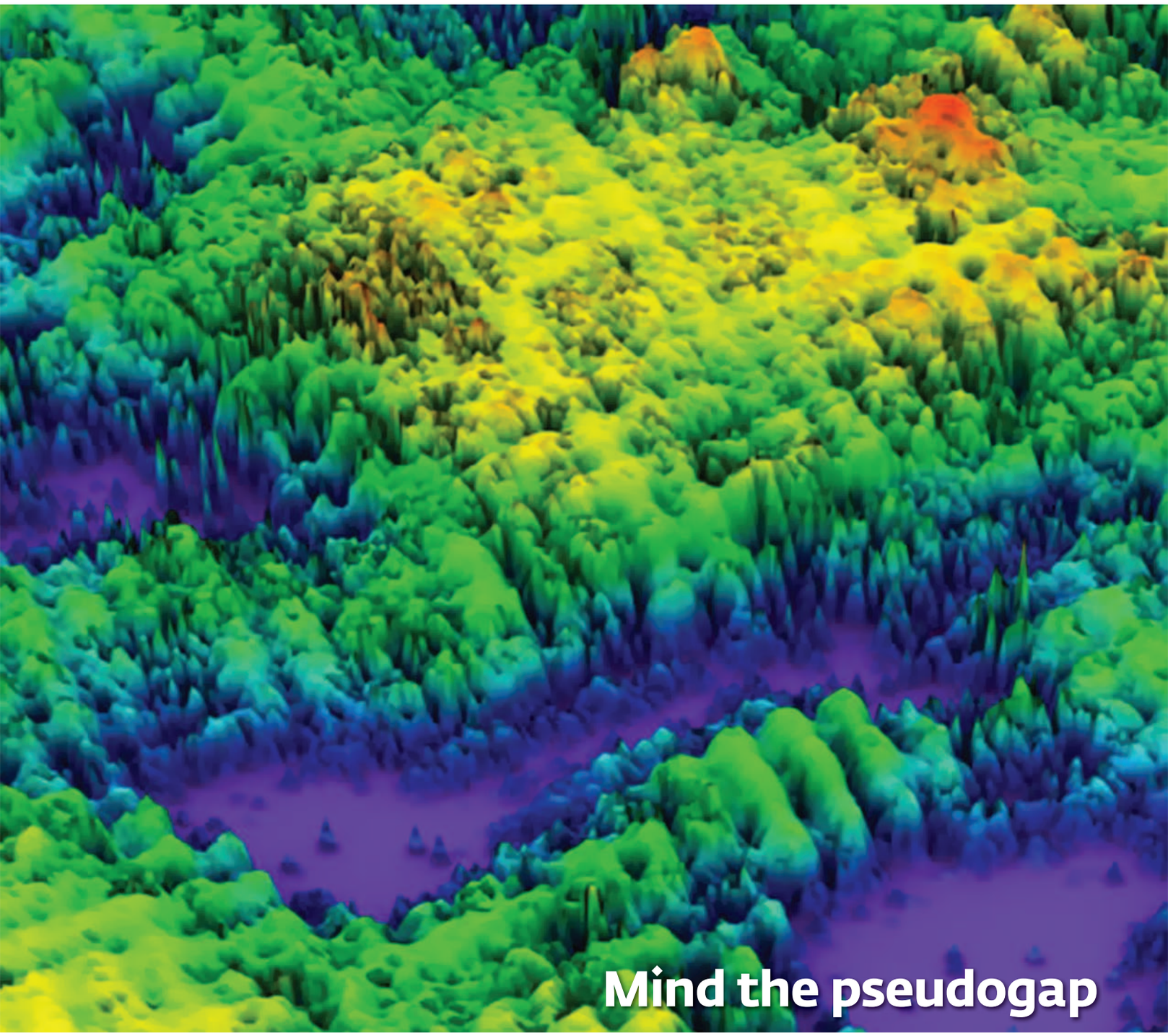

Mind the pseudogap

RIKEN

Biology

Combating cellular heat stress

When the body's exposed to heat shock, a recently identified 'firefighter' molecule transports heat-response proteins into the cell nucleus to prevent damage

Akin to sending firefighters in response to a house fire, cells deploy a brigade of heat shock proteins (HSPs) when exposed to environmental stresses, such as a rapid shift in temperature, or 'heat shock' (Fig. 1). Heat shock is a stressor that perturbs a cell's equilibrium, or homeostasis, by causing proteins to misfold and aggregate. HSPs are molecular chaperones that are essential for maintaining this homeostasis. In response to heat shock and other cellular stresses, HSP expression increases and these proteins are transported into the nucleus to protect cells from stress-induced misfolding.

Cell biologists think that the trafficking of most proteins into and out of the nucleus is controlled by the importin-β family proteins. However, the activity of the protein RanGTP, which provides the energy for importin-β-mediated nuclear trafficking, is reduced under heat shock stress conditions. Thus, the molecular mechanism facilitating the transport of HSPs into the nucleus has remained unclear.

Now, a team of cell biologists led by Naoko Imamoto at the RIKEN Advanced Science Institute, Wako, has solved this apparent paradox. In a study published recently in the journal *Cell*[1], Imamoto and her colleagues have identified a previously unknown nuclear import pathway that is triggered by heat-shock stress. Further, the pathway includes a hitherto unknown protein that binds to HSPs and carries them into the nucleus through pores in the nuclear membrane.

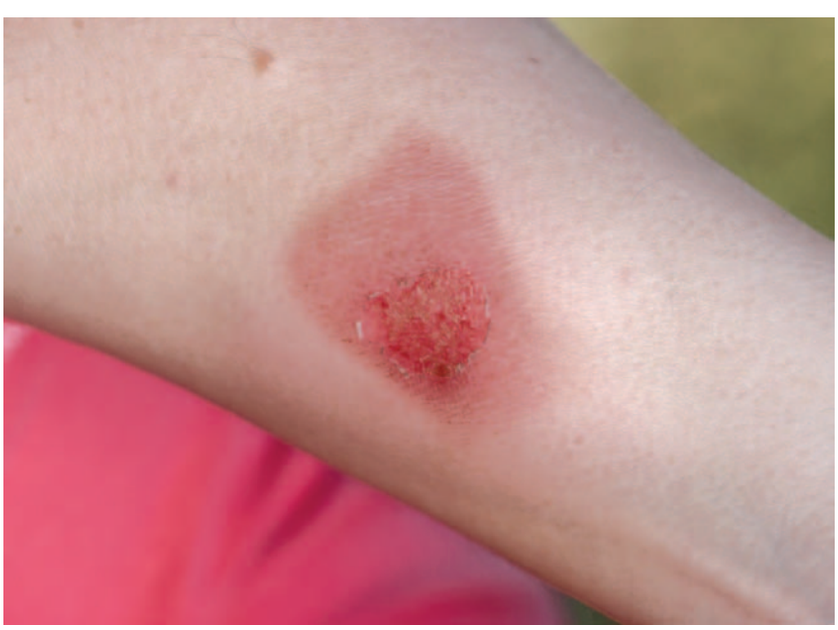

Figure 1: In response to heat shock, such as a steam burn to the forearm, cells respond to protect nuclear proteins from damage.

Understanding transport under heat stress

In one series of experiments, the researchers reconstituted the nuclear trafficking of HSPs in Petri dishes. Initially, they removed the fluid from normal and heat-shocked HeLa cells, which were originally derived from a human cervical tumor. They used it to make four different preparations, in which fluid taken from normal or heat-shocked cells was added to normal cells or those treated with heat shock, and then incubated with a fluorescently labeled HSP called Hsc70.

This revealed that Hsc70 is only transported into the nucleus in those preparations containing fluid removed from heat shock-treated cells. This occurred only when ATP, a ubiquitous molecule used by all cells as an energy source, was added to the Petri dishes (Fig. 2).

Proteins migrate into the nucleus through a protein assembly called the nuclear pore complex (NPC), which spans the membrane of the nucleus. NPCs are enormous structures consisting of some 30 different molecules called nucleoporins. The process of transporting proteins through NPCs involves several stages. First, the 'cargo' protein set for transportation binds to a carrier molecule. The carrier translocates through NPCs by interacting with the nucleoporins, and releases its cargo once inside.

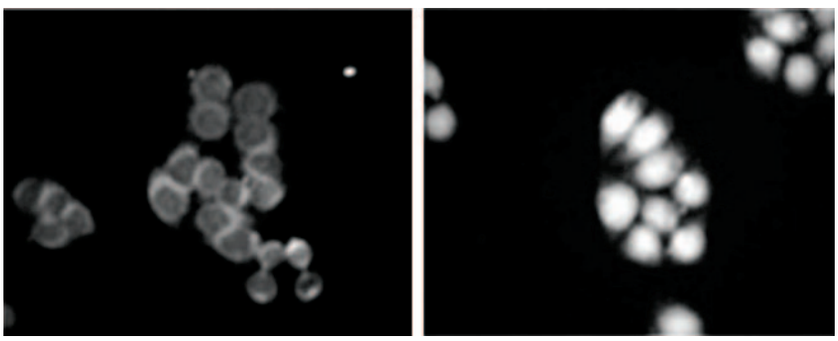

Figure 2: Fluorescently labeled Hsc70 localizes to the nucleus in reconstituted preparations from heat-shocked cells (right) but not normal cells (left).

"Nuclear transport is my main research topic," says Imamoto. "We identified importin-β and importin-α in 1995. The precise mechanism of how these carrier proteins translocate through the nuclear pore complexes (NPCs) is still unclear, but it is widely accepted to occur through interactions between the carriers and water-repellant regions of NPC components called nucleoporins."

Carrier proteins, such as importins, are strongly repelled by water. Imamoto and her colleagues exploited this fact to isolate the carrier that binds to Hsc70. They again removed the fluid from heat-shocked cells, and then ran it over a water-repellent column containing protein-binding beads. In this way, they identified a small protein that is encoded by a gene on chromosome 11, and named it Hikeshi, after the firefighters known as *hikeshi* during the Edo period in Japan.

"Nuclear transport is key for regulating cellular function, and there are diverse transport pathways mediated by different importin family in cells. We wondered whether different transport pathways function under different cellular conditions," Imamoto explains. "Nobody expected that a completely different nuclear transport pathway would exist in cells."

Further investigation revealed that: expression levels of the Hikeshi protein increase by 2- to 3-fold in response to heat shock; Hikeshi both binds to Hsc70 and interacts with nucleoporins; and binding of Hikeshi to Hsc70 is regulated by co-chaperones. Hikeshi binds only to Hsc70 molecules that have ATP attached to them, and the proteins dissociate from each other when ATP is broken down to ADP, a reaction that releases energy.

Based on this evidence, Imamoto and her colleagues propose that the energy needed for Hikeshi-mediated transport could come from the ATP–ADP cycle of Hsp70s, instead of that of RanGTP, as in the case of importin β family-mediated transport. The researchers also found that Hikeshi is sufficient and essential for the nuclear transport of Hsc70, and that it reverses the damage that heat shock causes to the nucleus. In fact, cells cannot survive heat shock treatment without it.

Solving one mystery reveals another

Imamoto and her team's findings show that cells activate distinct nuclear transport pathways under normal and heat shock stress conditions. When normal conditions prevail, the importin-β molecules mediate the transport of proteins into and out of the nucleus. This pathway is deactivated by heat shock stress, during which cells switch to the Hikeshi pathway, so that Hsc70 can be accumulated into the nucleus to counteract the cellular damages induced by heat-shock stress.

Exactly how cells switch from one nuclear transport pathway to the other, however, is not clear. It is possible that Hikeshi carries cargoes other than Hsc70 into the nucleus during heat shock stress conditions.

"Further study of the switching mechanism should provide new insights into the regulation of both the nuclear transport system and the molecular chaperone system," says Imamoto. "Another intriguing question is the molecular mechanism underlying activation of Hikeshi-mediated nuclear transport."

1. Kose, S., Furuta, M. & Imamoto, N. Hikeshi, a nuclear import carrier for Hsp70s, protects cells from heat shock-induced nuclear damage. *Cell* **149**, 578–589 (2012).

ABOUT THE RESEARCHER

Naoko Imamoto was born in Osaka, Japan. She received her Bachelor's degree in Biology from the Osaka University Faculty of Science, her Master's degree in Medical Science from Osaka University, and her PhD from Osaka University Medical School. Part of her initial work was the identification of importin α and importin β as nuclear transport receptors and carriers in 1995. In 2000, she became independent as an associate professor at the National Institute of Genetics. In 2002 Imamoto took up the position of chief scientist and established the Cellular Dynamics Laboratory at RIKEN. Her current research areas involve the mechanism and regulation of nuclear transport and the biogenesis of the nuclear pore complex. Nuclear transport affects various cellular processes, while nuclear pore complex biogenesis involves various cellular processes such as cell cycle, nuclear envelope, membrane lipid and chromatin. Imamoto aims to develop fusion domains as a new area of research.

Growing understanding of superconductivity

Atomic-resolution images provide fresh insights into a mysterious state found in superconducting materials

Superconductivity describes the state of certain materials when they conduct electric currents without any resistance. For superconductivity to develop, these materials generally have to be cooled to temperatures below roughly −140 °C, depending on the material. The family of materials that requires the least amount of cooling is known as cuprate superconductors. These compounds are therefore technologically interesting, but scientists are still working to understand the fundamental mechanism underlying superconductivity in these materials. In fact, determining what makes cuprate superconductors tick is one of the grand challenges in condensed-matter physics.

Now, an international research team, led by Yuhki Kohsaka and Hidenori Takagi from the RIKEN Advanced Science Institute, Wako, has provided fresh perspectives on the behavior of these systems[1]. The researchers, from Japan, the US and the UK, took atomic-resolution images of a cuprate material as it undergoes the transition from a 'normal' solid to a superconductor. A broad pool of data has been accumulated since the discovery of cuprate superconductors in 1986, but only a few studies provided microscopic details of how the superconducting state in cuprate materials emerges.

Using a unique setup, combining an exquisitely stable scanning tunneling microscope with high-quality samples, the researchers studied an enigmatic state known as the 'pseudogap state'. This state appears when the parent compound of their material, $Ca_2CuO_2Cl_2$—which is not a superconductor—is gradually doped with sodium atoms. Once the degree of doping is above a critical level, the material becomes superconducting. At intermediate levels of doping, however, the compound goes through the pseudogap state, whose role is the topic of intense debate among physicists.

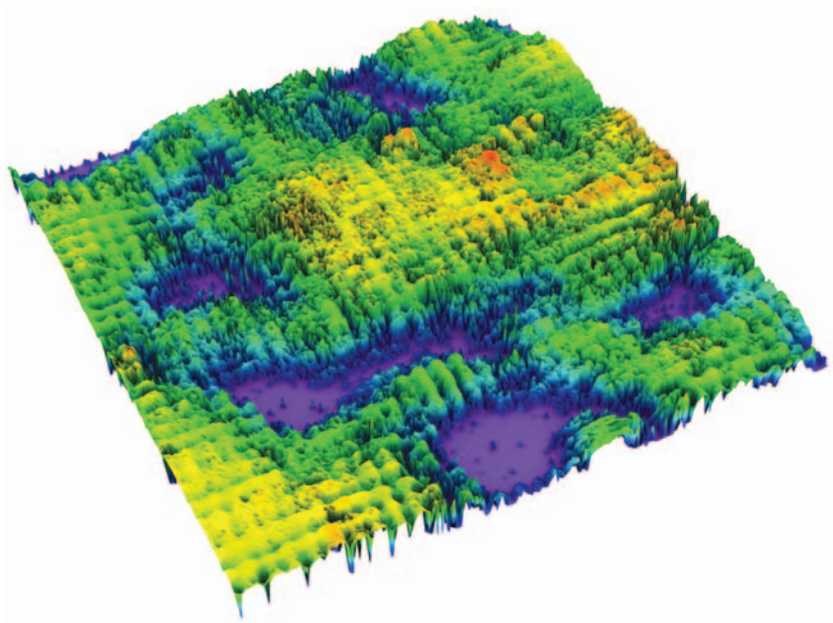

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Figure 1: This scanning-tunneling-spectroscopy image of $Ca_{2-x}Na_xCuO_2Cl_2$ shows the merging of clusters (in green and yellow) where the so-called pseudogap state has developed.

Kohsaka and colleagues found evidence that the pseudogap state may be helpful for emergence of the superconducting state. At very low doping levels, they saw the formation of distinct nanometer-scale clusters that are in the pseudogap state. As they added more dopant atoms, they observed that these clusters start to connect (Fig. 1). Intriguingly, full connection happens just as the material becomes a superconductor.

These are important new insights into the microscopic behavior of cuprate superconductors. But Kohsaka remains cautious: "We do not claim yet a local correlation between the pseudogap and superconductivity. We don't have experimental evidence strong enough to prove such a correlation. But establishing this connection will be an important direction of future study." ■

1. Kohsaka, Y., Hanaguri, T., Azuma, M., Takano, M., Davis, J. C. & Takagi, H. Visualization of the emergence of the pseudogap state and the evolution to superconductivity in a lightly hole-doped Mott insulator. *Nature Physics* **8**, 534–538 (2012).

Measuring microscopic magnets

A recently proposed technique could measure the movement of tiny magnets along magnetic wires with precision millions of times greater than the state-of-the-art method

At microscopic scales, magnetic materials are full of structure. Tiny magnets, called domains, order themselves in ways that control the magnetic properties of the bulk material. Technologies such as hard disk drives exploit this order by storing information in individual domain directions. In the past decade, however, engineers and scientists have focused increasingly on what happens between—rather than within—domains. They have also proposed new logic, memory, sensing and communication devices that manipulate the walls separating neighboring domains. Now, Shin'ichi Hikino from the RIKEN Advanced Science Institute, along with three Japan-based colleagues, has proposed a new and highly sensitive experimental technique for measuring domain wall motion[1].

The researchers adapted the technology behind one of the most precise known measures of voltage. The approach uses two superconductors separated by a thin layer of non-superconducting material. When the thin junction, called a Josephson junction, is exposed to microwave radiation, the voltage across the junction becomes a precise multiple of the microwave frequency. In fact, this multiple can be defined entirely in terms of fundamental physical constants, leading to a measurement precision so high that it is used to define the value of a volt by standards institutes around the world.

Hikino and colleagues realized that, if the non-superconducting layer was made from a ferromagnet containing two or more magnetic domains (Fig. 1), the motion of the domain walls could be measured with a similarly high precision. This is due to the fact that each moving wall has a magnetic moment, which affects the current and voltage across the junction. In their theoretically proposed experimental setup, a voltage applied to the ferromagnetic wire causes its domain walls to oscillate. Then, the measured current and voltage across the junction become a precise multiple of the walls' oscillation frequency, which plays the role of the microwave radiation frequency usually used.

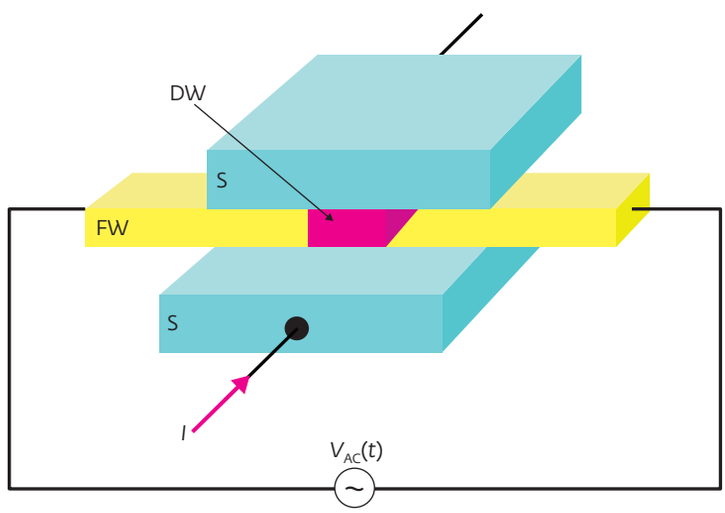


Figure 1: Schematic illustration of a ferromagnetic Josephson junction used for detecting magnetic domain wall motion. A domain wall (pink) inside a ferromagnetic wire (FW) between two superconducting electrodes (S) is driven into oscillatory motion by a voltage (bottom) applied horizontally across the wire. The current and voltage vertically across the junction become a precise multiple of the domain wall oscillation frequency.

As a result, the junction voltage can be used to measure the wall oscillation frequency with a precision six orders of magnitude better than conventional methods, which have errors of one part in a thousand or worse. The expected size of both the current and voltage across the junction are in the micro-ampere and micro-volt range for a ferromagnetic wire with a thickness of five nanometers. These quantities are easily measured, making the proposal experimentally feasible.

Hikino expects the technique to be applicable to uniform domain wall motion as well, and believes the approach could form the basis for a standard unit for magnetic dynamics, similar to the volt. ■

1 Hikino, S., Mori, M., Koshibae, W. & Maekawa, S. Towards precise measurement of oscillatory domain wall by ferromagnetic Josephson junction. *Applied Physics Letters* **100**, 152402 (2012).

How rattling around could save energy

More efficient energy use could result from a direct experimental observation of atomic behavior inside nanoscale cages

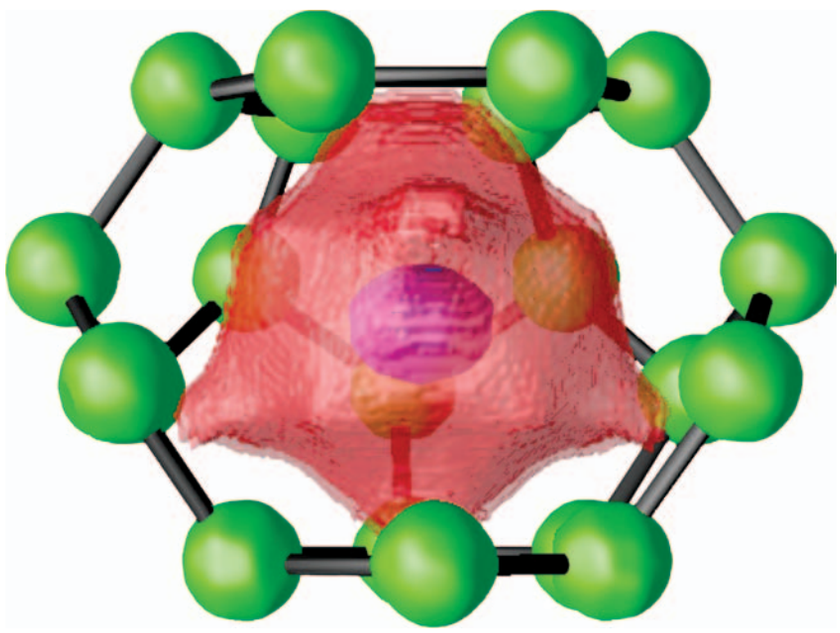


Figure 1: X-ray visualization of the interaction area (red) of a guest atom (pink) surrounded by the cage atoms (green). A larger interaction area results in a smaller thermal conductivity.

Thermoelectric generators can make better use of the excess heat generated by machines by converting temperature differences directly back into electricity. Now, Masaki Takata from the RIKEN SPring-8 Center, Harima, working in collaboration with colleagues at institutions across Japan, have shown how the thermoelectric properties of a class of materials known as clathrates are enhanced by their unusual atomic structure[1], thus demonstrating a potential route to more efficient energy usage.

Clathrates consist of a lattice-work of cages of 'host' atoms inside which loosely bound 'guest' atoms are trapped. Akin to a baby's rattle, the guest atoms are free to rattle around their confines. By using x-rays to visualize the electrostatic interactions between atoms, Takata and his colleagues' work provides a more complete understanding of how rattling influences the thermoelectric properties of clathrates. Their methodology enabled them to construct a quantitative relation between the degree of guest–atom motion and thermal conductivity, which will be useful for engineering future materials for thermoelectric applications.

When an ordinary solid material is heated, the thermal energy causes the atoms to vibrate. In response to this motion, neighboring atoms also start oscillating, thereby conducting heat through the medium. Scientists believe that the rattling in clathrates disrupts and scatters the flow of thermal energy. Consequently, the energy passes to neighboring atoms in a non-coherent way, thus inhibiting thermal conduction. Importantly, this inhibition occurs with little effect on the electrical conductivity of the material. "In thermoelectric generators, low thermal conductivity is preferable for keeping the temperature difference, whereas high electrical conductivity is essential for reducing loss of the generated electricity," explains Akihiko Fujiwara from the Japan Synchrotron Radiation Research Institute, a co-author of the paper.

Takata and his colleagues studied clathrates with polyhedron cages made of germanium and gallium, containing a guest atom of either barium or strontium. They fired beams of radiation at the samples and, from the way these beams were deflected, imaged the electrostatic potential in the cage, which clearly exhibited signs of the guest atom rattling. They observed that the clathrates with a larger electrostatic interaction area also displayed a commensurately reduced thermal conductivity (Fig. 1), confirming that rattling is responsible for the improved thermoelectric properties.

"Next we hope to extend our method for visualizing such invisible interactions to other electronic functions, such as superconductivity and ferroelectricity, which will be useful for designing future novel materials," says Fujiwara. ■

1. Fujiwara, A., Sugimoto, K., Shih, C.-H., Tanaka, H., Tang, J., Tanabe, Y., Xu, J., Heguri, S., Tanigaki, K. & Takata, M. Quantitative relation between structure and thermal conductivity in type-I clathrates $X_8Ga_{16}Ge_{30}$ (X = Sr, Ba) based on electrostatic-potential analysis. *Physical Review B* **85**, 144305 (2012).

Efficient and versatile chemistry

More pharmaceuticals could benefit from the stabilizing influence of fluorine atoms, thanks to an innovative reaction method for attaching them

Molecules with potent bioactivity alone are not suitable for new medicines. They must behave well inside the body, being easily absorbed, yet metabolically stable, once circulating through the blood stream. Adding fluorine atoms is a proven way to improve these properties. Now, this approach is set to expand thanks to a technique developed by Mikiko Sodeoka and her colleagues at the RIKEN Advanced Science Institute in Wako[1].

Sodeoka and her team's approach is to attach fluorine in the form of the trifluoromethyl group, $-CF_3$, a small side chain that adds three fluorine atoms in a single transformation. Since trifluoromethyl groups are hydrophobic, they help the drug to infiltrate the body and reach its site of action. The carbon–fluorine bond is also very strong, improving the drug's stability. "These factors are crucial for the development of therapeutic drugs and agrochemicals," Sodeoka says.

Despite the advantages offered by trifluoromethyl groups, the narrow range of substrates to which they can be attached currently has limited their use. While methods for attaching the group at a carbon–carbon double bond are well established, only a few approaches exist for attaching the group at other sites on the molecule.

Sodeoka's strategy was to first functionalize the target molecule with a silicon-based side chain to form an allylsilane, a well-understood functional group. The researchers showed that the allylsilane activates the molecule to react with a suitable trifluoromethyl source, a copper-activated form of a molecule known Togni's reagent. This generates a product in which the trifluoromethyl group is attached at a singly bonded carbon (Fig. 1).

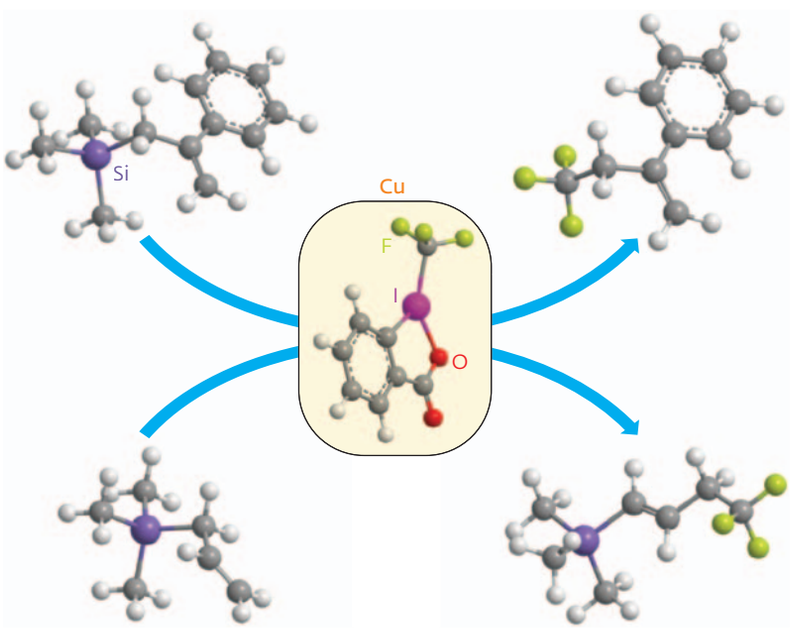


Figure 1: Togni's reagent (center) converts silicon-functionalized starting materials (left) into products incorporating the trifluoromethyl group (right).

"To date, these trifluoromethylated compounds have been difficult to make—our reactions provide a general and efficient method for constructing these types of compounds," Sodeoka says. "We hope that these compounds will contribute to the creation of new trifluoromethylated pharmaceutical drugs."

The process is versatile because the silicon group can be either eliminated or retained during the reaction, depending on the structure of the starting molecule. Leaving the silicon in place offers a way to further functionalize the molecule at that position, if required.

The team is currently investigating the reaction's exact mechanism, particularly regarding the trifluoromethyl source. Only certain copper species work, the researchers found, and the reasons why remain unclear.

Sodeoka and colleagues are also looking to push into new areas of trifluoromethylation chemistry. "We want to develop new types of trifluoromethylation reaction that could provide more complex and useful trifluoromethylated building blocks," she says. ■

1. Shimizu, R., Egami, H., Hamashima, Y. & Sodeoka, M. Copper-catalyzed trifluoromethylation of allylsilanes. *Angewandte Chemie International Edition* **51**, 4577–4580 (2012).

Water's triple play at membrane interfaces

Rapid-fire lasers reveal that water molecules adopt three distinct local structures around model lipid monolayers

Before taking effect, pharmaceuticals that target cell function must cross a crucial barrier: the complex assembly of lipids, proteins, and carbohydrates that make up biological membranes. Recently, scientists realized that besides a membrane's internal components, thin water layers at its inner and outer surfaces may also play important roles in ion and small molecule transport. However, most characterization methods cannot distinguish between bulk and surface water, so identifying water structures around membranes is challenging.

Tahei Tahara and colleagues from the RIKEN Advanced Science Institute in Wako have overcome this problem by developing a technique that uses ultra-short laser pulses to probe interfacial water[1]. Using this technique, known as heterodyne-detected vibrational sum frequency generation (HD-VSFG), the team has now discovered that water adopts three structures at a lipid interface containing atoms with both negative and positive charges[2], providing critical new evidence of 'local' hydration arrangements around different membrane components.

Every human body cell contains a type of lipid called phosphatidylcholines. Their long, saturated hydrocarbon 'tails' enable them to self-assemble into membrane films at water interfaces, held in place by hydrophilic 'head' groups. Unlike most molecules, phosphatidylcholines are zwitterions—they contain separate but oppositely charged components; in this case, a head-group with a negatively charged phosphate and a positively charged amine. Researchers suspected that water behaves in unexpected ways at zwitterionic lipid interfaces, and now they have the quantitative details.

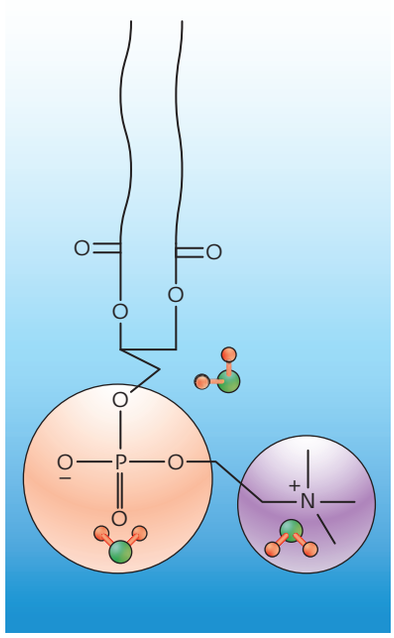


Figure 1: A schematic illustration of three water structures at a model cellular membrane interface: an 'H-up' orientation close to a negative phosphate (bottom left), an 'H-down' arrangement at a positive amine (bottom right), and a structure in a lipid chain (center).

Tahara and colleagues used their HD-VSFG technique to make only surface water molecules vibrate at a phosphatidylcholine interface. Instead of the broad oxygen–hydrogen (OH) bond-stretching vibrations normally seen in water, they spotted a distinct, double-peaked feature with both positive and negative components. Tahara notes this is clear evidence of two different surface water structures: an 'H-down' orientation, with water's hydrogen atoms pointing away from the lipid interface, and the opposite 'H-up' arrangement.

To pinpoint how these two structures arranged at zwitterionic interfaces, the researchers mixed together pure anionic and cationic lipid surfactants in varying amounts. Then using HD-VSFG, they revealed that H-up structures attached to negative phosphates through hydrogen bonding, while H-down structures bonded to positively charged amines—a new concept for phosphatidylcholine interfaces (Fig. 1).

The researchers also saw a third distinct OH vibrational signal: a water structure with H-up orientation in the hydrophobic lipid tail. "We think that insights from this study can lead to better understanding and predictions of protein structure as well as electron transfer processes within cellular membranes," Tahara concludes. ■

1. Nihonyanagi, S., Yamaguchi, S. & Tahara, T. Direct evidence for orientational flip-flop of water molecules at charged interfaces: A heterodyne-detected vibrational sum frequency generation study. *Journal of Chemical Physics* **130**, 204704 (2009).
2. Mondal, J.A., Nihonyanagi, S., Yamaguchi, S. & Tahara, T. Three distinct water structures at a zwitterionic lipid/water interface revealed by heterodyne-detected vibrational sum frequency generation. *Journal of the American Chemical Society* **134**, 7842–7850 (2012).

Extra-active click catalysts

A self-assembling polymeric copper catalyst accelerates a broadly applied chemical reaction to unprecedented speeds

Few recently discovered chemical reactions have proven as powerful as the copper-catalysed Huisgen 1,3-dipolar cycloaddition between azides and alkynes—a transformation better known as a 'click reaction'. The process gets its nickname from the robust, reliable way that the azide and the alkyne organic functional groups 'click' together. From materials science to biochemical applications, this dependable method for joining molecules together has been exploited widely in the decade since its discovery. Now, Yoichi Yamada, Shaheen Sarkar and Yasuhiro Uozumi at the RIKEN Advanced Science Institute in Wako have developed a new form of heterogeneous copper catalyst that promises to make the click reaction more efficient than ever[1].

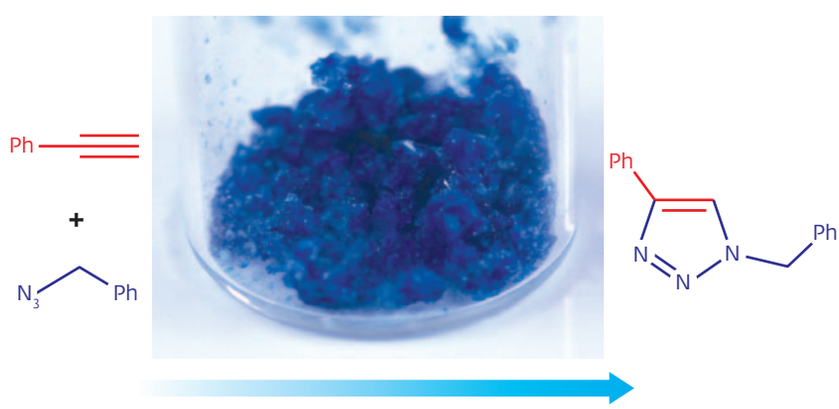


Figure 1: The blue copper catalyst drives the click reaction between an alkyne (left, red) and an azide (left, blue) to form a triazole (right).

Heterogeneous catalysts do not dissolve into the reaction mixture; they remain as a solid inside the reaction flask, offering a catalytic surface on which the reaction can take place. The key advantage of these catalysts is that they can easily be recaptured for re-use at the end of a reaction, often by simple filtration. Their disadvantage is that they are less intimately dispersed with the reactants than catalysts that dissolve, slowing the reaction.

The researchers overcame this disadvantage by embedding their copper within a self-assembled two-component polymer. The polymer backbone is made of a material called isopropylacrylamide, which has a hydrophobic sub-section and a hydrophilic sub-section. Overall, the material acts as an 'amphiphilic sponge': it readily draws in reactants and substrates regardless of their hydrophobicity, Yamada says.

The second polymer component is an imidazole, an electron-donating material that stabilizes and activates the copper to accelerate the click reaction. "The catalytic copper species within the sponge instantaneously react with substrates and reactant to give the products and to regenerate the catalyst (Fig. 1)," Yamada explains.

The material's performance is the best yet reported for a heterogeneous click catalyst, he adds. The best previous materials had turnover numbers below 1,000 before the catalyst would become deactivated, whereas the team's catalyst had a turnover number of 209,000. The catalyst's turnover frequency was also fast, turning reactants into product at a rate of 6,740 conversions per hour.

The re-usable catalyst should find a host of applications, Yamada says. "The catalyst will be applied to the synthesis of pharmaceutical compounds and functional organic materials." The next step for the researchers is to incorporate the catalyst into a 'flow system', in which the catalyst is immobilized within a cartridge through which substrates and reagents are continually pumped, generating a continuous steady stream of product.

1. Yamada, Y. M. A., Sarkar, S. M. & Uozumi, Y. Amphiphilic self-assembled polymeric copper catalyst to parts per million levels: click chemistry. *Journal of the American Chemical Society* **134**, 9285–9290 (2012).

Making sense of misfolded proteins

Synthetic proteins that are improperly folded by design shed light on how protein folding is regulated in the body

The endoplasmic reticulum of cells provides a pivotal quality-control system that eliminates improperly folded, or misfolded, glycoproteins, such as antibodies and hormones. The UDP-glucose:glycoprotein glycotransferase (UGGT) enzyme is central to this system: it binds to incompletely folded proteins and facilitates biochemical reactions that lead to their proper folding. However, the rules governing UGGT's reactivity remain unclear. Now, a synthetic approach is available for biochemists to relate this reactivity with protein folding. The method, which produces a series of intentionally misfolded glycoproteins to probe UGGT's selectivity, was developed by a team led by Yasuhiro Kajihara of Osaka University, working with the Japan Science and Technology Agency's ERATO Glycotrilogy Project, directed by Yukishige Ito of the RIKEN Advanced Science Institute, Wako[1].

Previous investigations relied on denatured glycoproteins obtained from natural sources, but the carbohydrate portion of these compounds usually displayed heterogeneous structures and compositions, making UGGT's properties difficult to assess precisely.

Kajihara, Ito and colleagues synthesized a homogeneous, artificial glycoprotein using a protein known as interleukin-8, which has a well-understood architecture. Interleukin-8 contains sulfur-based functional groups that form so-called disulfide linkages, allowing the researchers to introduce folds at desired positions either within or between protein strands and generate structures with varying folded geometries, or isomers.

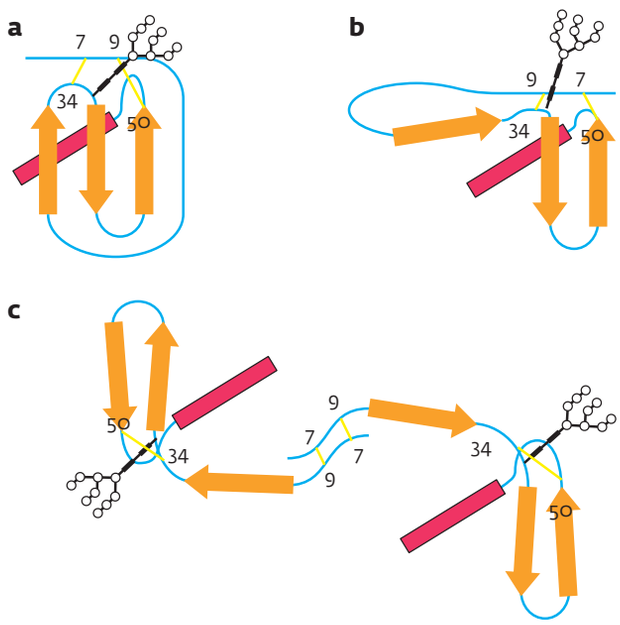


Figure 1: Structures of the properly folded glycoprotein: a) a misfolded isomer, b) the dimer, and c) substrates for UGGT. Disulfide linkages are highlighted in yellow.

To obtain the model glycoprotein, the researchers divided interleukin-8 into two segments and grafted a carbohydrate pendant to one of the segments before joining the resulting molecules together. In the presence of cysteine and cystine amino acids, the glycoprotein folded correctly (Fig. 1). The absence of cysteine and cystine produced three supplementary misfolded isomers: two structures containing two disulfide linkages and a dimer. The dimer consisted of two strands, bearing one intramolecular disulfide, which were bridged by two additional intermolecular disulfides.

Evaluation of UGGT's reactivity revealed that the enzyme was active only when incubated with misfolded glycoproteins. Furthermore, the dimer was the most reactive isomer. Improper folding exposes water-repelling patches on protein surfaces, leading to speculation that UGGT's specificity may originate from this hydrophobicity. "Our analyses unambiguously demonstrated that the isomer with the highest surface hydrophobicity, namely the dimer, also exhibited the highest activity, matching this hypothesis," says Ito.

"We are now eager to uncover the mechanistic details involving UGGT, especially how this enzyme deals with a variety of glycoproteins," he adds. The team is also interested in reconstituting the folding process at the laboratory level. "Complete understanding of this process will be beneficial in the production of bioactive and medicinally important glycoproteins," says Ito. ■

1. Izumi, M., Makimura, Y., Dedola, S., Seko, A., Kanamori, A., Sakono, M., Ito, Y. & Kajihara, Y. Chemical synthesis of intentionally misfolded homogeneous glycoprotein: a unique approach for the study of glycoprotein quality control. *Journal of the American Chemical Society* **134**, 7238–7241 (2012).

Upping the odds of developing emphysema

Lower levels of a key lung protein increase the risk of developing cigarette-smoke-induced emphysema

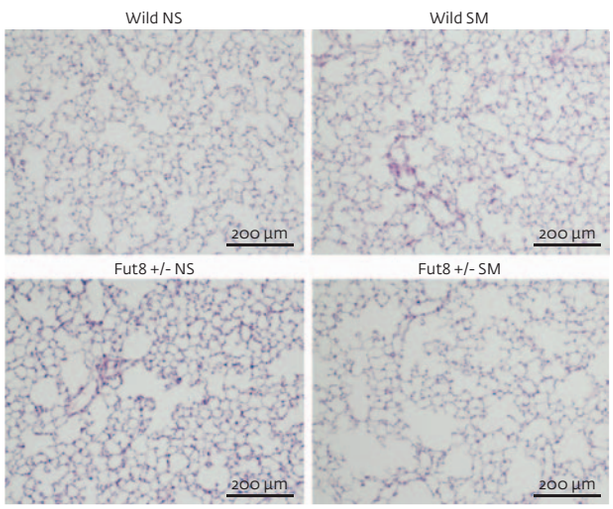


Figure 1: Compared with normal animals (top), mice with mutations in the *Fut8* gene, and exposed to cigarette smoke (SM) (right), have enlarged airspaces (NS, no smoke).

Cigarette smoking is a major risk factor for emphysema, one of the leading symptoms of chronic obstructive pulmonary disease (COPD). Each year, this deadly condition kills more than three million people worldwide. Yet, despite the irreversible cell damage wrought by tobacco smoke, around only one in five lifelong smokers develop the degenerative lung disease. This indicates that some people have genetic factors that predispose them to the condition, whereas others harbor DNA variants that offer protection. Now, a research team from the RIKEN Advanced Science Institute in Wako, Japan, has discovered one gene implicated in vulnerability to smoke-induced emphysema[1], a finding that could help doctors identify those cigarette smokers at highest risk of end-stage chronic lung disease.

Led by Naoyuki Taniguchi, the research team previously showed that eliminating a gene called alpha1,6-fucosyltransferase (*Fut8*) led to lung-destructive characteristics in mice[2]. *Fut8* is involved in making a simple sugar structure known as 'core fucose', which helps to maintain alveolar structure in the lung. Although the animals typically died within days of birth, the work suggested that perturbation of this gene could lead to emphysema.

To investigate this possibility further, Taniguchi's team, together with collaborators at Japan's Gunma University, exposed mice with only one functional copy of the *Fut8* gene to cigarette smoke. These animals experienced a rapid influx of inflammatory cells in the lungs and developed emphysema after only three months. In contrast, wild-type mice needed at least six months of smoke inhalation to trigger the disease (Fig. 1). The researchers also found that core fucose controls proteins involved in the so-called 'Smad pathway', which in turn regulate the activity of enzymes involved in breaking down the extracellular matrix along the lung alveolar wall.

To validate the work in people, Taniguchi and his colleagues teamed up with clinicians at the Nippon Medical School and measured FUT8 protein levels in the blood of ex-smokers. They found that people with lower FUT8 activity had worse lung function on average and experienced more acute exacerbations of COPD than people with elevated FUT8 levels[3]. An independent Japanese team also reported last year that a particular genetic variant in the *FUT8* gene was significantly associated with COPD.

Together, these results make a compelling case for measuring FUT8 levels in blood to help predict rates of disease progression in COPD patients. "Our study clearly indicated FUT8 enzyme activity is a biomarker for COPD exacerbations," Taniguchi says, "and provides useful information for personalized medicine in terms of COPD exacerbations." ■

1. Gao, C., Maeno, T., Ota, F., Ueno, M., Korekane, H., Takamatsu, S., Shirato, K., Matsumoto, A., Kobayashi, S., Yoshida, K., *et al*. Sensitivity of heterozygous α1,6-fucosyltransferase knock-out mice to cigarette smoke-induced emphysema: implication of aberrant transforming growth factor-β signaling and matrix metalloproteinase gene expression. *Journal of Biological Chemistry* **287**, 16699–16708 (2012).
2. Wang, X., Inoue, S., Gu, J., Miyoshi, E., Noda, K., Li, W., Mizuno-Horikawa, Y., Nakano, M., Asahi, M., Takahashi, M., *et al*. Dysregulation of TGF-beta1 receptor activation leads to abnormal lung development and emphysema-like phenotype in core fucose-deficient mice. *Proceedings of the National Academy of Sciences USA* **102**, 15791–15796 (2005).
3. Kamio, K., Yoshida, T., Gao, C., Ishii, T., Ota, F., Motegi, T., Kobayashi, S., Fujinawa, R., Ohtsubo, K., Kitazume, S., *et al*. α1,6 Fucosyltransferase (Fut8) is implicated in vulnerability to elastase-induced emphysema in mice and a possible non-invasive predictive marker for disease progression and exacerbations in chronic obstructive pulmonary disease (COPD). *Biochemical and Biophysical Research Communications* **414**, 112–117 (2012).

How large enzymes get hitched

In atomic detail, advanced computer simulations reveal the conformational changes of an enzyme anchoring to its substrate

Pathogenic bacteria bristle with surface proteins that help them to infect their host. From chemical hooks that anchor the bacteria in place to cloaking proteins that hide them from their host's immune system, each protein is clipped into place by an enzyme called sortase. Kei Moritsugu and his colleagues at the RIKEN Research Cluster for Innovation in Wako have now shown, in atomic detail, how this enzyme functions[1]. The work could lead to new antibiotics that work by disrupting these functions.

Sortase is a difficult enzyme to study because it incorporates a highly flexible section within its structure known as an intrinsically disordered region (IDR). Previous research showed that the enzyme performs its protein-tethering function with the help of a calcium ion, but the molecular mechanism by which calcium interacts with the IDR to assist this process remained unclear.

To find answers, Moritsugu and his team developed a computational simulation technique that they called multi-scale enhanced sampling (MSES). Conventional conformational sampling methods at the atomic scale are only applicable to the smallest of enzymes, so are not suitable for studying sortase. "The MSES simulation has overcome this problem," Moritsugu explains. The new simulation technique uses a multi-scale approach: it couples a detailed 'all-atom' structure with a coarse-grained model of the enzyme in a way that enhances sampling at the main point of interest, the IDR.

Using this approach, the researchers could calculate how sortase's structure changes as the protein substrate and the calcium ion bind to it. Their results showed that each binding partner induces a disorder-to-order transition in one part of the IDR, so that with both partners in place the entire IDR becomes ordered (Fig. 1). The simulation also showed that with the calcium ion bound, the enzyme's structure shifts to better bind the protein substrate, explaining calcium's beneficial effect.

"Our discovery has medical implications," says Moritsugu, because blocking the enzyme's function could offer a new way to treat drug-resistant pathogens such as Methicillin-resistant *Staphylococcus aureus* (MRSA). "Sortase enzymes play a crucial role in virulence, infection, and colonization by pathogens. Elucidating the sortase function at atomistic resolution is expected to allow the computational design of inhibitors," he says.

The team is planning to use the MSES simulation technique for other biomolecules that have proven too large for conventional simulation techniques. Using the K computer, a powerful new supercomputer which has been jointly developed by RIKEN and Fujitsu, even multi-enzyme complexes could be studied using the technique, says Moritsugu. ■

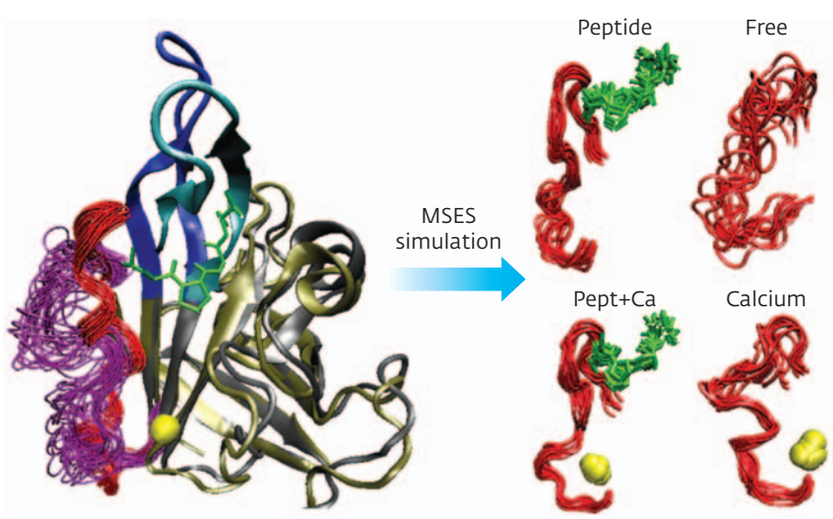


Figure 1: MSES simulations reveal that the disordered section of sortase (left, purple and red section) changes conformation when binding to either or both of its peptide and calcium ion binding partners (right).

1. Moritsugu, K., Terada, T. & Kidera, A. Disorder-to-order transition of an intrinsically disordered region of sortase revealed by multiscale enhanced sampling. *Journal of the American Chemical Society* **134**, 7094–7101 (2012).

A new twist on protein misfolding

Cellular accumulation of misfolded protein clumps may be a survival advantage rather than a liability under certain environmental conditions

Most proteins have a single 'correct' way to fold; typically, improperly folded proteins are promptly eliminated by cells. However, certain misfolded proteins have a tendency to aggregate in dense fibrous clumps known as amyloid plaques. In humans, this accumulation is often a pathological feature, as observed in Alzheimer's or Huntington's disease.

Surprising findings from Motomasa Tanaka's group at the RIKEN Brain Science Institute in Wako, however, have demonstrated that amyloid formation may also be beneficial for cells under certain conditions[1]. They conducted an assay to identify yeast prions—proteins with the capacity to misfold in a manner that induces similar misfolding in other molecules, resulting in amyloid formation. Their screen revealed 66 candidate yeast prions, but Tanaka and colleagues focused on Mod5, a particularly interesting protein.

The Mod5 enzyme is normally responsible for introducing chemical modifications to the transfer RNA molecules that regulate synthesis of new proteins. The researchers demonstrated that it can also be induced to assemble into amyloid aggregates (Fig. 1). This misfolding is also 'contagious': introduction of Mod5 amyloids into yeast cells that express only the soluble form of the protein ([*mod*$^-$] cells) gave rise to cells that instead produce the aggregating prion form of Mod5 ([*MOD*$^+$] cells).

This shift proved to have important physiological consequences. Mod5 normally makes use of a chemical called DMAPP, which is also used in production of the membrane lipid ergosterol.

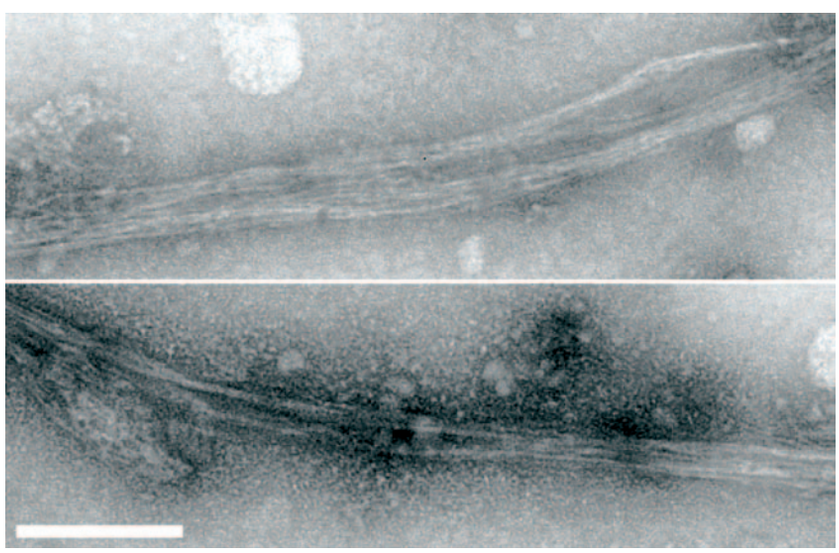


Figure 1: Under the right conditions, as shown in both images, Mod5 can be induced to misfold and accumulate as fibrous aggregates—a typical characteristic of prion proteins (scale, 100 µm).

The reduced levels of functional Mod5 in [*MOD*$^+$] cells therefore leave more DMAPP available for erogsterol production. "[*MOD*$^+$] cells may have thicker cell membranes, which would protect the yeast cells from attack by antifungal drugs," says Tanaka. Several experiments confirmed this protective effect, and prion-forming [*MOD*$^+$] cells proved more resistant to antifungal agents such as fluconfazole than their soluble [*mod*$^-$] counterparts.

This resistance comes at a cost. "[*mod*$^-$] yeast have a growth advantage in the absence of antifungal drugs," says Tanaka. As such, the [*mod*$^-$] state is selected for unless the presence of drugs makes the reduced growth rate the only alternative to poisoning. These findings demonstrate an unexpected functional importance for protein 'misfolding'. Tanaka notes that there is even evidence to suggest that the amyloids formed in Alzheimer's and other neurodegenerative diseases may accumulate as part of a protective mechanism triggered by cellular stress.

Future studies should reveal more about how this surprising defense mechanism is triggered and regulated. "We would like to learn more about how protein aggregation can regulate a cell's stress response," says Tanaka. ■

1. Suzuki, G., Shimazu, N. & Tanaka, M. A yeast prion, Mod5, promotes acquired drug resistance and cell survival under environmental stress. *Science* **336**, 355–359 (2012).

ERINA KURANAGA

Team Leader
Laboratory for Histogenetic Dynamics
Center for Developmental Biology
RIKEN Kobe Institute

Exploring morphogenesis: How an organism develops its shape

"For all of us, life begins with a single fertilized egg. This fertilized egg undergoes a series of mitotic cell divisions to form our bodies in all their complexity. It's amazing, isn't it?" says Erina Kuranaga, team leader of the Laboratory for Histogenetic Dynamics of the Center for Developmental Biology at the RIKEN Kobe Institute. "Cells form tissues and organs not just by dividing and increasing in number. They exhibit dynamic changes; for example, they exchange information and various substances, they migrate, and they die. How do cells migrate to form the body of an organism? This is what I am investigating." Kuranaga is researching the mechanisms underlying morphogenesis by using live imaging to observe the migration patterns of individual cells.

'Seeing' the renewal of epidermal cells

The fertilized egg of *Drosophila melanogaster*, the main subject of Kuranaga's research, typically measures about 0.5 mm in length and 0.1 mm in width. The larva that hatches from the egg develops into a pupa in about five days, and then into an adult with a body length of around 2 mm in a further four days. "The larva's rod-like body differentiates into a head, chest and abdomen, and metamorphoses to become an adult that has a totally different body shape, with legs and wings, as it emerges from the pupa. I was bowled over by this astonishing process."

Kuranaga became intrigued by *Drosophila* epidermal cells; during the larval stage, the body is soft, resembling jelly, and has relatively large epidermal cells that secrete a bactericidal substance. In contrast, the adult's body is hard; its epidermal cells secrete substances that produce a hard coating and are smaller than those at the larval stage. "In the pupa, larval epidermal cells are replaced by adult epidermal cells. In the past, textbooks have described larval tissue as 'melting' or 'decaying'. However, if the epidermis and other coverings of the body melt, the insect is no longer capable of maintaining its body shape. So, how are the epidermal cells renewed? I was intent on observing the changes in epidermal cells with my own eyes."

On the question of whether it is actually possible to 'see' inside a living pupa, Kuranaga affirms: "It is indeed possible, by using live imaging." By targeting molecules or cells of interest using fluorescent probes such as green fluorescent protein (GFP), live-imaging analyses enable observation of living cells in real-time.

Figure 1 shows *Drosophila* pupa epidermal cells using a live-imaging technique. This live-imaging analysis was conducted by Kuranaga while serving as assistant professor at the Department of Genetics, School of Pharmaceutical Sciences at the University of Tokyo under Prof. Masayuki Miura, in collaboration with a former graduate student Yuichiro Nakajima, currently a postdoctoral research associate at the Stowers Institute for Medical Research, US. It is notable that the cell-cell boundaries were made visible with the fluorescence of DE-cadherin, a type of protein known for its cell adhesion properties. Kuranaga explains: "You can see larger cells on the left and smaller cells on the right. The larger ones are larval epidermal cells and the smaller ones become adult epidermal cells."

Further observation revealed that the adult epidermal cells divided one after another to increase in number. Meanwhile, the larval epidermal cells decreased both in size and population. Eventually, all of the larval epidermal cells disappeared, leaving only adult epidermal cells. "The larval epidermal cells died through a process known as apoptosis. It is important to note, however, that the larval epidermal cells did not disappear altogether due to cellular death—instead, cell death and adult epidermal cell proliferation occurred at the same time, keeping a good balance."

To Kuranaga, the larval epidermal cells appeared to shrink, and 'fall' under the epidermis, where specialized cells appeared to be lying in wait and engulfed the fallen dead cells. The areas previously occupied by larval epidermal cells were filled by adult epidermal cells, so that the space was never empty.

"It was almost as if the larval and adult epidermal cells were replacing each other by 'communicating' with one another," says Kuranaga. Upon further examination of the relationship between cell death and proliferation, other clues began to be evident. "Among many of the molecular agents that cause cell death is an enzyme called caspase. I examined caspase activity using live fluorescence imaging." Kuranaga found that larval epidermal cells with activated caspase were in contact with proliferating adult epidermal cells. "This led us to believe that proliferating adult epidermal cells are capable of 'switching on' cell death. I aim to continue studying how adult epidermal cells activate caspase in larval epidermal cells."

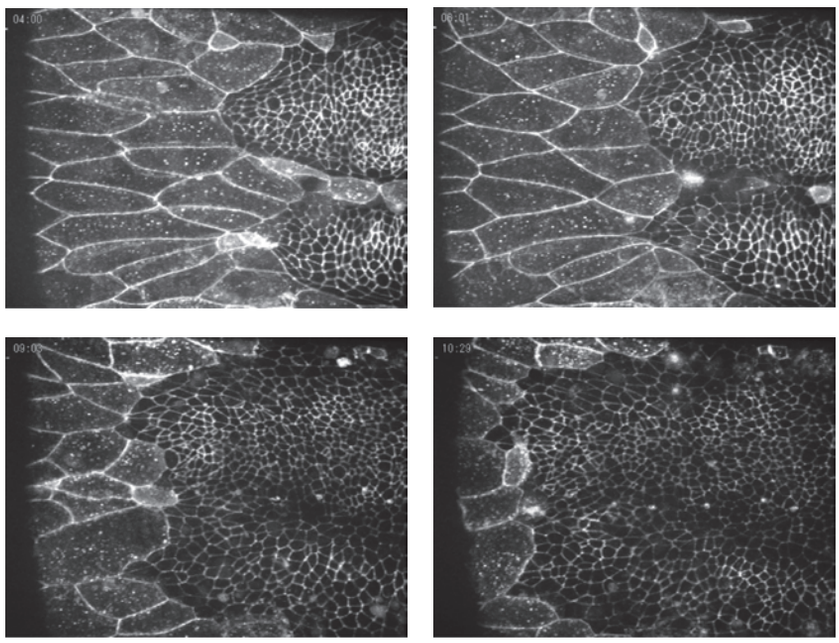


Figure 1: Epidermal cell renewal in pupa

As small adult epidermal cells on the right proliferate, large larval epidermal cells on the left gradually shrink, 'falling' below the epidermis, where they are processed. (Photographs courtesy of Yuichiro Nakajima)

Seeing is believing

Kuranaga's research also involves studying the male terminalia of *Drosophila* (see Fig. 2(A)). Located at the posterior end of the pupa body, the terminalia are a set of cells that develop into the male reproductive system, and can be viewed externally. "The male terminalia rotate during the developmental process," says Kuranaga. The process has been described in an anatomy textbook published in the 1930s; however, these depictions had only existed in the form of a series of sketches. "In 2005, we succeeded for the first time in visualizing the process," says Kuranaga. "Even though the images we obtained were of low quality as part of a sequence of photographs taken every hour using a fluorescence stereomicroscope, we were very impressed by the fact that the male terminalia were actually rotating."

Kuranaga recounts that she had been doubtful of whether the male terminalia rotated until she actually saw the sequence photographs: "Seeing is believing. I attempted to observe the male terminalia of pupa by using live-imaging techniques. In this way, I was able to obtain clear images showing how the terminalia rotated 360 degrees clockwise over a time period of 12 to 14 hours (see Fig. 2(B))."

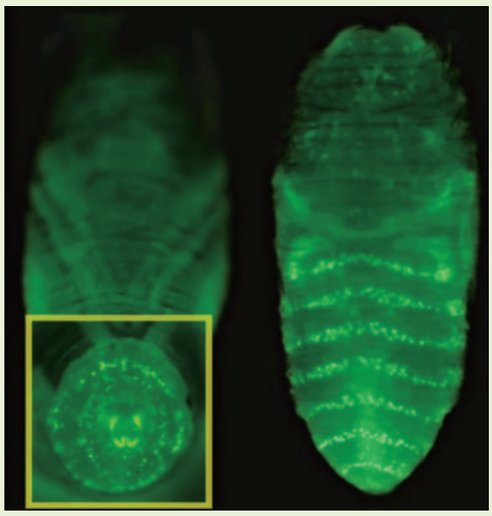
Contemplating why the male terminalia rotate, Kuranaga says: "Although the reason remains unclear, the rotation is thought to produce a long spermiduct for transporting spermatozoa to the testis." An adult *Drosophila* has a body length of about 2 mm, and each spermatozoon can measure as long as 1.8 mm. The spermiduct also has a considerable length of around 1.6 mm, and is contained within the body in a coiled structure. Production of the long spermiduct may require the rotation of the male terminalia at the pupal stage—in fact, insufficient rotation has been linked with shorter spermiducts.

In March 2011, Kuranaga published a set of results she had obtained using live imaging. "A research group in France is similarly working on live imaging of the *Drosophila* male terminalia, and had published their results in October 2010. I was slightly regretful that we failed to become the world's first to achieve the task." The French team's techniques for live imaging differed from Kuranaga's, for example, in terms of how to keep the pupa moist. The French publication also did not include the study of adults emerging from the pupa. "Using our method, the adult can emerge from the pupa, and so we are able to obtain more naturalistic observations of *Drosophila* metamorphosis," says Kuranaga.

Does cell death accelerate male terminalia rotation?

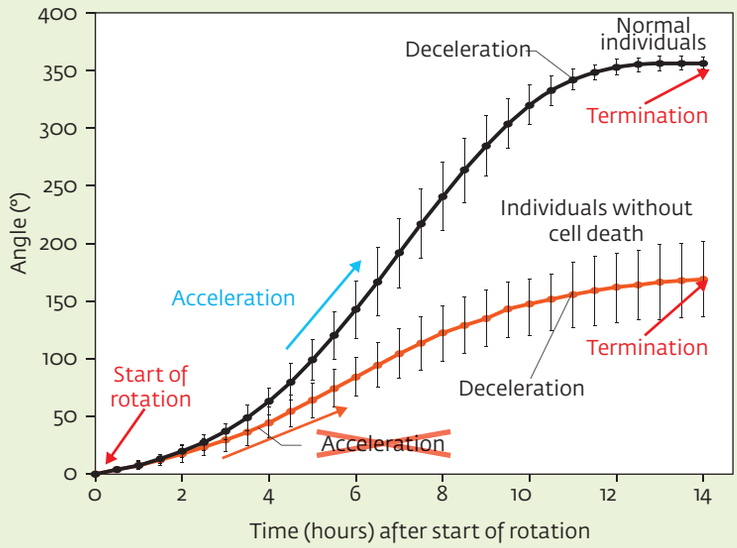
Kuranaga chose to focus on the male terminalia due to her interest in uncovering the mechanisms involved in cell death. "In 1991, it was reported that any mutation in a certain protein that causes cell death would cause adult male *Drosophila* to develop abnormally-angled terminalia. This abnormal angle may be attributable to premature termination of the rotation of the terminalia. The process of rotation and cell death may be interrelated. Based on these considerations, I attempted to observe how male terminalia rotate using live imaging."

After Kuranaga succeeded in visualizing the rotation of the male terminalia by live imaging, she observed: "I was puzzled by the riddle of male terminalia rotation. What are the mechanisms that



A: A *Drosophila* pupa expressing a fluorescent protein. The image on the left shows a ventral view and the one on the right shows a dorsal view. The male terminalia are found in the tail end (as marked in the square).

B: Rotation of the male terminalia. The *Drosophila* male terminalia are located at the posterior end of the pupa. They rotate 360 degrees over 12 to 14 hours.



C: Comparison of terminalia rotation rates. The horizontal axis indicates time after the start of rotation. The vertical axis represents the angle of rotation. In normal individuals, there are four steps: initiation, acceleration, deceleration, and termination. About two hours after the start of rotation, acceleration occurs; the terminalia rotate 360 degrees over 12 to 14 hours. In individuals without cell death, the rotation does not accelerate; the rotation lasts for 12 to 14 hours and the angle is only 180 degrees.

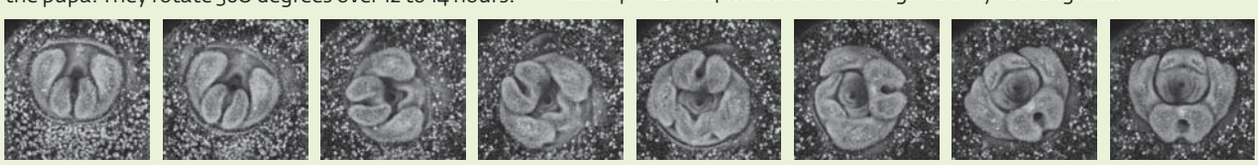


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Figure 2: **Rotation of the male terminalia.**

control the rotation? What are the boundaries between migrating cells and non-migrating cells? What is actually going on there? And why does the rotation cease at exactly 360 degrees? There is much that remains unknown."

By making the best use of live imaging techniques, Kuranaga began to tackle the mechanisms by which the male terminalia rotate. First, she attempted to measure the rotation rate. "In normal *Drosophila*, the male terminalia begin to rotate about 24 hours after pupa formation, and the rotation continues all the way up to 360 degrees over 12 to 14 hours. I had thought that the male terminalia rotated at a constant rate from beginning to end, but when it came to actually measuring the rotation rate, I was astonished by what we found," says Kuranaga.

The results of the measurement are shown in Fig. 2(C). At the beginning, the rotation speed is slow, and about two hours later, it begins to accelerate. Some nine hours later, the rotation begins to decelerate, and terminates 12 to 14 hours later. Kuranaga says, "The rotation was accelerating, rather like a gear change in an automobile; this was far beyond my expectations."

Next, Kuranaga examined the live imaging of *Drosophila* with premature termination of rotation of the terminalia. This revealed another intriguing finding. "I predicted that the rotation would be slow at the beginning, or that the rotation rate would be slow," she says. However, rotation did not differ from that in normal *Drosophila* for up to two hours after the onset of rotation. The difference was in how the rotation accelerated. In addition, the rotation lasted for 12 to 14 hours just as in normal individuals, but the angle of rotation was only 180 degrees. "In individuals with abnormally rotating male terminalia, the 'gear change' does not occur. In these individuals, epidermal cells do not undergo cell death due to the expression of a protein that inhibits the caspase mentioned earlier. I suspected that cell death contributes to the mechanism of acceleration."

The 'moving walkway' analogy

The male terminalia in *Drosophila* consist of three parts: A8, A9 and A10 (see Fig. 3, left panel). In male *Drosophila*, the ring-shaped A8 section differentiates into the tergite; the reverse-headphone-shaped A9 into the male terminalia and spermiduct; and the centrally located A10 into the anus.

"At first glance, the A9 and A10 areas of the male terminalia appear to rotate. On further examination, however, it can be observed that the ring-shaped A8 around A9 and A10 also migrates. Suspecting that this might be the key to the riddle of acceleration, I proceeded to investigate A8 in detail."

Kuranaga monitored the migrations of cells in A8 using live imaging one by one, and found that there were three patterns (Fig. 3, right panel). In the first pattern, cells on the inside of A8 begin to migrate at the same time as A9, and at the same speed (blue). In the second pattern, cells on the outside of A8 begin to migrate with a time lag behind the migration of A9 and

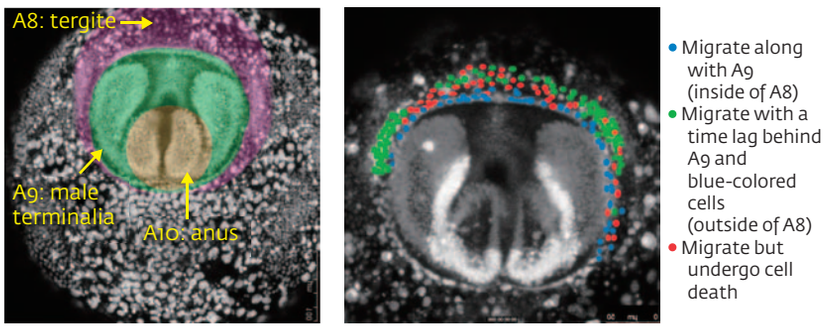


Figure 3: Structure of the male terminalia and cell migration

The male terminalia consists of A8 for tergite, A9 for male terminalia and spermiduct, and A10 for anus. The lower panel is a photomicrograph of A8 cells classified by migration rate. The migrating speed of each cell was determined by live imaging, and the cells were color-coded.
Images reproduced with permission from *Development*: Kuranaga, E. *et al.* Apoptosis controls the speed of looping morphogenesis in *Drosophila* male terminalia. *Development* **138**, 1493-1499 (2011).

blue-colored cells, and their terminalia prematurely terminate rotation at 180 degrees (green). In the third pattern, cells migrate but die (red). "I had long pondered the meaning of this finding. One day I was struck with an idea—the cells might be migrating based on the same principle as a moving walkway."

The cells on the outside of A8 (green) begin to migrate with a time lag behind A9, and this is synchronous with the acceleration of A9. Therefore, Kuranaga observed, "This cellular migration may be associated with the acceleration of the rotation. When we get on a moving walkway, we can progress faster than we usually walk. Likewise, as the cells on the outside of A8 (green) migrate, the cells on the inside of A8 (blue) and A9 are accelerated, and their apparent speed increases."

To investigate what occurs in individuals that do not undergo cell death, examination of the migration of the cells on the outside of A8 revealed that they hardly migrated. This, in turn, may be the reason why the cells on the inside of A8 and A9 do not undergo acceleration during their migration. "Remember the fact that in individuals that do not undergo cell death, the male terminalia rotates only 180 degrees," says Kuranaga. "Essentially, A9 rotates only 180 degrees. Combining these rotations, the *Drosophila* male terminalia rotates 360 degrees in total."

Cell death, migration, and body formation

The following question then arises: How is it possible to explain the association between acceleration of migration speed and cell death? On the outside of A8, a correlation was found between the frequency of cell death and the change in migration speed. Kuranaga says, "Although further experiments will be necessary, as with the epidermal cells, when cells die, other cells are mobilized to fill the space. When cells die on the outside of A8, other cells in the same layer may begin to migrate and accelerate the rotation of the inside of A8 and A9. Therefore, cell death may serve as an engine to drive the 'moving walkway'. Alternatively, cell death may occur at the boundaries between the rotating layer and the non-rotating layer, and 'braking' cells are eliminated. Thus, the male terminalia are able to rotate smoothly. Cell death may be switching on the moving walkway. There may also be many other possible interpretations." Kuranaga is planning to continue extensively examining the relationship between cell migration and cell death.

It is generally accepted that cell death essentially represents the elimination of cells as they no longer become necessary. Kuranaga says, "This notion may need to be changed. Cell death might influence the cells around the dead cells, for example, by promoting cell migration, and prompting the cells to be actively involved in body morphogenesis. A new functionality of cell death may be discovered by clarifying the mechanism behind the rotation of the male terminalia."

In January 2011, Kuranaga established the Laboratory for Histogenetic Dynamics at the RIKEN Center for Developmental Biology (CDB). "Research into the rotation of *Drosophila* male terminalia is quite challenging as it involves a wide variety of cells migrating in a complex way, and I often found the research to be frustrating. In order to advance my research, I thought it would be necessary to conduct collaborative research and hold discussions with experts in a broad range of fields, including cellular development, cell death, image analysis, and theoretical biology. Here at RIKEN, I can do all of this. In fact, new things are being discovered every day." Joint research with Atsushi Mochizuki, chief scientist in the Theoretical Biology Laboratory at the RIKEN Advanced Science Institute and Tatsuo Shibata, leader of the Research Unit for Physical Biology at the CDB, is already underway. "Working jointly with theoretical researchers could lead to the discovery of new mechanisms for cell migration that might never have been revealed just by observing individual cells. Now, I am really enjoying my research."

Epidermal cell renewal is not exclusively seen in *Drosophila*. For example, similar processes occur in human skin and the intestine. It is possible to conduct basic research into disease mechanisms using *Drosophila* as it carries more than 70% of human disease-related genes. Kuranaga says, "I hope to one day link this *Drosophila*-based research to vertebrate, mammalian and possibly even human investigations." She adds, "I think *Drosophila* are just wonderful—they are lovely creatures!" ■

ABOUT THE RESEARCHER

Erina Kuranaga was born in Kumamoto, Japan, in 1974. She graduated from the Faculty of Agriculture, Kyushu University, in 1997 and received her doctorate in medical science from the Department of Cell Biology and Neuroscience at the Osaka University Graduate School of Medicine in 2004. From 2000 to 2004, she was a junior research associate at the RIKEN Brain Science Institute. She moved to the University of Tokyo as assistant professor in the Department of Genetics at the Graduate School of Pharmaceutical Sciences. In 2006, she was promoted to associate professor in the same department, where she remained until she joined the RIKEN Center for Developmental Biology as team leader in 2011. Her research focuses on the physiological function and regulatory mechanisms of apoptosis during morphogenesis and the mechanical interaction among cells during organogenesis.

YUKIHIKO KUSHIDA

Manager
General Affairs Section
RIKEN Advanced Institute for Computational Science

Making a mark in management

How and when did you join RIKEN?

I originally worked at the Japan Nuclear Cycle Development Agency (JNC, now the Japan Atomic Energy Agency, or JAEA) and came to the RIKEN International Cooperation Office (ICO) on a transfer assignment. The initial plan was for me to be there for two years, but after my stay was extended several times I received a request to fully transfer from the JAEA to RIKEN. At first I was a bit hesitant to move to RIKEN because I enjoyed the challenge of working with nuclear energy and was comfortable with my job situation at the time. However, I could tell that I was needed, and even the JAEA encouraged me to take the position. I finally decided to join RIKEN in April 2009.

Please tell us about your work at RIKEN.

I worked abroad through the JNC for almost 5 years, which was why I was first assigned to the ICO at RIKEN, where I helped with visits from overseas guests and research cooperation with international institutions. My next position, in the General Affairs Section, gave me the opportunity to welcome distinguished visitors from within Japan and overseas, along with other diverse projects.

I was later transferred to the Evaluation Section of the Policy Planning Division, where I was part of the secretariat for the RIKEN Advisory Council, which handles operations for external evaluations of RIKEN. I was involved whenever RIKEN received government evaluations and assisted with the administration of external award nominations, and also helped run a research information database.

In May 2012 I began my current position as manager of the General Affairs Section of the Research Support Division at the Advanced Institute for Computational Science (AICS) in Kobe. My duties now include overseeing general administrative tasks, human resources, employee benefits, facility management and office IT.

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

A memorable experience for me was the time when in October 2006 the Emperor and Empress of Japan visited the Wako Campus. Planning started months in advance, and the day of the visit required very tight security and a minute-by-minute breakdown of the schedule. As the RIKEN security coordinator, I worked with the police to ensure that everything went smoothly. The preparation was difficult, but I still remember the satisfaction I felt when the visit was successfully completed without any problems.

What is the best thing about working at RIKEN?

Working at RIKEN gives administrative staff the opportunity to interact with Japan's leading researchers and learn about the country's current scientific research and its future direction.

What would you say to other people considering joining RIKEN?

RIKEN's offices do more than just the "glamorous" work of research planning, public relations and international affairs. There are also "behind-the-scenes" tasks related to contracts, accounting and general administrative work. I think it is important to enjoy this kind of work too, and look forward to working together with those eager to do so.

CONTACT INFORMATION

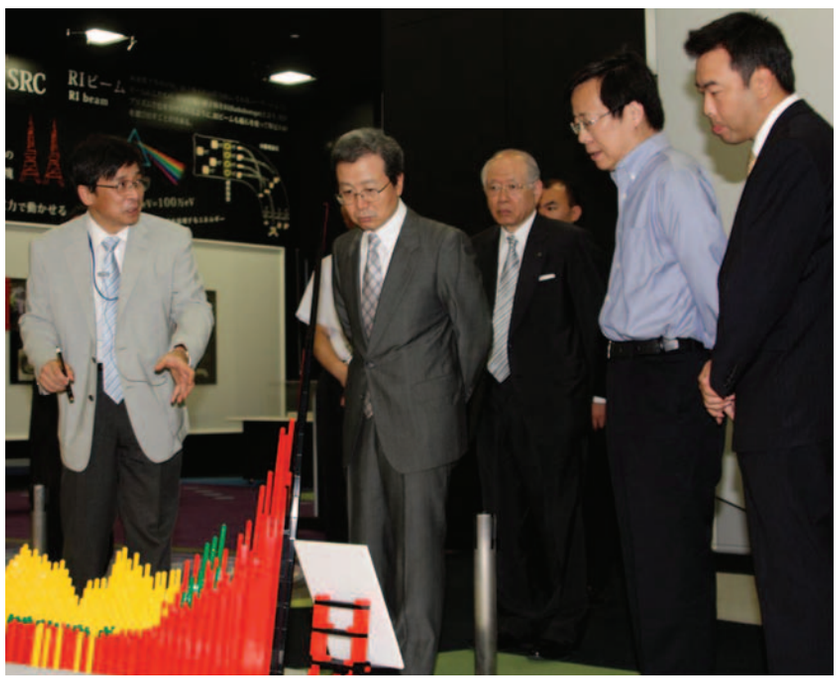
For details about working at RIKEN, please contact the RIKEN Global Relations Office:
Tel: +81-(0)48-462-1225
E-mail: gro-pr@riken.jp

Chinese ambassador visits RIKEN

In July 2012, RIKEN welcomed His Excellency Cheng Yonghua, the Chinese Ambassador to Japan, to its Wako campus in Saitama on the outskirts of metropolitan Tokyo. Following a formal welcome and greeting by RIKEN President Ryoji Noyori and an explanation of the history and activities of RIKEN, the two parties held talks on the furthering of research cooperation between the two countries.

Cheng expressed his hope for a strengthening of relations with RIKEN as a leading Japanese research center and thanked the authorities and staff at RIKEN for the contribution it has made to the development of science in China. Over 130 researchers from China work at RIKEN constituting almost a quarter of its non-Japanese scientific staff. Responding, Noyori reaffirmed RIKEN's commitment to working as a bridge between Japanese researchers and the international scientific community. He also acknowledged the importance of deepening RIKEN's research links with Chinese institutions, an area in which the RIKEN president is personally already highly active—Noyori is one of only 64 foreign members of the Chinese Academy of Sciences (CAS), the pre-eminent national body for natural sciences research in China.

During his stay the ambassador visited the RIKEN Nishina Center for Accelerator-Based Science (RNC) and toured the Radioactive Isotope Beam Factory facility in the company of RNC director Hideto En'yo and subsequently visited the RIKEN Brain Science Institute, another of the three major research centers located at the Wako campus.



His Excellency Cheng Yonghua (center left) toured several RIKEN institutes accompanied by RIKEN President Ryoji Noyori (center right).

The visit of the Chinese ambassador was lent additional weight by the fact that 2012 marks the fortieth anniversary of the renormalization of diplomatic ties between Japan and China and is the latest in a series of recent initiatives aimed at strengthening research cooperation with Chinese institutions. In June 2011, RIKEN signed a memorandum of agreement with the Department of International Cooperation of the Chinese Ministry of Science and Technology followed by a joint symposium with the CAS in May 2012. ■

RIKEN exhibits at ESOF 2012 in Dublin

A delegation of RIKEN officials traveled to Dublin, Ireland, to attend the Euroscience Open Forum (ESOF) held over 11–15 July 2012. The ESOF is a biennial gathering which takes place in different cities across Europe and aims to discuss the state and future of science and science policy in Europe. ESOF 2012 attracted researchers and students from all over the continent with its high-profile lineup of speakers including Nobel laureate James Watson, philanthropist and former rock star Bob Geldof, CERN Director General Rolf-Dieter Heuer, and NASA Administrator Charles Bolden.

The RIKEN delegation, including members of the Global Relations Office, Public Relations Office, and Advanced Research Promotion Division, ran a large promotional booth to increase public awareness of the RIKEN brand and to create interest among young researchers in coming to RIKEN to work or to conduct research toward their doctorates.

There was a constant stream of visitors to the booth, some asking questions about how to get a position within RIKEN or about living conditions in Japan, and other visitors just wanting to learn more about the broad array of research undertaken at RIKEN. Visitors from a number of countries expressed interest in initiating or expanding research cooperation with RIKEN. ■

Prospective researchers visited the RIKEN booth at ESOF 2012 held in Dublin, Ireland.

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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.

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

www.rikenresearch.riken.jp