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Harmonic microscopy

A new microscopy technique significantly increases imaging resolution far beyond classical optics

As a consequence of the wave nature of light, the minimum spot size a light beam can be focused for use in optical instruments corresponds approximately to its wavelength. Now researchers at RIKEN's Advanced Science Institute (formerly the Discovery Research Institute) in Wako, in collaboration with researchers from Osaka University, have developed a new microscopy method based on conventional fluorescence microscopes that is easy to use and allows for almost arbitrary enhancements in imaging resolution beyond the classical limit.

The diffraction limit

The operation of classical optical instruments is limited by diffraction, a phenomenon that occurs by the bending of light waves caused by obstacles in the light beam. Diffraction originates from the interference of the different light paths reflected from the obstacle, and as such is a direct consequence of the wave properties of light.

Since the limits to imaging resolution are related to the wavelength of light, shorter wavelengths have to be used to achieve high spatial resolution. For example, in the semiconductor industry, the lithographic patterning of increasingly smaller devices necessitates the use of extremely short wavelengths in the ultraviolet region of the spectrum.

However, the use of shorter wavelengths is not always an option, particularly for imaging, and a number of other techniques—beyond those used in classical optics—have been developed to achieve a much higher spatial resolution. This is because the diffraction limit is merely a consequence of the wave nature of light rather than a fundamental law.

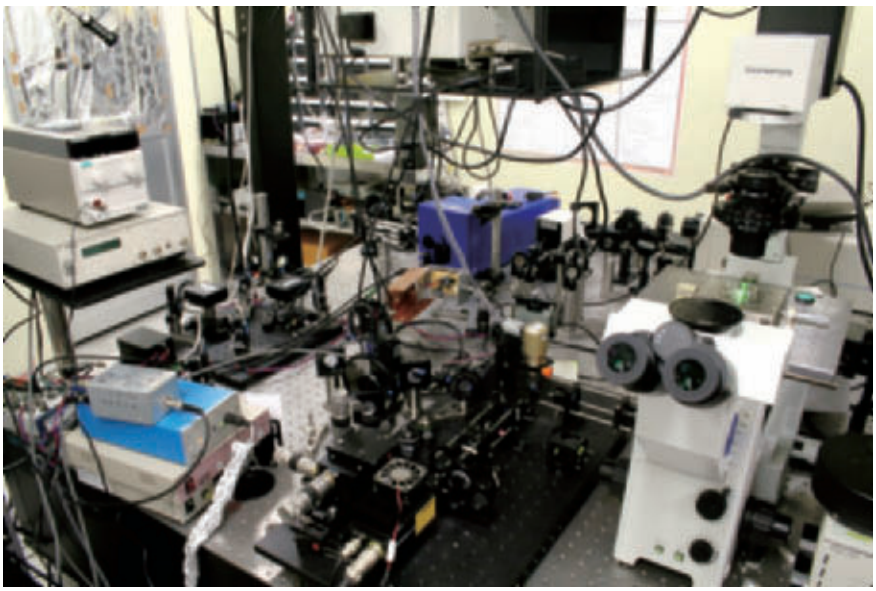

Figure 1: High-resolution microscopy. High-resolution imaging can be achieved by a conventional optical microscope (right). Fluorescence from the sample is excited by a green laser (visible at the object holder). The laser is modulated at a certain frequency. The higher harmonics of this frequency are used for super-resolution imaging. The left part of the image depicts the modulation and detection apparatus.

One well-known example is scanning probe microscopy, where a sharp tip scans the surface of a material and allows for imaging with atomic resolution. However, many of the techniques that achieve 'super-resolution' suffer from practical drawbacks: scanning probe microscopy, for example, requires a close contact with the sample, and therefore only allows for planar imaging.

Laser fluorescence microscopy

The new microscopy technique developed by the Japanese research team, led by Osaka University's Katsumasa Fujita and RIKEN's Satoshi Kawata, is practical and offers super-resolution beyond the diffraction limit. "The major advantage of our method is that no special skills are required to achieve super-resolution," says Kawata emphatically.

Published in the *journal Physical Review Letters*[1], the team's technique is based on the fluorescence that a sample emits under optical excitation by a laser beam matched to the excitation energy of the sample. In biological imaging small fluorescent molecules are used to mark processes within a cell. This emission is collected by a standard optical microscope (Fig. 1).

Although powerful instruments such as the electron microscope can achieve a much higher imaging resolution, they are unsuitable for biological imaging because the high-intensity electron beam damages the samples. An optical microscope, however, allows continuous three-dimensional imaging of biological processes from inside a living cell, without damage to the sample.

In order to detect small fluorescent signals and therefore enable high spatial resolution using laser fluorescence microscopy, the beam intensity of the excitation laser has to be increased. However, if excitation intensities are too high, the light emission from the fluorescent markers saturates. Similar to an overexposed photograph, details of the sample structure are lost in the bright emission.

Higher harmonics

The method developed by the researchers now turns this disadvantage into an advantage. Although a saturated emission is too bright to be suitable for conventional microscopy, it also carries components that the researchers have used for super-resolution imaging.

Fujita, Kawata and colleagues modulated the optical excitation of the specimen by the excitation laser at a certain frequency. The emission from the fluorescent molecules follows this modulation and oscillates at the same frequency. At sufficiently high emission intensities, the molecules also emit light at multiples of this fundamental frequency, the so-called overtones or higher harmonics.

Similar to musical instruments, the higher harmonics generated by the fluorescent molecules are much weaker in magnitude than the fundamental signal. Even though the intensity of the fundamental signal is saturated and blurred, the higher harmonics are not. Therefore, if the light emission of the fundamental emission is filtered out and only light of a specific harmonic modulation frequency is considered, a much better spatial resolution can be achieved (Fig. 2).

Almost boundless imaging

Theoretically, the imaging resolution by this method is without bounds. As higher and higher harmonics are used, the better the spatial resolution of the system becomes. The only limit to this method is the increasingly lower fluorescence intensity at the higher harmonic frequencies that eventually becomes impossible to detect against the noise level of the detector.

Therefore, to further improve the detection limit, the signal-to-noise ratio of the detector needs to be improved. Indeed, Fujita asserts that "in this work, we used a very slow modulation frequency. To further suppress the noise level we just need to increase the modulation frequency of the excitation laser beam to enhance the high-resolution component." Changes to the imaging apparatus itself may also bring significant improvements in the detection of low signals, and further increase resolution.

Kawata is convinced that this practical method will have a big impact on biology and biomolecular sciences, since researchers require three-dimensional images of samples with nanometer resolution. A particular advantage of this approach is that this method is a straight extension of conventional fluorescence microscopy. Unlike more complex alternative approaches based on a sophisticated apparatus, no special skills are required to operate high-resolution microscopy instruments. Efforts are underway to commercialize this technique. ■

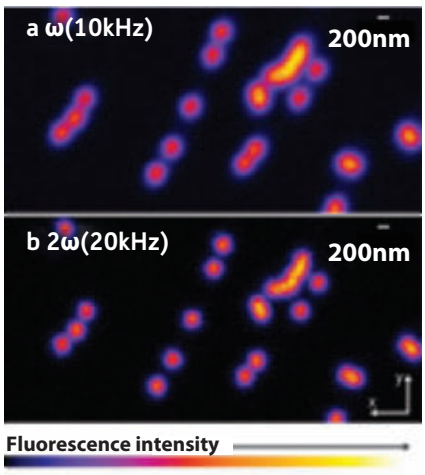

Figure 2: The benefits of higher harmonics. (a) A conventional microscope image of small fluorescent peaks excited with light modulated at a frequency ω of 10 kHz. (b) If the detection frequency is twice the original frequency, a much better resolution is achieved.

1. Fujita, K., Kobayashi, M., Kawano, S., Yamanaka, M. & Kawata, S. High-resolution confocal microscopy by saturated excitation of fluorescence. *Physical Review Letters* **99,** 228105 (2007).

About the researcher

Satoshi Kawata received his BSc, MSc, and PhD, all in Applied Physics, from Osaka University, Japan, in 1974, 1976, and 1979, respectively. In 1979, he joined the Electrical Engineering Department of the University of California, Irvine, as a research associate, and then in 1979 he became a faculty member of the Department of Applied Physics, Osaka University. Since 1993, he has been the professor for Applied Physics at Osaka University and the director of the Photonics Advanced Research Center of Osaka University. Since 2002, he has been working for RIKEN as head of the Nanophotonics Laboratory and as chief scientist. He is an editor for *Optics Communications*, ex-president of the Spectroscopical Society of Japan, and a fellow of SPIE, OSA, and IOP. His research interests include nanophotonics, plasmonics, biophotonics, and spectroscopy. He was awarded a number of prizes, including the medal with Purple Ribbon awarded by Japan's emperor for his academic contribution to society.

Scatter and spin

The spin Hall effect in platinum is mainly caused by scattering within the wire

When a wire carrying electric current is placed in a magnetic field, the flowing electrons are deflected by a force at right angles to the field. This phenomenon, called the Hall effect, has found applications in many types of electronic sensors.

Now scientists hope to build 'spintronic' devices using a similar phenomenon called the spin Hall effect, which results from a particle's intrinsic angular momentum, or spin, rather than its electric charge. Yoshichika Otani and Takashi Kimura at the University of Tokyo and the RIKEN Advanced Science Institute (formerly the Frontier Research System) in Wako have explained why the spin Hall effect is particularly strong in platinum[1].

Any moving charged particle generates a magnetic field because of its spin. In the spin Hall effect, these spin magnetic moments are deflected by a right-angled force when they are placed in an electric field—the opposite case to the classical Hall effect. If the spins are all pointing in the same direction—a so-called 'spin-polarized' current—the spins accumulate at one edge of the wire. This opens up the possibility of generating and detecting spin currents without needing magnetic fields.

Many researchers have looked at the spin Hall effect in semiconductors, because they are easily compatible with current technology. But the effect is small in semiconductors, especially compared to platinum, which has the largest spin Hall effect so far measured[2].

"For platinum, there is a lot of discussion concerning the origin of the spin Hall effect," says Otani. "One [idea concerns an] intrinsic origin due to the electric band structure, another is an extrinsic origin due to defects or impurities."

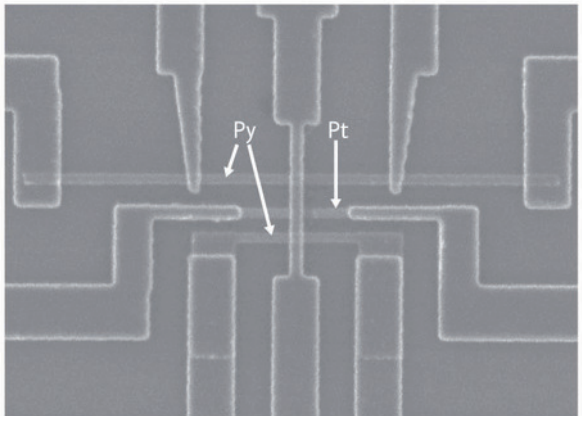

Figure 1: Scanning electron microscope image of the modified lateral spin valve for measuring the spin Hall effect in platinum. Permalloy (Py) and platinum (Pt) wires are indicated, all other components are copper.

Otani and colleagues modified a tiny spintronic device called a lateral spin valve[3,4] to measure the spin Hall conductivity in platinum wires of varying thicknesses and at different temperatures (Fig. 1). They found that the spin Hall effect decreased for thicker wires because the spins could relax back to more random orientations, losing their polarization. The results also showed that the spin Hall effect is mainly caused by scattering within the metal.

This new knowledge could allow greater control over the spin Hall effect. Unfortunately, the effect is largest at very low temperatures that are not practical for everyday devices—a problem the researchers hope to overcome in future. "At the moment, the observed spin Hall effect [at room temperature] is too small," says Kimura. "The challenge will be how to enlarge the effect." ■

1. Vila, L., Kimura, T. & Otani, Y. Evolution of the spin Hall effect in Pt nanowires: size and temperature effects. *Physical Review Letters* **99,** 226604 (2007).
2. Kimura, T., Otani, Y., Sato, T., Takahashi, S. & Maekawa, S. Room-temperature reversible spin Hall effect. *Physical Review Letters* **98,** 156601 (2007).
3. Kimura, T., Otani, Y. & Levy, P.M. Electrical control of the direction of spin accumulation. *Physical Review Letters* **99,** 166601 (2007).
4. Kimura, T. & Otani, Y. Large spin accumulation in a permalloy-silver lateral spin valve. *Physical Review Letters* **99,** 196604 (2007).

Spins in nickel stand together

Theorists extend a simple model to explain ferromagnetism in transition metals

Physicists have solved various exotic problems yet the familiar phenomenon of magnetism in metals, such as iron and nickel, has remained difficult to explain.

Now Ryotaro Arita of the the RIKEN Advanced Science Institute (formerly the Discovery Research Institute) at Wako and his colleagues Shiro Sakai and Hideo Aoki at the University of Tokyo have shown the importance of two elements in predicting magnetism in transition metals[1]—lattice structure and multiple electron orbitals (or energy levels), on the magnetic ions. "This work will have an impact on a long-standing condensed matter physics problem," explains Arita. "Now we know how electrons can conduct current with their spins aligned at the same time."

The group based their calculations on an extension of the Hubbard model, one of the simplest solid state physics models that takes into account interactions between electrons in a material. Previous work showed that the Hubbard model can explain certain magnetic states, but not ferromagnetism—the state in which all spins are aligned—in real metals.

In its simplest form, the Hubbard model describes a lattice of atoms with one orbital per atom. If only one electron occupies the orbital on an atom, there is no cost in energy, but if two electrons occupy the same orbital there is a large energy cost. Moreover, the spins of two electrons on the same orbital cannot point in the same direction because of the so-called Pauli exclusion principle. The final arrangement of the electrons—and their spins—depends on the balance between the 'repulsive' energy between electrons on the same site and the kinetic energy that electrons gain by moving around the lattice.

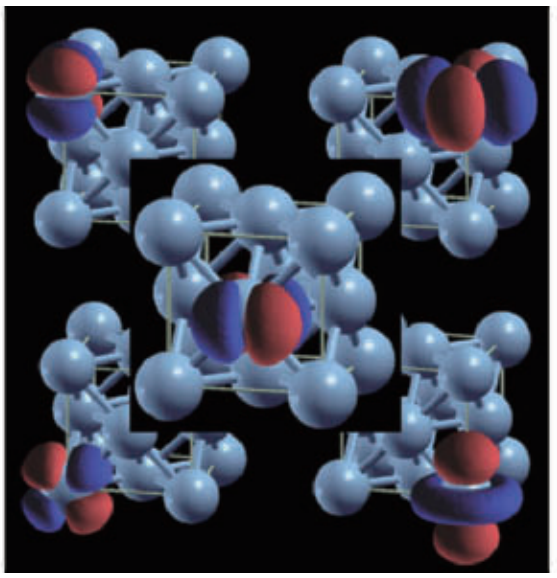

Figure 1: The face-centered cubic (FCC) lattice structure of atoms (blue spheres) is a common structure of pure metals, such as nickel. Each of the five images shows one of the possible multiple orbitals (red and blue lobes) for the 3*d* transition metal ions (found in the fourth row of the periodic table). For clarity, each orbital is drawn on a different FCC lattice.

The Hubbard model is attractive for theorists because it provides a clear microscopic picture of magnetism. Arita and colleagues therefore built on the 'simple' Hubbard model, to see if it could predict ferromagnetism in transition metals. In their model, each atomic site contains multiple electron orbitals (Fig. 1). Since electrons on the same atom can occupy distinct orbitals, their spins can point in the same direction without violating the Pauli exclusion principle. The group shows this effect is important for obtaining ferromagnetism in a metal.

Arita and colleagues also considered the role of lattice structure. They showed their model predicts ferromagnetism in transition metals with a 'face centered cubic' structure (Fig. 1) but not a 'simple cubic' structure.

The team's model is successful at explaining ferromagnetism in nickel and may ultimately be applied to more complex materials. ■

1. Sakai, S., Arita, R. & Aoki, H. Itinerant ferromagnetism in the multiorbital Hubbard model: A dynamical mean-field study. *Physical Review Letters* **99**, 216402 (2007).

Splitting up cell fates during brain development

The orientation of the axis of progenitor cell division in the brain dictates whether daughter cells will maintain the parental cell fate

New findings indicate that the way in which the contents of progenitor cells called neuroepithelial cells are distributed during division strongly influences the fate of the resulting progeny. During division, the mitotic spindle is responsible for separating and allocating chromosomes into daughter cells. The spindle position dictates the orientation of the plane through which the parent cell will split.

Depending on the placement of the plane of division, progenitor cells can divide symmetrically—producing two daughter cells with fates identical to that of the parent cell, or asymmetrically—producing daughter cells having cell fates distinct from each other and/or from the parent cell. Daughter cells of neuroepithelial progenitor cells can either maintain the 'parental' progenitor fate, or embark along a developmental pathway leading to the neuron cell fate.

A team led by Fumio Matsuzaki at the RIKEN Center for Developmental Biology in Kobe conducted experiments to find out whether mitotic spindle orientation influenced the fate of neuroepithelial progenitors, as these cells are highly polarized with two distinct regions referred to as apical and basal endfoots. Their work was recently published in *Nature Cell Biology*[1].

By tracking the positions of fluorescently labeled chromosomes in neuroepithelial progenitors in developing mouse forebrains, the researchers noted that the majority of divisions were symmetric, or planar, along the plane perpendicular to the apicobasal axis (Fig. 1). To manipulate mitotic spindle orientation in neuroepithelial progenitors, the team ablated and enhanced expression of the proteins LGN and mInsc, respectively; both LGN and mInsc can shift spindle positioning.

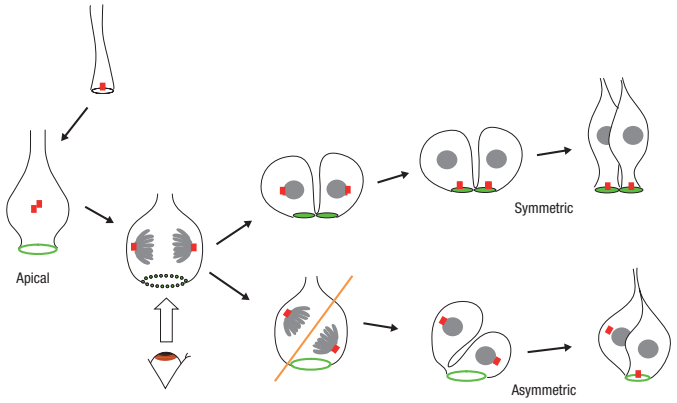

Figure 1: Neuroepithelial progenitor cell undergoing mitosis in the brain. Plane of division is parallel to the apicobasal axis and yet mitosis produces daughter cells with distinct cell fates. Green, chromosomes; red, cytoplasm.

Deletion of LGN and over-expression of mInsc resulted in asymmetric division occurring parallel to the apicobasal axis, and unequal distribution of apical and basal endfoots. Detailed study of individual dividing parent cells revealed that inheritance of both apical and basal endfoots was essential for adoption of the parental progenitor fate. Daughter cells inheriting either the apical or basal endfoots assumed the fate of cells on their way towards differentiating into neurons.

LGN ablation, which resulted in randomization of mitotic spindle positioning, led to depletion of neuroepithelial progenitors. Remarkably, however, overall neuron production remained unaltered.

Future study is needed to identify the cellular 'cargo' localized in the apical and basal endfoots of neuroepithelial cells that is required to bestow upon daughter cells a parental neuroepithelial progenitor cell destiny.

"There has been long-lasting controversy regarding mitotic spindle orientation during neuroepithelial progenitor divisions," says Matsuzaki. "Our study provides a firm answer to this issue, and raises the fundamental question of how neurons are born from these planar divisions." ■

1. Konno, D., Shioi, G., Shitamukai, A., Mori, A., Kiyonari, H., Miyata, T. & Matsuzaki, F. Neuroepithelial progenitors undergo LGN-dependent planar divisions to maintain self-renewability during mammalian neurogenesis. *Nature Cell Biology* **10**, 93–101 (2007).

Controlling the message

New research reveals how cells prevent sensitive protein-encoding messages from falling into the wrong hands

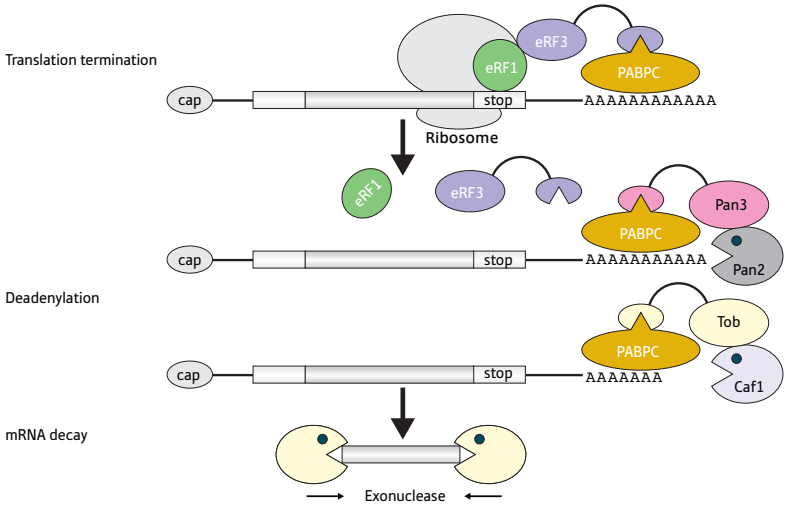

Figure 1: A schematic of the transition from mRNA translation to degradation. mRNA is translated into protein by a cellular machine called a ribosome. When the ribosome reaches the 'stop' signal within the mRNA being translated, it comes into contact with a termination complex consisting of eRF1 and eRF3. eRF3 interacts with PABPC1 bound to the poly(A) tail. After termination, eRF3 dissociates from PABPC1, freeing up a binding site subsequently used by Pan2–Pan3 and Caf1–Ccr4 complexes, which remove the poly(A) tail in two stages. Once this tail is gone, exonuclease enzymes eliminate the rest of the transcript. Tob is a scaffold protein that the Caf1 complex binds to as an intermediary for the interaction with PABPC1.

Like a spy receiving a coded secret message, cells typically destroy mRNA transcripts once they have been successfully translated into protein. As with the spy, this is for the cell's own protection; the longer a transcript survives, the less control there is over the amount of protein generated from it.

This process requires precise coordination, and Yuji Funakoshi, a researcher in Masafumi Tsujimoto's group at the RIKEN Advanced Science Institute (formerly the Discovery Research Institute) in Wako, explains that "mRNA decay is intimately linked to and regulated by translation." Until recently, however, the details of that linkage were unclear.

A key step in mRNA decay involves the removal of a long stretch of adenine nucleotides at the end of the transcript—a stabilizing structure known as the poly(A) tail—in a process called deadenylation. Previous work by Shin-ichi Hoshino's group at Nagoya-city University has demonstrated that eRF3, a factor involved in translational termination, appears to mediate deadenylation via interaction with PABPC1, a protein that binds the poly(A) tail[1]. Now Funakoshi, Tsujimoto, and Hoshino have followed up on this work to examine how this interaction facilitates post-translational mRNA decay[2].

In eukaryotic cells, mRNA degradation is managed by two specialized multi-protein complexes, Pan2–Pan3 and Caf1–Ccr4. By selectively disrupting the activity of these complexes in yeast and mammalian cells, Hoshino's group demonstrated that eRF3 relies on both of them to trigger mRNA decay. This appears to be a two-stage process—Pan2–Pan3 causes slow but steady shortening of the tail, after which Caf1–Ccr4 induces rapid removal of the rest of the tail and subsequent transcript degradation.

Both complexes bind to PABPC1, which activates their enzymatic activity. Importantly, Pan2–Pan3 and Caf1–Ccr4 both interact with the same subdomain of PABPC1 as eRF3. This suggests a model where transcript decay is initiated when eRF3 recognizes that translation is complete and dissociates itself from PABPC1, freeing up this site for sequential binding by the two deadenylation complexes (Fig. 1).

"The most important finding in this research is that we have clarified the molecular mechanism that triggers mRNA decay," says Funakoshi. "This mechanism plays a fundamental role in gene expression." These findings also suggest other roles for eRF3, and the Hoshino group is now examining eRF3's involvement with nonsense-mediated decay (NMD), a process that eliminates faulty mRNAs in which random mutations have introduced inappropriate 'stop translation' signals into a transcript. "eRF3 could play a pivotal role in NMD," concludes Funakoshi. ■

1. Hoshino, S., Imai, M., Kobayashi, T., Uchida, N. & Katada, T. The eukaryotic polypeptide chain releasing factor (eRF3/GSPT) carrying the translation termination signal to the 3′-Poly(A) tail of mRNA. *Journal of Biological Chemistry* **274,** 16677–16680 (1999).
2. Funakoshi, Y., Doi, Y., Hosoda, N., Uchida, N., Osawa, M., Shimada, I., Tsujimoto, M., Suzuki, T., Katada, T. & Hoshino, S. Mechanism of mRNA deadenylation: evidence for a molecular interplay between translation termination factor eRF3 and mRNA deadenylases. *Genes & Development* **21,** 3135–3148 (2007).

Synchronized spiking

Oscillating neurons knock their neighbors into step to produce signals that can survive the journey through the nervous system

Many natural and artificial systems are made up of oscillators that have to be synchronized. Tomoki Fukai and co-workers at the RIKEN Brain Science Institute in Wako have described how oscillating neurons in the brain work together to produce strong electrical signals[1].

"In response to a constant stimulus, neurons typically generate near-periodic action potentials or spikes," says Fukai. "In the brain, rhythmic neuronal activity is often linked to cognitive behavior. These periodic spiking neurons can be regarded as oscillators."

Neurons relay information to other neurons via the synapses that connect up the nervous system. A spike from a single neuron may not be strong enough to activate a neuron further down the line, because the signal power is dissipated in the synapses. Therefore it is important that the periodic spikes from several neighboring neurons can be synchronized into one stronger signal (Fig. 1).

For example, synchronization in the frequency range 30–70 Hz is believed to be crucial for paying attention, while the range 3–8 Hz may play a role in learning and memory. "However these issues are still in debate," says Fukai.

The behavior of each neuron is dominated by its phase response curve (PRC)—which determines how its output spike is affected by the phase of the input spike. A previous theory called the Kuramoto model has shown how these phase shifts can lead to synchronized states, but it assumes that the PRCs of neurons are all fairly similar. In reality PRCs vary considerably from neuron to neuron, especially across different layers in the cerebral cortex—a point noted by the RIKEN team[2].

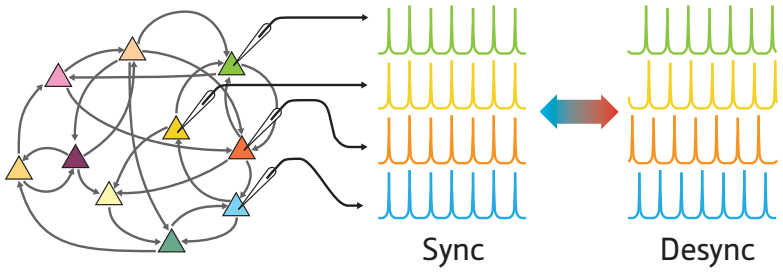

Figure 1: Schematic showing neurons connected by synapses (left). The voltage spikes from the neurons are usually out of phase, or desynchronized (right) but can become synchronized (centre) to produce a much stronger signal.

"In our [new] model, we extend the Kuramoto model to the case where the…phase response curves are heterogeneous in an oscillator network," says Yasuhiro Tsubo, a member of the team. "Our method is important since in the real world no system is exactly homogeneous."

Two types of neurons are known: Type-I, in which the phase of oscillation can only be advanced, and Type-II, in which the phase can be advanced or delayed by a signal. This extra flexibility means that Type-II neurons are more likely to become synchronized. The researchers predicted three possible states for the heterogeneous system: perfect synchronization, partial synchronization or perfect desynchronization.

The new model successfully revealed the novel dynamical states in neuron data from the cerebral cortex of rats. It could even be applied to diverse oscillator systems such as semiconductor devices, heartbeats, laser arrays or the luminescence of fireflies. ■

1. Tsubo, Y., Teramae, J. & Fukai, T. Synchronization of excitatory neurons with strongly heterogeneous phase responses. *Physical Review Letters* **99,** 228101 (2007).
2. Tsubo, Y., Takada, M., Reyes, A.D. & Fukai, T. Layer and frequency dependences of phase response properties of pyramidal neurons in rat motor cortex. *European Journal of Neuroscience* **25,** 3429–3441 (2007).

Sturdy synapses

Researchers show that brain cell signaling is regulated by phosphate

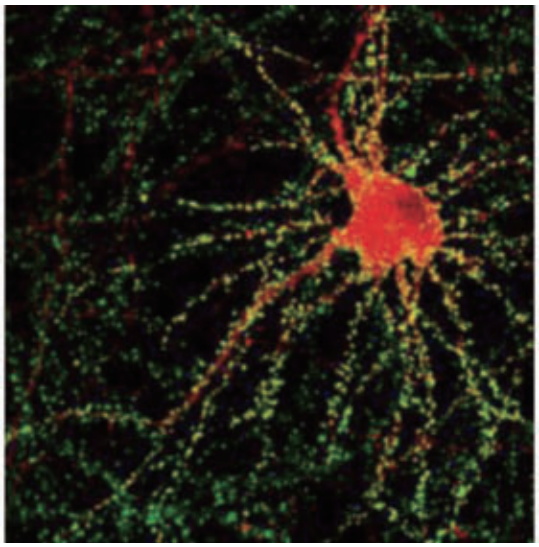

Figure 1: The effects of PSD-95 Ser-295 mutants on surface neurotransmitter receptors. Phosphomimic mutants of PSD-95 were transferred into brain cells, which were stained for surface receptors (green) and PSD-95 (red). The area of concentrated receptors coincides with the area containing the phosphomimic mutant of PSD-95.

Neuroscientists in the US and UK have revealed that brain cell communication is regulated by modification of the synaptic protein PSD-95, helping it to anchor signal receptors.

Synapses are junctions at which nerve cells transfer information to one another or to muscle or gland cells. Electrical impulses from the pre-synaptic neuron, or nerve cells of the brain, are converted into chemical signals called neurotransmitters that travel across the gap between cells and are received by receptors on the opposite side. Immediately behind the membrane of the post-synaptic cell is an elaborate mix of proteins called the post-synaptic density (PSD). These proteins serve to anchor and traffic neurotransmitter receptors to the cell membrane and secure various receptor-modulating molecules.

Because PSD-95 helps to strengthen and mature synapses, it has been of specific interest to Morgan Sheng and his team at the RIKEN-MIT Neuroscience Research Center in Cambridge, Massachusetts, The Picower Institute for Learning and Memory also at MIT, US, and the University of Bristol, UK. In the brain, PSD-95 is modified by the introduction of a phosphate group, a process known as phosphorylation, on one of its amino acid residues, serine-295. The researchers studied how phosphorylation regulates synaptic function. As reported recently in *Neuron*[1], they found that the addition of a phosphate group ushers the protein to part of the synapse where it can stabilize receptors that receive neurotransmitters and activate the post-synaptic cell.

Sheng and his team created mutant proteins that either mimicked phosphorylated PSD-95 or prevented phosphorylation and examined their effects in brain cells. The 'phosphomimic' mutant increased the level of neurotransmitter receptors on the surface of the post-synaptic cell compared to normal brain cells, whereas the non-phosphorylated mutant failed to increase receptor levels. The phosphomimic mutant was targeted to a particular area of the synapse that allowed it to promote the surface expression of receptors (Fig. 1).

The researchers also describe, for the first time, a mechanism for the regulation of serine-95 phosphorylation. They found it is mediated mostly by an enzyme called Jun N-terminal kinase1 (JNK1) and dependent on another protein, Rac1. Phosphorylation is vital to synaptic targeting of the PSD-95 protein, whereas dephosphorylation is important for long-term depression of synaptic strength, which, for example, is important in learning and memory. "Boosting serine-295 phosphorylation should increase the amount of PSD-95 in synapses and might help to prevent the synapse weakening or synapse loss that is associated with certain neurodegenerative diseases, such as Alzheimer's disease," says Sheng. ■

1. Kim, M. J., Futai, K., Jo, J., Hayashi, Y., Cho, K. & Sheng, M. Synaptic accumulation of PSD-95 and synaptic function regulated by phosphorylation of serine-295 of PSD-95. *Neuron* **56,** 488–502 (2007).

The key to controlling DNA copying

Researchers use beamlines at RIKEN's SPring-8 Center to glean new insights into how replication is regulated

Molecular biologists in Japan have unraveled structural details of a key molecular process which regulates DNA replication—the basis of reproduction.

The researchers used as their model the F-plasmid—a small piece of genetic material which operates independently to the chromosome—associated with sexual reproduction in the bacterium *Escherichia coli*. They believe, however, that while the fine detail of control of DNA replication may differ in other locations and organisms, the general pattern will be the same.

Previous work has shown that the protein RepE plays an essential role in regulating the copying of the F-plasmid, ensuring there are only one to two plasmids in each individual bacterial cell. Single RepE molecules, or monomers, join together to generate a double form, or dimer, which is the predominate form in the cell. The dimer binds to the promoter/operator of the *repE* gene preventing its transcription and repressing replication, whereas the monomer binds to a small repeated DNA sequence to initiate replication.

The conversion between the two forms is known to be mediated by the DnaK system of three chaperone proteins, but the structural details of how this might occur were unclear because no dimeric structure had been published for RepE. In a recent paper published in the *Proceedings of the National Academy of Sciences*[1], the researchers from Kyoto University and RIKEN detail how they used the beamlines at RIKEN's SPring-8 Center in Harima to generate the first structure of the RepE dimer and then compared it with the monomer.

They successfully prepared the dimer under high salt conditions and obtained crystals of the complex with the *repE* operator DNA. The monomer consists of two independent regions at each end known as domains—a C-terminal domain and an N-terminal domain. They are joined by a linker region. In the domains the conformation, or 3-D disposition of atoms to each other, remains constant in both monomer and dimer. But the orientation of the domains to each other is strikingly different between the two forms, exhibiting structural changes in the linker regions connecting them (Fig. 1).

The researchers found that sequences in the linker regions provide convenient binding points for the molecular chaperones that regulate the splitting of the dimer into two monomers thereby activating DNA replication. "Now we will try to prepare the RepE-chaperone complex," says team-member, Akira Nakamura from Kyoto University. "It should provide good information on the detail of the activation process." ■

1. Nakamura, A., Wada, C. & Miki, K. Structural basis for regulation of bifunctional roles in replication initiator protein. *Proceedings of the National Academy of Sciences USA* **104,** 18484–18489 (2007).

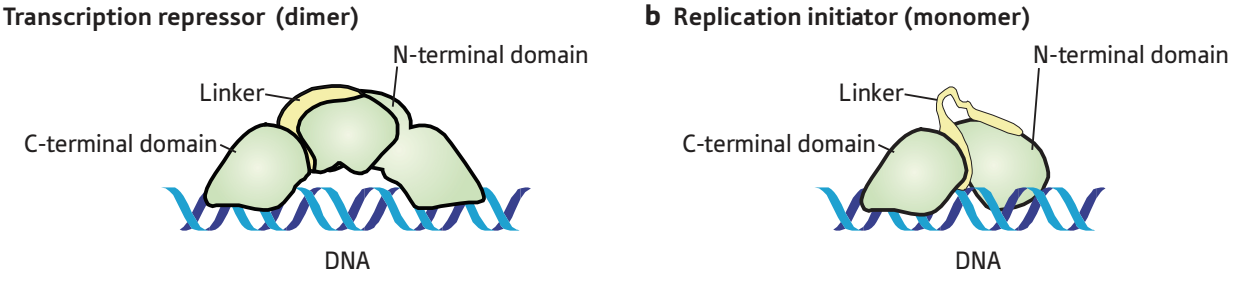

Figure 1: Schematics of the structural difference in the dimeric (left) and monomeric (right) RepE protein.

A cure for the cold

A recent structural biology study reveals how one protein helps keep bacteria running through a cold spell

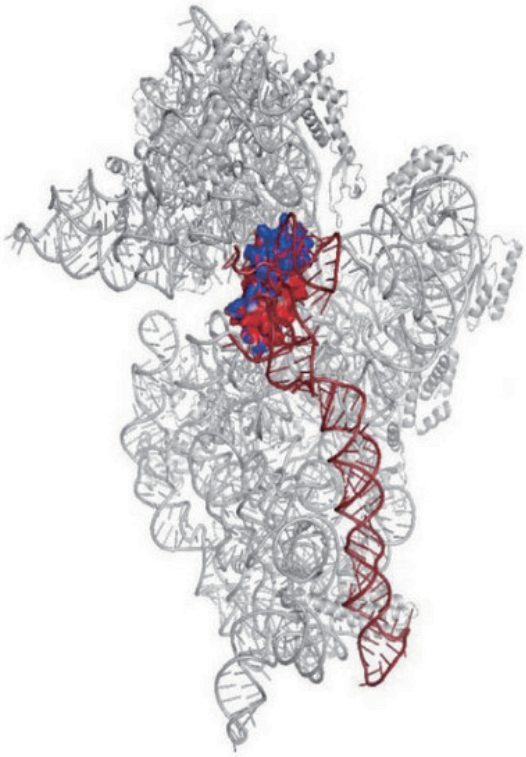

Figure 1: The structure of the RbfA-30S complex, revealing the interaction of the RbfA (red and blue) with helices (brown) on 30S involved in ribosomal assembly and RNA translation.

The translation of RNA into protein is managed by cellular machines known as ribosomes. A ribosome consists of two subunits, each composed of an RNA-based scaffolding with a number of specialized proteins attached.

The formation and assembly of ribosomes within a living cell seems to be a complicated process, which can be strongly affected by environmental conditions. For example, extreme cold has a very negative impact on bacterial protein production. "Cold shock results in an increase in the level of non-translating ribosomes, and produces a temporary cessation of bacterial growth," explains Shigeyuki Yokoyama of the RIKEN Systems and Structural Biology Center in Yokohama. "Growth is then restored through the action of a set of cold-shock response proteins."

Previous studies have implicated the bacterial protein RbfA as an important component of this cold-shock response, and Yokoyama's team recently joined up with German and American scientists to explore the RbfA-ribosome interaction in order to better understand the mechanism of this process[1]. RbfA binds to the smaller 30S subunit of the bacterial ribosome, and so the researchers began by acquiring detailed structural data for the subunit alone and bound to RbfA.

These data showed that RbfA binds in the immediate vicinity of helix h1, a specific domain of 30S that is known to fold improperly under cold-shock conditions. They also revealed a dramatic conformational change in 30S after RbfA binding, affecting a separate ribosomal domain directly involved in the RNA decoding process as well as assembly of the large and small ribosomal subunits (Fig. 1). Subsequent comparison of the RbfA binding site on the ribosome against the binding sites used by other proteins associated with 30S subunit maturation indicated that these various proteins interact with many of the same ribosomal domains, suggesting mechanistic overlap.

Collectively, these results indicate that the binding of RbfA to 30S serves a dual purpose: enabling proper formation of the ribosomal subunit, as well as ensuring that mRNA transcripts do not associate with premature, incompletely folded subunits. The findings from this work should be broadly applicable with regard to understanding ribosomal maturation, for although RbfA production is markedly increased at temperatures where ribosome formation becomes inefficient, the protein is generally present at low levels under other cellular conditions. "Our results not only provide insight into the role of RbfA during maturation of the 30S subunit," says Yokoyama, "but they also suggest how RbfA confers a translational advantage to cells under conditions of cold shock." ■

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Sizing up plants

Scientists link a component of the DNA replication machinery with the control of organ size in the mustard plant, *Arabidopsis thaliana*

The size of plant organs, such as flowers, roots, leaves and stems, is determined by the size and number of cells each contains, which in turn can be regulated by cell expansion and cell proliferation, respectively. Cell expansion in plants is known to result when successive rounds of DNA replication occur without cell division—a process called endoreduplication. Indeed, researchers studying the genetics of plant size have revealed novel factors in endoreduplication, further underlying its involvement in the cell expansion process.

Now, Keiko Sugimoto-Shirasu at the RIKEN Plant Science Center in Yokohama, along with colleagues in Japan, the UK, and the US, have shown that the small size of a dwarf strain of *Arabidopsis* (*bin4*) is due to reduced endoreduplication, which results in a defect in cell expansion[1]. When the researchers increased the DNA content of this mutant plant using a chemical treatment, the size of the leaves increased (Fig. 1). No rescue occurred, however, when other growth factors that do not affect DNA replication were used.

The team has also shown that the gene product encoded by *BIN4*, which is expressed in endoreduplicating cells, is a new member of the DNA replication machinery that prevents DNA damage. Thus, the deficiency of this gene product in the mutant plant results not only in reduced endoreduplication but also more DNA damage in those cells.

As a result of the increased DNA damage in the endoreduplicating cells, DNA-damage response genes are activated as is a gene, *CYCB1;1*, whose gene product is known to regulate the cell cycle. The expression of *CYCB* genes is normally reduced in plant cells undergoing endoreduplication, so the increased level of *CYCB1;1* in the dwarf plants may prevent successive rounds of endoreduplication from occurring, thus leading to their smaller size.

Similar to her peers, Sugimoto-Shirasu previously thought that endoreduplicating cells would have no mechanisms to prevent a build-up of DNA damage because these cells do not divide or separate chromosomes again. Thus, there is not a high risk of passing on damaged DNA. However, she points out that "[our] studies indicate that endoreduplicating cells do protect themselves against the damage" by increasing the expression of DNA damage response genes and *CYCB1;1*. While it is still unclear why plants would go through the energetic expense to respond in this way, Sugimoto-Shirasu suggests that they may do so to maintain their proper size. ■

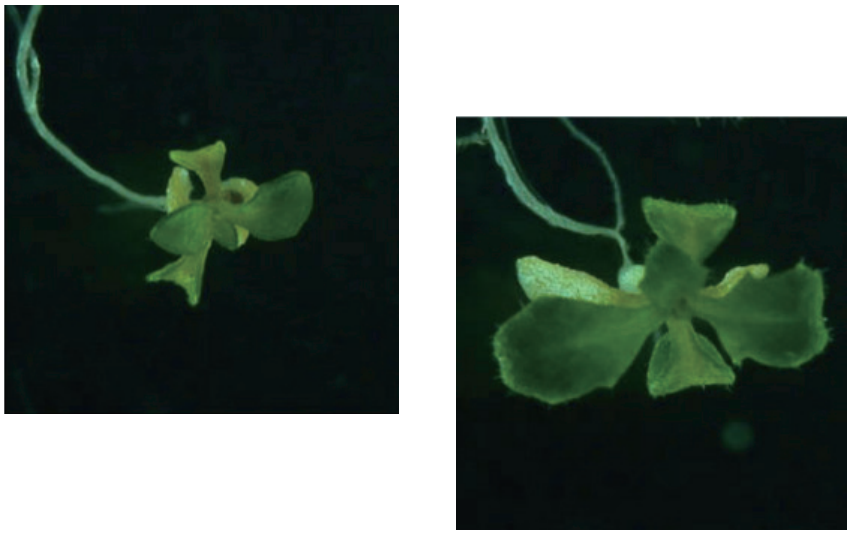

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Figure 1: An increase in DNA content increases plant size. An untreated mutant dwarf seedling (*bin4*) has smaller leaf size (left) than the same strain treated with a chemical that doubles DNA content (right). Both images are at the same magnification.

1. Breuer, C., Stacey, N.J., West, C.E., Zhao, Y., Chory, J., Tsukaya, H., Azumi, Y., Maxwell, A., Roberts, K. & Sugimoto-Shirasu, K. BIN4, a novel component of the plant DNA topoisomerase VI complex, is required for endoreduplication in *Arabidopsis*. *The Plant Cell* **19**, 3655–3668 (2007).

How plants resist attack by disease

More of the mystery of immunity solved with insights on the proteins needed for resistance in plants

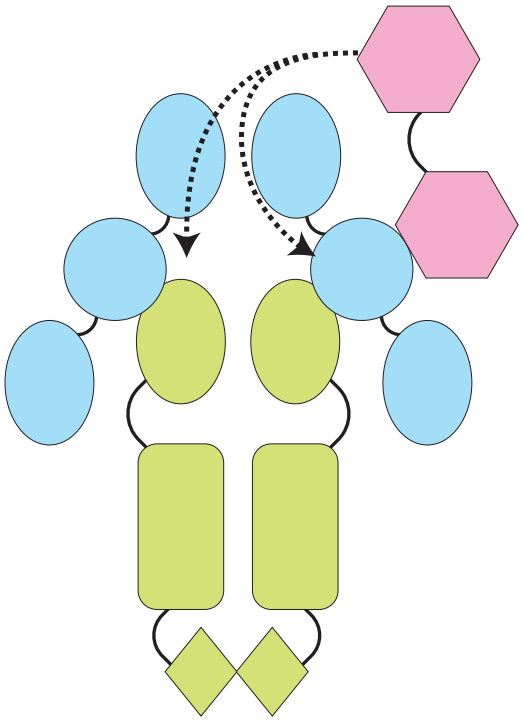

Figure 1: Proposed model for the interaction between HSP90–RAR1–SGT1. SGT1 (light blue) binds HSP90 (light green) and RAR1 (pink) on opposite sides of the CS domain.

An international research team led by a RIKEN molecular biologist has revealed a significant part of the complex mechanism of plant disease resistance. The team has found that the interaction of a highly conserved protein, SGT1, with the molecular chaperone HSP90 (heat shock protein 90) is essential for plant immunity. Understanding the molecular details of plant immunity is not only important for the protection of crop plants against disease, but can also provide insight into the human immune system where many of the same compounds and mechanisms are used.

Previous work showed that disease organisms or pathogens are detected in the cells of higher plants by the interaction of compounds they secrete with resistance (R) proteins. These interactions somehow activate a system which results in cell death, thereby limiting the spread of the pathogen. Recent research demonstrated that R proteins require HSP90 to function properly. It is also known that HSP90 interacts with the proteins SGT1 and RAR1 to regulate plant disease resistance. These two proteins can also interact with each other, and are required to stabilize many R proteins.

In order to find out more about the complex interplay between all these elements, a research team with members from RIKEN's Plant Science Center in Yokohama, research institutes in France and the UK and two British universities investigated the structure and function of SGT1 and its interaction with HSP90 in particular. The team published their results recently in *The Plant Cell*[1].

The researchers generated a series of random point mutations in the SGT1 gene. They found that the gene essential for the resistance of the thale cress, *Arabidopsis thaliana*, to *Potato virus X*, and that all of the mutations resulting in loss of resistance were located in two key domains of the SGT1 protein that interact with other proteins. One of those domains, CS, binds both HSP90 and RAR1. On the basis of nuclear magnetic resonance-based surface mapping, the researchers determined that the two compounds were bound on opposite sides of the domain (Fig. 1). But only interaction with HSP90 was required for *Potato virus X* resistance. Other experiments suggested that RAR1 may enhance the interaction with HSP90.

"We would now like to know how this complex interacts with R proteins," says project leader Ken Shirasu. "Our next step is to investigate the structure of a complex of the three proteins. The more challenging task is to solve the structure of the R proteins." ■

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Creating new molecules and contributing to drug discovery and biology

Mikiko Sodeoka

Chief Scientist
Director of the Synthetic Organic Chemistry Laboratory
Advanced Science Institute

The Synthetic Organic Chemistry Laboratory at the RIKEN Advanced Science Institute is working to create new molecules that act on living organisms. This research has two major objectives. One is to develop new chemical reactions for efficient production of desired molecules and to apply them to drug discovery and similar applications. The other is to use them to create new molecules and elucidate unknown mechanisms behind biological phenomena. Now the research has begun to explore biological phenomena from chemical perspectives.

Two influential events

"I studied pharmaceutical science at university because I was interested in biology," begins Mikiko Sodeoka, Chief Scientist of the Synthetic Organic Chemistry Laboratory, looking back on her student days. "But I was not good at experiments with mice," she laughs, adding, "In those days, I viewed biological phenomena as things in a black box and biology as a discipline involving memory work. In contrast, chemistry seemed to allow me to clearly understand the mechanisms of reactions. So I gradually became interested in chemistry."

Sodeoka chose to specialize in synthetic organic chemistry. Around the time she completed her Master's course, two significant events happened to her that led to her current work.

"One day, my hand reddened due to peripheral vasodilation caused by a very small amount of a molecule I had synthesized myself. I was struck by my body's acute response to the synthetic molecule. 'Why is my body influenced so much by a trace amount?' I wanted to find the mechanism behind it. In those days, however, a limited number of analytical methods were available for examining biological phenomena chemically, so I could not get on with such biological research immediately."

The other event happened while Sodeoka was performing an experiment to selectively synthesize isomers. Isomers refer to a set of molecules that share the same composition and number of atoms, but have different steric bonds and atomic configurations. Sodeoka explains that some isomers have totally different effects on the body despite close similarity in terms of their chemical and physical properties. "On one occasion, I needed to synthesize one of the two isomers; one is effective even in only trace amounts, whereas the other is far less effective. I attempted to use a catalyst containing chromium, a kind of transition metal, and successfully synthesized only the desired isomer very efficiently." She adds, "I was very impressed by the power of the transition-metal catalyst."

Selective production of left- and right-handed isomers

Since then, Sodeoka has been engaged in developing chemical reactions that are efficient in the selective production of isomers. At present, she is working mainly to develop a catalyst containing palladium, and to selectively produce

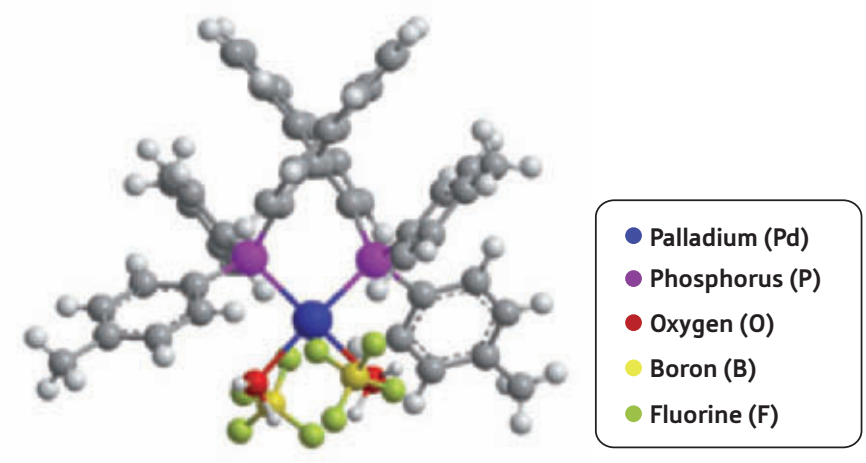

Figure 1 : Palladium catalyst for the selective production of optical isomers.

optical isomers (enantiomers). The left- and right-handed versions of optical enantiomers are mirror images of each other that appear to be the same, but their configurations cannot actually be superposed on each other. A well-known episode concerning functional differences in optical isomers is the tragedy of thalidomide, a drug once used as a sedative hypnotic in the 1960s. The dextral (right-handed) molecule of thalidomide is pharmacologically effective, whereas the sinistral (left-handed) molecule has been proposed to have an adverse effect that causes fetal malformation. Technology for selectively producing optical isomers is of paramount importance in the context of drug discovery and other areas of research. "RIKEN's president, Ryoji Noyori, received the Nobel Prize in Chemistry for developing a catalyst that allows the selective production of optical isomers by manipulating carbon–hydrogen bonds," says Sodeoka. "We are working to produce optical isomers selectively with a focus on carbon–carbon, carbon–nitrogen, and carbon–fluorine bonds."

Sodeoka's laboratory has succeeded in developing a palladium-based catalyst that allows the selective production of optical isomers in a ratio of more than 99 to 1 (Fig. 1).

Chemical biology

Another research theme for the Synthetic Organic Chemistry Laboratory is to create new molecules and use them to elucidate unknown mechanisms behind biological phenomena.

When a bioactive molecule enters the body, it binds to a particular target protein and causes a biological reaction. "For a while after graduating from university, I was studying bioactive molecules while not knowing the shape of their target proteins," Sodeoka continues. "I wanted to conduct research on designing molecules that recognize the shape of target proteins and selectively bind to them, and to explore how these molecules cause biological phenomena by using chemical approaches."

At the beginning of the 1990s, her career reached a turning point. "I had the opportunity to study abroad at the Chemistry Department of Harvard University in the USA, where I was engaged in research on the structural analysis of a protein." She explains that the molecules that are normally synthesized in her laboratory have low molecular weights. On the other hand, proteins are polymeric molecules with high molecular weights. "I had previously considered the two entities from very different viewpoints. However, while studying at the laboratory abroad, I realized that analytical techniques for low-molecular weight substances were basically applicable to polymers such as proteins, and that advanced techniques for examining the tertiary structures of proteins were available."

On the floor above her laboratory was the laboratory of Stuart Schreiber. Around that time, Schreiber proposed 'chemical biology', a new discipline that aims to elucidate biological phenomena from chemical perspectives. "In the early 1990s, the technical basis for research on life science from a chemical viewpoint began to be established," says Sodeoka. "After coming back to Japan, I started some chemical biology research, and when I assumed my current post at RIKEN in 2004, I became more involved in chemical biology research." She adds that the big advantage at RIKEN lies in the fact that researchers in all fields, including biologists, are readily available, so that it is easy to ask an expert in the relevant field if help is needed. "The interdisciplinary barrier is low and there are active personal exchanges. This is why RIKEN provides the perfect environment to conduct chemical biology as a fusion of chemistry and biology."

Creating molecules that prevent cell death

Described below are some studies in chemical biology that are being conducted by Sodeoka and her colleagues.

One study concerns necrosis, the passive form of cell death that occurs in response to severe cell injury. In contrast, the other type of cell death is called apoptosis—the form of cell death in which cells that are aged or no longer necessary during developmental processes are actively killed.

"More than 10 years ago, I heard from a biologist that he had discovered a phenomenon in which necrosis is suppressed by a molecule called BM I. This was the beginning of this study," says Sodeoka. The molecule BM I was developed as an inhibitor of protein phosphorylases (protein kinases). "However, I thought that the suppression of necrosis by BM I was probably unrelated to the inhibitory effect on protein kinases," she explains. This was because BM V, a derivative of BM I, has a weak but measurable necrosis-suppressive effect, although it does not inhibit protein kinase. "Hence, I attempted to create molecules that

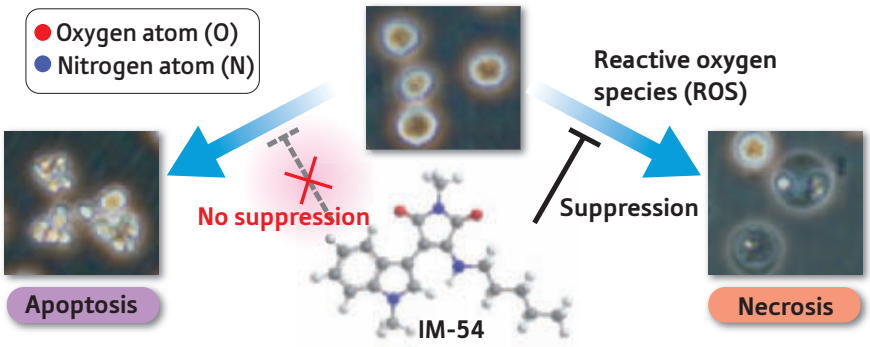

Figure 2 : A molecule that suppresses cell death.
The IM-54 molecule created by Sodeoka and her colleagues suppresses necrotic cell death, but it does not suppress apoptotic cell death caused by anticancer agents, for example.

strongly and selectively inhibit necrosis, without inhibiting protein kinases."

Sodeoka and her colleagues succeeded in creating IM-54, which strongly and selectively suppresses necrosis, by modifying BM I (Fig. 2).

Sodeoka continues, "If blood vessels in the brain or heart are clogged and then the blood circulation is restored, the cells in the affected organ are damaged by oxidative stress and necrosis occurs." She explains that when a heart blood vessel in a mouse is occluded and then re-opened, the animal dies within 10 minutes owing to heart failure. However, if the IM derivative is administered in advance, the mouse survives the situation.

During a heart transplant, while being taken out of the donor's body and transported to the recipient, the heart becomes ischemic and the heart cells are damaged. "In this case as well, the damage can be reduced by adding the IM derivative to the preservative solution for the heart. At present, we are exploring the mechanism behind the suppression of necrosis by the IM derivative."

Sodeoka and her colleagues found that the IM derivative binds to proteins that exist in mitochondria (Fig. 3). They are also investigating which protein is directly related to the necrosis. Although necrosis research lags behind apoptosis research, it is anticipated that the important proteins involved in necrosis and the mechanism of the function will be elucidated, with IM-54 and other molecules that selectively suppress necrosis as the key.

Molecules that selectively bind to target proteins

Sodeoka and her colleagues are also conducting research that will create molecules that bind to important target proteins, and explore the functions of the target proteins using them. A protein phosphatase called PP2B is known to play a key role in the signal transduction of the immune system. "The tertiary structure of PP2B has been solved," says Sodeoka. However, she adds that it is difficult to create molecules that bind to PP2B merely on the basis of the known protein structure. "We took note of a natural product that binds to and inhibits another protein phosphatase that has a very similar structure to that of PP2B. It is a component in the poison of the Chinese blister beetle. We modified it to create a molecule that binds selectively to PP2B and inhibits its function."

Such molecules are expected to find applications as immunosuppressants, and also as a powerful tools for exploring the role of PP2B and the mechanism of signal transduction by the immune system.

To date, research has been undertaken to examine the functions of genes and proteins by suppressing or over-expressing the genes that bear information on the proteins of interest. For example, knockout mice, prepared by suppressing the function of a particular gene, provide an important tool for investigation in biology. However, knocking out a gene sometimes causes the death of the animal during development, and so it cannot be used for experimentation. In other cases, many other genes are expressed in compensation for the function that is no longer active in the gene. Therefore, it cannot immediately be concluded that any event occurring in knockout mice is associated directly with the knocked-out gene.

On the other hand, if you use molecules that suppress the function of a particular protein, you can examine the function in a more straightforward way by suppressing it in a particular type of cell at a given time.

Additionally, proteins such as enzymes occur in two states: active and inactive. Each state has its own steric structure. If you create a molecule that binds only to an enzyme in the active state and label the molecule with a radioisotope marker, you can watch where and how the activated enzyme functions in the body using positron emission tomography (PET). This approach to visualizing the behavior of biomolecules, such as

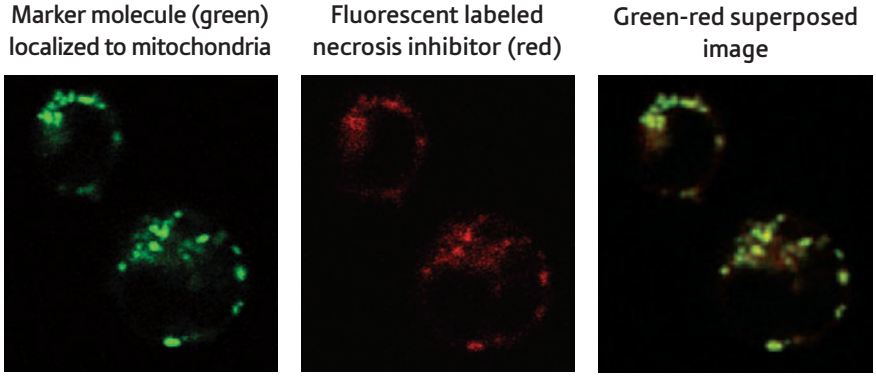

Figure 3 : Intracellular localization of necrosis inhibitor.
A target protein for the necrosis inhibitor is localized in mitochondria.

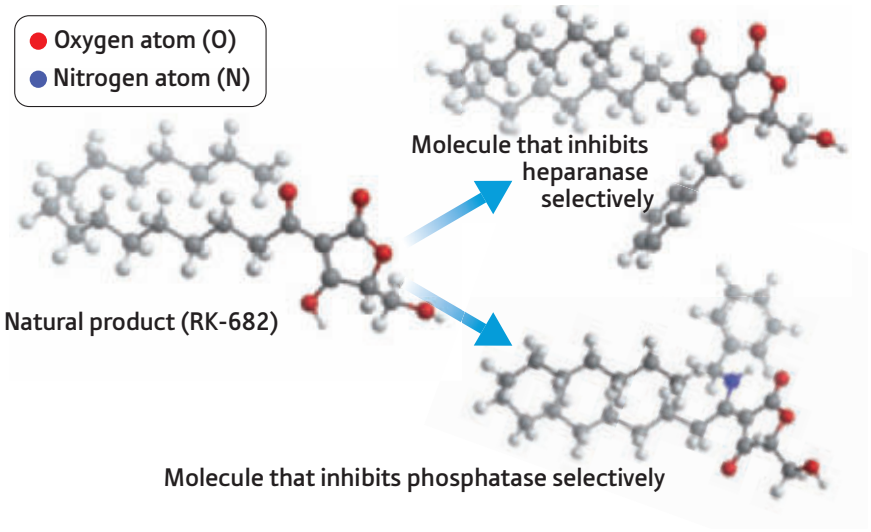

Figure 4 : New molecules created from a natural product.

proteins in the body, is called molecular imaging and will serve as an important tool for medicine and biology in the near future. As such, molecular imaging cannot evolve and advance without the development of molecules that bind selectively to particular proteins.

RIKEN, the center for chemical biology research

At the RIKEN Advanced Science Institute, the Chemical Biology Department is working under the leadership of Hiroyuki Osada, the director in the Antibiotics Laboratory. "As a research institution, RIKEN plays the central role in chemical biology research in Japan," says Sodeoka. "As such, RIKEN is promoting research following a different strategy to that in the USA."

Schreiber of the USA, who proposed the term chemical biology, and others are conducting investigations focusing on creating easily synthesizable molecules among which useful molecules can be discovered. This approach requires a great deal of money and human resources.

"On the other hand, Osada and others employ a strategy to collect a library of natural products and produce useful molecules from them efficiently," says Sodeoka. "Our laboratory is cooperating with this project."

For example, Osada and co-workers discovered RK-682, a natural product that inhibits protein phosphatase. They found that the molecule also inhibits the function of heparanase, an enzyme that is active during cancer cell metastasis. "We have succeeded in creating molecules that selectively inhibit phosphatase, and molecules that conversely inhibit heparanase only, by modifying the molecular structure of the natural product," Sodeoka continues (Fig. 4).

In outlining future prospects, Sodeoka says, "I want to create molecules that will open a way to elucidating the black box of biological phenomena." She adds, "My ultimate goal is to do joint research with biologists using a molecule that we have created ourselves, which will later be proven to be essential in elucidating the mechanism behind biological phenomena."

Thus chemists are now striving to lead biology to a new stage (Fig. 5).

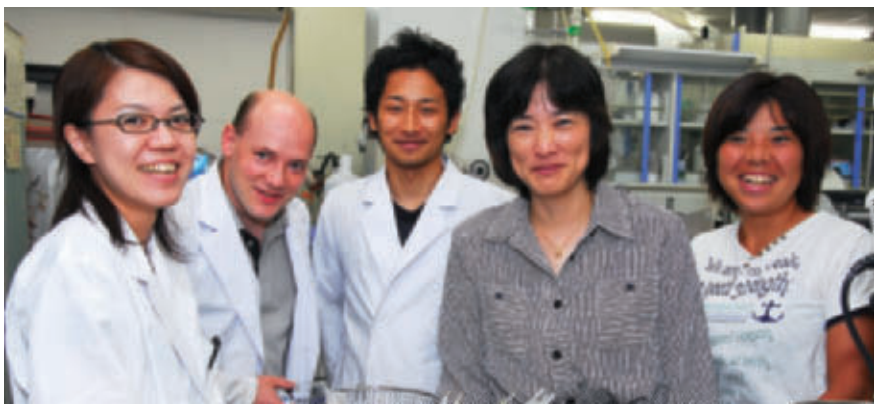

Figure 5 : Chief scientist Sodeoka and her co-workers.
At present, the Synthetic Organic Chemistry Laboratory has 21 researchers. "Good luck and accidents are also important for successful research in chemistry. You cannot receive the good luck that will lead to the creation of a new molecule unless you do your own experiments. Strange things that happen in experiments are of great interest. I hope that the students studying in my laboratory think so, too."

Background Information

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About the researcher

Mikiko Sodeoka received her BS (1981), MS (1983) and PhD (1989) degrees in the pharmaceutical sciences from Chiba University. After working at Sagami Chemical Research Center (1983–1986), she joined the Faculty of Pharmaceutical Sciences, Hokkaido University as a research associate (working with Masakatsu Shibasaki). After working as a post-doctoral fellow at Harvard University (with Elias Corey and Greg Verdine), she moved to the University of Tokyo (1992). She became a group leader at Sagami Chemical Research Center in 1996, and an associate professor at the University of Tokyo in 1999. In 2000, she moved to Tohoku University as a full professor. Since 2004 she has been the chief scientist and director of the Synthetic Organic Chemistry Laboratory at RIKEN. Her research interests are in the areas of synthetic organic chemistry and chemical biology. Her current research focuses on enantioselective catalysis using transition-metal complexes and the development of intracellular signal transduction modulators. She has been awarded the Encouraging Award of the Pharmaceutical Society of Japan (1993), the Takeda Chemical Industry Award in Synthetic Organic Chemistry, Japan (1999), the Chemical Society of Japan Award for Creative Work (2003), and the Nagoya Medal Prize, Silver Medal (2007).

Database of mechanical properties of tissues for mechanical simulations of the human body

RIKEN researchers have created a database of the nonlinear mechanical properties of soft tissues of the human body. The database will help researchers perform more accurate mechanical simulations of the human body. In addition, doctors will be able to use it for simulations to help estimate more accurately the damage to musculature and the internal organs of an injured patient, improve medical treatment, and better forecast the progress of disease and healing.

RIKEN created the database in cooperation with the Shizuoka Prefecture Industrial Research Institute, and opened it up for public use in March. Additional data is being solicited from researchers around the world to further develop the database.

The database includes information on the hardness and other mechanical characteristics of 17 internal organs, including the liver and pancreas. Most internal organs that make up the body, in particular soft internal organs such as skin and muscle, show nonlinear mechanical characteristics. The research consortium aims to construct a database of nonlinear physical properties for the simulation of the mechanical attributes of the human body.

The development work is being carried out at the RIKEN Advanced Science Institute's Living Matter Simulation Research Group, in cooperation with the Research and Development Group for Next-generation Integrated Living Matter Simulation and the Bio-research Infrastructure Construction Team of the VCAD System Research Program.

The database will allow researchers to forecast how an applied force will spread to various parts of the body such as the bones, muscles, and internal organs. The aim is to eventually have a model of the human body that includes both mechanical and shape information. This will be invaluable in the development of safety and prosthetic equipment, medical treatment technologies, and 'universal design' products. ■

RIKEN releases joint database of rice and thale cress cDNA

RIKEN, the National Institute of Agrobiological Sciences (NAIS), and the Research Institute for Biological Sciences, Okayama, have teamed up to develop an online database to provide information of phenotypes on gain-of-function mutants of Arabidopsis thaliana (Thale cress, a model plant).

The new database allows quick cross-referencing of the phenotypes with introduced rice full-length cDNA (complimentary DNA). It includes data on approximately 18,000 of the 24,000 mutant lines of Arabidopsis thaliana produced for this project. It is also possible to reference the introduced rice full-length cDNA in addition to morphological features of a broad range of traits, and a wide variety of non-visible traits, profiles of hormones and metabolites, elements, photosynthesis characteristics, resistances against heat and salt stresses, and so on.

This is the first time such a large and comprehensive amount of data has been collected for reference of traits of mutants in a single system. Useful features to enable quick searches of the database have also been incorporated.

The database will help in the development of functional genomics in plants. ■

SPring-8 researchers observe crystallization process in real time

A team of researchers at the RIKEN SPring-8 Center in Hyogo Prefecture has, for the first time, observed in real time the phase change of a material used in super-fast optical recording. The achievement, the first such observation in the world, is expected to further speed up the development of high-speed optical recording media.

The RIKEN researchers, along with scientists from the Japan Synchrotron Radiation Research Institute, the Japan Science and Technology Promotion Agency, Matsushita Electric Industrial Co. and University of Tsukuba, investigated the material using the X-ray diffraction capabilities of SPring-8's BL40XU high-flux beamline, and observed the crystallization process at the nanosecond level for compounds comprising germanium, antimony, and tellurium, and silver, indium, antimony, and tellurium.

The crystal growth process observed is much faster than previously thought, and the new technique is expected to be of great help in designing next-generation phase-change materials. The rewritable phase-change type DVD-RAM disc is an indispensable memory medium used in mass data recording, in widespread use in consumer and office electronics.

Optical data is recorded by heating the crystals of the recording medium with a laser, then cooling them suddenly, forming a pattern of reflective and non-reflective areas that can be detected by a read laser. When rewriting, the film is made to change to an amorphous phase, and then it is crystallized again, changing its reflectivity. When reading the data, the laser does not use enough power to cause the amorphous phase to change its reflectivity. In this state, the material can remain stable for decades, preserving the data.

Since the data must be accurately encoded on an extremely small scale, the writing system must be extremely precise. In order to enhance control over this process, the researchers wanted to observe this high-speed phase change at the atomic level, so they used 40-picosecond X-ray pulses from SPring-8 to perform pinpoint structural measurements and observe the changes in the material's crystal structure. ■

In the May issue Roundup 'Sakura Workshop hears about nuclear probes from high-energy' the end of the title was omitted in error. The title should have been 'Sakura Workshop hears about nuclear probes from high-energy accelerators'.

Dr. Tohru Motobayashi
Chief Scientist
Heavy Ion Nuclear Physics Laboratory
RIKEN Nishina Center for Accelerator-Based Science
Wako-city, Saitama, Japan.

Dear Dr. Motobayashi,

I clearly remember my first stay in Japan in 2001 when I met you at Rikkyo University. We had our first personal discussion there about a RIKEN experiment performed in a joint effort with my home institute, ATOMKI, in Hungary. Your field of interest, nuclear structure studies with radioactive ion beams, was relatively new to me at that time. I was truly impressed by your warm welcome and scientific intelligence, which inspired me to spend my postdoctoral years under your auspices.

I started my two-year Japan Society for the Promotion of Science (JSPS) fellowship in your newly established laboratory at RIKEN in 2002. Those first days were memorable because we had to build everything from scratch. I will never forget how much you and your colleagues helped me to collect the necessary items in order to furnish a small workshop to fabricate about 300 CsI(Tl) detectors for our commonly developed array. I was also introduced to the details of the routinely used techniques and methods, which I appreciated very much. This was so successful that I was given the opportunity to direct my own radioactive ion beam experiments with the invaluable assistance of the laboratory fellows. These measurements led to important discoveries in the areas of 'island of inversion', changing of 'magic' numbers in exotic nuclei and valence neutron decoupling phenomenon near the neutron drip line.

Not only did my scientific career get a boost in Japan, but socially my wife and I also experienced a different world. From the beginning, we were surrounded by a polite and responsive environment, particularly when we needed help or were hungry for information about travel, food, and home electronics. We found real friends in Japan. We treasure the time we could spend hiking in the beautiful mountains, eating never-tasted and never-imagined food, exploring the remains of ancient Japan and watching sumo wrestling in the arena. Since returning to Hungary in 2004, we badly miss these pieces of our Japanese life.

Fortunately, since returning to ATOMKI, you and I have been able to work in close cooperation and continue our fruitful projects. Due to the recent upgrade of the RIKEN Accelerator Facility to a so-called Radioactive Ion Beam Factory, unprecedented possibilities have opened up. With the abundant number of exotic beams of isotopes of world-leading intensities, we can extend our investigations to further unexplored regions of nuclei. The RIKEN–ATOMKI collaboration agreement signed recently provides us with a solid base to further deepen our relationship in the future.

With my best regards,
Zoltan Elekes
Institute of Nuclear Research (ATOMKI)
Debrecen, Hungary

www.riken.jp

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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 48 467 9443
FAX: +81 48 462 4715
E-Mail: rikenresearch@riken.jp

www.rikenresearch.riken.jp