



RIKEN RESEARCH

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Finding the bug in the aphid genome

HIGHLIGHT OF THE MONTH

Measuring unconventionality

RESEARCH HIGHLIGHTS

- Carbon wears a halo
- Semiconducting sandwich filling retains its mystery
- Better connections through green catalysis
- Joined at the genes?
- Making the right contacts to get ahead
- Wired for sight
- Time to grow
- New progress from a 'joint' venture
- Illuminating the genetic alphabet
- Projecting pain relief

FRONTLINE

Studying cell signaling using single-molecule imaging

ROUNDUP

Cementing the partnership with Xi'an Jiaotong University in China

POSTCARDS

Dr Poshak Gandhi (Institute of Space and Astronautical Science, Japan Aerospace Exploration Agency, Sagami-hara, Kanagawa, Japan)

Measuring unconventionality

Interference patterns made by wave-like electrons reveal that tiny atomic magnets are critical to iron-based superconductors

Achieving superconductivity at room temperature has represented one of the holy grails of physics for decades. A practical material with zero electrical resistance would not only represent a major advance in physics, but also revolutionize technologies from power grids to electric motors. However, the mechanism behind so-called 'high-temperature' superconductors, which are superconducting above approximately -240 Celsius, has been unclear, and the highest temperature at which superconductivity has been observed remains at a frigid -108 Celsius.

Now, the mechanism responsible for superconductivity in an important class of high-temperature superconducting materials, discovered in 2008, has been revealed by Tetsuo Hanaguri and colleagues at the RIKEN Advanced Science Institute, the Japan Science and Technology Agency (JST), The University of Electro-Communications in Tokyo, and The University of Tokyo¹.

Pairing up

The researchers studied the mechanism behind a key property of all superconductors: electron pairing. In an ordinary material, electrons travel independently and their motion is regularly disrupted, or scattered, by defects and by vibrations (or phonons) of the atomic lattice they are traveling through. This leads to electrical resistance, so that any flowing current must be 'pushed' along by an applied voltage. In superconductors, electrons travel in pairs, rather than individually, making them less prone to scattering. A minimum amount of energy called the 'superconducting gap' energy must then be expended to break an electron pair. Since this energy is unavailable at low temperatures, the motion of the

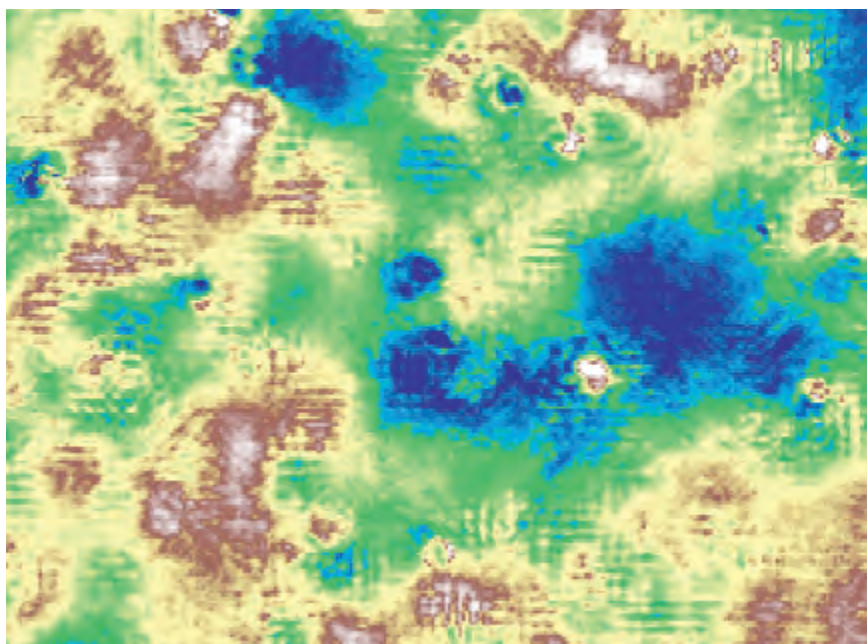


Figure 1: An image of the interference pattern made by wave-like electron pairs in a high-temperature, iron-based superconductor as measured using a phase-sensitive scanning tunneling microscope.

electron pairs remains unperturbed, and the material's resistance is zero. This means a current can flow perpetually without any applied voltage.

Hanaguri and colleagues focused on understanding how electron pairing occurs in iron-based superconductors, one of the two major classes of high-temperature superconductors. In conventional, low-temperature superconductors, electrons are paired because phonons create attractions between them, overcoming the natural repulsion the electrons have as a result of their identical negative charges. In iron-based superconductors, however, superconductivity is associated with a particular ordering of the atomic magnets found in the materials. This generated speculation among physicists that these tiny magnets, or spins, may be involved

in the pairing mechanism. The work by Hanaguri and colleagues provides strong evidence that these spins are indeed responsible for electron pairing in iron-based superconductors.

Out of phase

The researchers leveraged their expertise with scanning tunneling microscopes (STMs) to gather this evidence. Traditionally used to map the shapes of nanostructures and atoms, these microscopes measure the current between a sharp nanoscale tip and a surface just beneath it. They can also be used to measure the momentum of electrons traveling across a surface. Just before the discovery of iron-based superconductors, Hanaguri had developed a method at RIKEN in Hidenori Takagi's laboratory

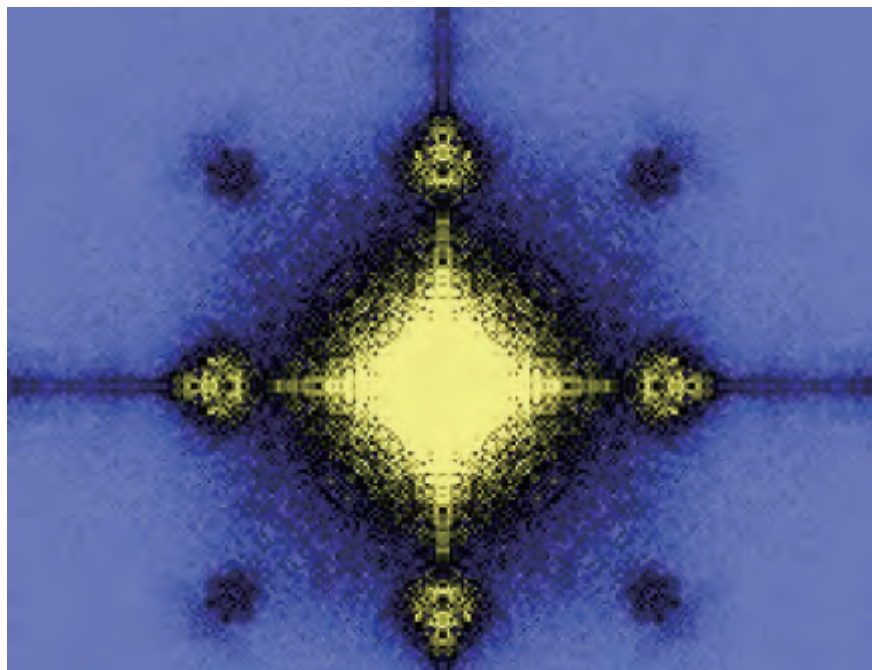


Figure 2: An image of the interference pattern shown in Fig. 1 that has been converted numerically from real space (with axes representing left/right and up/down) to momentum space, pictured here (with axes representing momentum to the left/right and up/down). This alternate view of the interference data yields information about how electron scattering depends on electron momentum.

to use STMs to measure the phase of electrons, and this capability was the key to their work on superconductors.

Hanaguri and colleagues were able to measure the interference pattern of electron pairs by purposefully scattering them from magnetic vortices that they created in the superconductor Fe(Se,Te) using an applied magnetic field. Electron pairs behave like waves at very small scales so, like all waves, they have a phase. For example, two water waves traveling across a pond at the same speed have different phases if one wave is slightly behind the other. If they collide, they make an interference pattern that is affected by the phase difference between them. Similarly, the interference pattern made by electron pairs is affected by the phase difference between those pairs (Fig. 1).

The researchers measured and interpreted these interference patterns to understand iron-based superconductors. After initial measurements on high-quality crystals grown by their collaborator Seiji Niitaka, they began the task of data interpretation. Unfortunately, they made an early mistake with the coordinate system that stymied their progress until

Kazuhiko Kuroki from The University of Electro-Communications realized the error at a presentation. Kuroki later joined the collaboration and helped interpret the measured interference patterns.

The team found that the patterns could be explained by assuming that the phase of an electron pair, and its associated superconducting gap, depends on the momentum of the pair (Fig. 2). This telltale sign of spin-mediated electron pairing had been predicted theoretically but never realized experimentally. By confirming the role of spins in iron-based superconductors, the team's data lay the foundation for an understanding of superconductivity that is not based on lattice vibrations unlike more conventional superconductors.

Past and future

Hanaguri says his group was in a lucky position at the outset. "My 'aha!' moment came when I realized that the phase-sensitive STM technique that I had already developed could be applied to iron superconductors, which had just been discovered." He also counts openness as a key to the success of the work: had

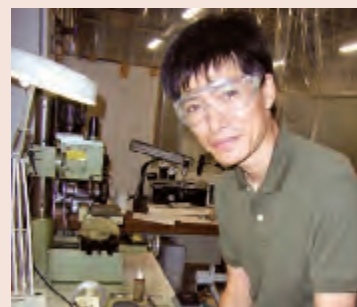
Hanaguri not comprehensively described his preliminary results at a conference, Kuroki would not have identified his mistake. "My policy is that all the data, techniques and plans that I have must be as open as possible," Hanaguri says.

Hanaguri also notes that the phase-sensitive scanning tunneling microscope developed by his team yielded a significant result in only its first years of operation, and can be expected to produce important results in other realms of physics, including magnetism. Ultimately, Hanaguri would be most satisfied by finding something completely new. "Our equipment is capable of studying matter under extreme conditions, and it is under extreme conditions that many new physical phenomena have been discovered," he explains. "To discover a new phenomenon would be much more exciting than the elucidation of an existing phenomenon's mechanism." ■

1. Hanaguri, T., Niitaka, S., Kuroki, K., Takagi, H. Unconventional *s*-wave superconductivity in Fe(Se,Te). *Science* **328**, 474–476 (2010).

About the researchers

Tetsuo Hanaguri was born in Tokyo, Japan, in 1965. He graduated from the Department of Applied Physics at Tohoku University in 1988, and received his PhD in applied physics from the same university in 1993. He then worked as a research associate and associate professor at The University of Tokyo until he joined RIKEN. Since 2004, he has held the position of senior research scientist in the Takagi Magnetic Materials Laboratory at RIKEN. He works in the field of experimental condensed-matter physics at low temperatures, and his current research focus is on spectroscopic imaging scanning tunneling microscopy of complex electron systems including superconductors and topological insulators. He is also interested in measurement science and technology and enjoys building scientific apparatus.



Carbon wears a halo

Discovery of the heaviest known Borromean nucleus provides a new testing ground for fundamental nuclear models

In addition to lithium-11 and beryllium-14, the neutron-rich isotope carbon-22 (^{22}C) could also be a Borromean ‘halo’ nucleus, a team of researchers from Japan has reported in *Physical Review Letters*¹. The finding will allow physicists to test fundamental nuclear models in nuclei containing a high ratio of neutrons to protons.

To a good approximation, the atomic nucleus is a uniformly dense distribution of protons and neutrons packed into a spherical drop a few femtometers (10^{-15} m) in radius. However, isotopes that contain more than two to three neutrons for every proton start to ‘leak’ neutrons. For a very few of these neutron rich nuclei, one or two excess neutrons form a loosely bound orbit—or halo—about the nuclear core.

The two-neutron halo nucleus is a special quantum three-body system: if one of the neutrons in the halo is removed, the remaining part falls apart. This interdependent system of two neutrons and a core is called a ‘Borromean’ nucleus, because of its similarity to the three, interlocked Borromean rings (Fig. 1).

“Previously, only the instability of ^{21}C suggested that ^{22}C might be a Borromean nucleus, and hence have a two-neutron halo,” explains Kanenobu Tanaka from the RIKEN Nishina Center for Accelerator-Based Science in Wako. “To study whether ^{22}C has the halo structure, we assembled a large-scale collaboration among institutions with expertise on many techniques. For example, special detector settings had to be prepared and creating the beams of carbon isotopes required careful tuning.”

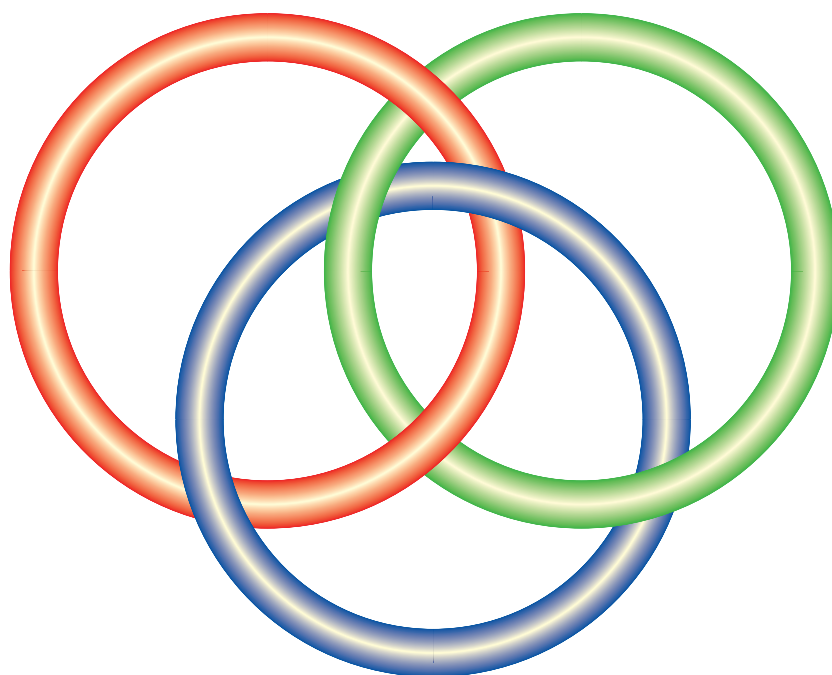


Figure 1: An example of ‘Borromean’ rings. If just one ring is cut, the whole structure will fall apart.

Nuclei with a high neutron-to-proton ratio are unstable and can only be made artificially. Using the RIKEN projectile fragment separator (RIPS) the researchers produced three isotopes of carbon— ^{19}C , ^{20}C and ^{22}C —from the fragments of a high-energy beam of argon that impinged on a tantalum target. They then bombarded the carbon nuclei against a target cell for liquid hydrogen. Since larger nuclei are more likely to strike the hydrogen protons in the liquid, the researchers could determine the size of each carbon isotope by measuring its frequency of collision.

Tanaka and colleagues found that the radius of the ^{22}C was about 5.4 fm, which

is more than 50% larger than theoretical predictions, providing strong evidence that ^{22}C is a halo nucleus and making it the heaviest Borromean nucleus ever observed.

“This finding opens the possibility to find halo nuclei in a more extended region of the nuclear chart and will give us greater insight into the mechanism of halo formation,” says Tanaka. ■

1. Tanaka, K., Yamaguchi, T., Suzuki, T., Ohtsubo, T., Fukuda, M., Nishimura, D., Takechi, M., Ogata, K., Ozawa, A., Izumikawa, T. *et al.* Observation of a large reaction cross section in the drip-line nucleus ^{22}C . *Physical Review Letters* **104**, 062701 (2010).

Semiconducting sandwich filling retains its mystery

Solving part of the long-standing puzzle of the electronic properties of an enigmatic temperature phase of a titanium oxide may yield new clues

Oxides of transition metals such as titanium are of interest for applications, such as hydrogen gas sensors or as catalysts, and have intriguing fundamental physical properties. In particular, the origin of an intermediate temperature phase of the compound Ti_4O_7 has puzzled scientists for decades. By studying the different electrical phases of Ti_4O_7 , researchers from the RIKEN SPring-8 Center in Harima, along with colleagues from other institutions in Japan, have now taken important steps towards understanding the fundamental differences between the compound's electrical conductivity at room and low temperatures, and the enigmatic phase that forms at intermediate temperatures¹.

At room temperature and down to temperatures of 154 K, Ti_4O_7 is an excellent conductor, as it allows fast transport of electrical charges. At temperatures below 142 K, the compound is an electrical insulator. Between 142 K and 154 K, however, the mysterious intermediate temperature phase sets in where the compound is semiconducting. Both, the metallic and the insulating phases are well understood by classical theories. The semiconducting phase, however, is very strange and complex; its origin is particularly interesting because it is sandwiched by two such well-known phases, explains Munetaka Taguchi from the research team.

To elucidate the origin of the semiconducting phase, the researchers studied the electronic phases at the top of the valence band and bottom of the conduction band that are responsible for the electrical conduction. They employed

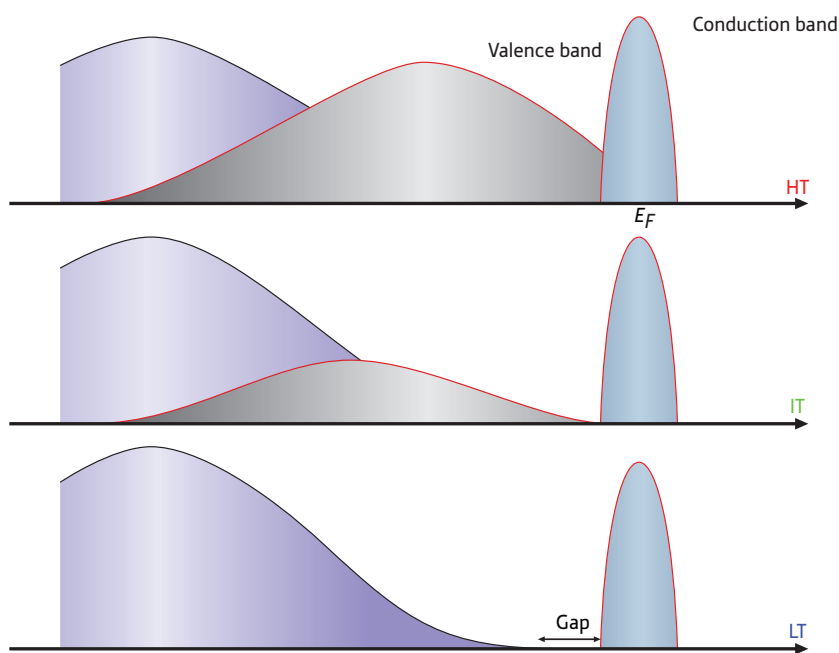


Figure 1: The electronic phases of Ti_4O_7 . In the high-temperature (HT) range (top), coherent electrons from the valence band (gray) reach the conduction band so it is metallic. In the intermediate-temperature (IT) range (middle), only a few coherent electrons remain, which are a remnant of the metallic state. In the low-temperature (LT) range (bottom), the gap in the electronic phases means it is an insulator.

the techniques of electron photoemission and x-ray absorption, which combined provide a detailed picture of the electronic phases.

In the high-temperature phase, Taguchi and colleagues found that so-called ‘coherent valence electrons’ extended as far as the conduction band, making it a metallic conductor. In the insulating phase, there is a gap in the electronic band structure and no electrons are available in the conduction band. For the intermediate regime, however, a small number of coherent electronic phases remain close to the conduction band and explain the measured electrical conductivity (Fig. 1).

While it is clear that the small number of coherent electrons is a remnant of the

metallic phase, the transformation path—from the semiconducting state to both the room-temperature metal and the low-temperature insulating phase—remains unclear, notes Taguchi. With such crucial fundamental questions still unsolved, more work is needed to study the nature of the coherent electronic phases, which Taguchi hopes “will provide us [with] vital clues to a more complete understanding of phase transitions.” ■

1. Taguchi, M., Chainan, A., Matsunami, M., Eguchi, R., Takata, Y., Yabashi, M., Tamasaku, K., Nihino, Y., Ishikawa, T., Tsuda, S. *et al.* Anomalous state sandwiched between fermi liquid and charge ordered Mott-insulating phases of Ti_4O_7 , *Physical Review Letters* **104**, 106401 (2010).

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Better connections through green catalysis

Finely tuned nickel complexes combine important biomolecular precursors with high efficiency and low environmental impact

In chemistry, the nitro group (O-N=O) has a formidable reputation. The high reactivity that makes some nitro-bearing molecules potent explosives—think nitroglycerin or trinitrotoluene (TNT)—also enables NO₂ to be extremely versatile in organic synthesis. Chemists can transform nitro groups into numerous other functionalities, such as biologically important amines or carbonyl compounds, providing a constant demand for new, efficient reactions involving these compounds.

Now, researchers led by Mikiko Sodeoka from the RIKEN Advanced Science Institute in Wako have developed an innovative way to connect organic molecules known as nitroalkenes and α -ketoesters together with precisely controlled geometries¹. Because this synthesis uses an ‘environmentally friendly’ catalytic system, it can help create a broad range of molecules, including therapeutic natural products, under mild conditions.

Typically, reactions between nitroalkenes and α -ketoesters require hazardous liquids, generous quantities of catalysts, and very low temperatures to be successful. Instead, Sodeoka and her team were able to complete this chemical transformation at room temperature, with a non-toxic propanol solvent, by using small amounts of a nickel acetate catalyst (Fig. 1)—an advance with significant cost-saving and environmental-hazard reducing potential.

According to Yoshitaka Hamashima, a co-author of the paper, this discovery originated in the team’s previous finding that certain palladium complexes are

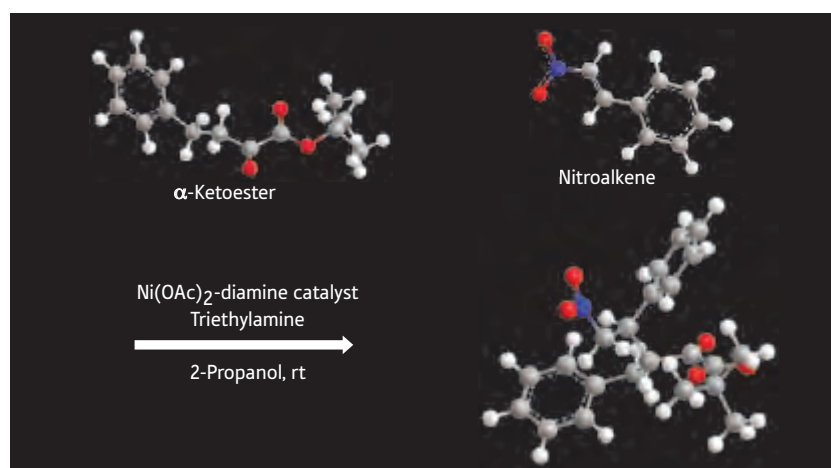


Figure 1: Nickel complexes catalyze the addition of α -ketoesters to nitroalkenes under extremely mild, environmentally non-harmful conditions.

stable and active catalysts, even in water². After several trials, the researchers determined that nickel catalysts, which share similar properties to palladium materials, allowed the α -ketoesters to add to nitroalkenes with high yields and purity; over 90% of the final product corresponded to a specific stereoisomer, a molecule with a hard-to-achieve, geometrically distinct structure.

Hamashima explains that the nickel complexes are particularly effective because they recognize specific carbon atoms on the α -ketoesters and chemically activate them, generating products with precise frameworks. Furthermore, nickel has the right properties to maintain a delicate catalytic balance. “Nickel has a reasonable—not too strong, but not too weak—affinity towards nitro groups,” says Hamashima. “This affinity enabled the facile dissociation of the product from the catalyst, allowing high catalytic turnover.”

The high selectivity of this process, when combined with the mild reaction

conditions, allowed the researchers to perform similar reactions on a broad range of molecules—including a highly efficient synthesis of the natural product kainic acid analog, a chemical that can bind to glutamate receptors within neuronal cells.

“Such selective activations are key to the success of our reaction,” says Hamashima. “Otherwise, undesired side reactions would occur when compounds with various functional groups are used as substrates.”

1. Nakamura, A., Lectard, S., Hashizume, D., Hamashima, Y. & Sodeoka, M. Diastereo- and enantioselective conjugate addition of α -ketoesters to nitroalkenes catalyzed by a chiral Ni(OAc)_2 complex under mild conditions. *Journal of the American Chemical Society* **132**, 4036–4037 (2010).
2. Sodeoka, M. & Hamashima, Y. Chiral Pd aqua complex-catalyzed asymmetric C–C bond-forming reactions: a Brønsted acid–base cooperative system. *Chemical Communications* 5787–5798 (2009).

Joined at the genes?

Genomic data reveal surprising insights into the essential partnership between a widespread plant pest and a symbiotic bacterium dwelling within its cells

Aphids leave behind a trail of damaged crops in pursuit of their diet of sap, and a better understanding of the roughly 5,000 known aphid species could prove invaluable in developing safeguards against this agricultural menace. Unfortunately, they represent a particularly complicated family of insects. For one thing, even genetically identical individuals can manifest dramatically different traits depending on environmental conditions. Furthermore, aphids cannot survive on sap alone and depend on the presence of symbiotic bacteria to sustain them.

Accordingly, when the International Aphid Genome Consortium (IAGC) decided to determine the first complete aphid genomic sequence, they targeted the pea aphid, *Acyrtosiphon pisum*—a reasonably well-understood species (Fig. 1). “As this species has long been used for research, a relatively large amount of biological and genetic information is available,” explains Atsushi Nakabachi, a researcher at the RIKEN Advanced Science Institute and IAGC project leader.

IAGC scientists recently published a ‘first draft’ genome containing 464 million bases of assembled sequence data from *A. pisum*, and have tentatively identified over 34,000 putative genes—although many are purely hypothetical¹. Fortunately, Nakabachi and others have also compiled a library of over 50,000 full-length cDNAs—clones directly derived from expressed genes—which should greatly facilitate genome annotation and characterization of gene function².

The IAGC has also gathered genomic



Figure 1: The pea aphid, *Acyrtosiphon pisum*, is a crop-destroying pest found throughout the world.

data from *Buchnera aphidicola*, the primary pea aphid endosymbiont, and Nakabachi’s team has been steadily working to untangle the complicated relationship between bug and bacterium³. Over the past 100 million years, *Buchnera* and the aphid have forged a highly interdependent relationship; today, these bacteria lack numerous essential genes whose function appears to be compensated for by their host species, raising questions of whether these genes were incorporated into the aphid genome over the course of evolution.

Careful analysis of the aphid genome revealed at least eight functional aphid genes of apparent bacterial origin, seven of which are highly expressed in symbiont-containing bacteriocyte cells. Unexpectedly, however, all of these appear to originate from non-*Buchnera* bacteria, suggesting that these symbionts are being sustained by contributions from other species. “Researchers have hypothesized that at least part of ancestral *Buchnera* genes have been transferred to

the host aphid genome, reminiscent of organelles such as mitochondria,” says Nakabachi. “The present study rules out this hypothesis and reveals that the case is more complicated.” He now hopes to uncover more practical details of this genetic partnership, which could eventually reveal useful vulnerabilities to exploit for pest control. ■

1. The International Aphid Genomics Consortium. Genome sequence of the pea aphid *Acyrtosiphon pisum*. *PLoS Biology* **8**, e1000313 (2010).
2. Shigenobu, S., Richards, S., Cree, A.G., Morioka, M., Fukatsu, T., Kudo, T., Miyagishima, S., Gibbs, R.A., Stern, D.L. & Nakabachi, A. A full-length cDNA resource for the pea aphid, *Acyrtosiphon pisum*. *Insect Molecular Biology* **19**, 23–31 (2010).
3. Nikoh, N., McCutcheon, J.P., Kudo, T., Miyagishima, S., Moran, N.A. & Nakabachi, A. Bacterial genes in the aphid genome: absence of functional gene transfer from *Buchnera* to its host. *PLoS Genetics* **6**, e1000827 (2010).

Making the right contacts to get ahead

A set of mutant yeast strains allows researchers to identify structural elements that help motor proteins to get moving

Cells are crisscrossed by microtubules, protein cables that provide essential infrastructure and serve as ‘highways’ for moving molecular cargoes. Motor proteins, such as kinesin that travels along microtubules via a multi-step ‘walking’ mechanism, effectively drive this transport. The broad strokes of this process are well understood generally, but new work from Etsuko Muto and Seiichi Uchimura of the RIKEN Brain Science Institute in Wako in collaboration with physicists at Waseda University, Tokyo, has revealed valuable new details about how microtubule interactions facilitate kinesin movement¹.

Kinesin is associated with the nucleotide molecule adenosine diphosphate (ADP) when it first binds microtubules, after which it undergoes a structural change that triggers release of ADP and enables interaction with adenosine triphosphate (ATP). Subsequent enzymatic processing of ATP into ADP triggers additional structural changes, causing kinesin to move forward along the microtubule while also returning the protein to its initial ADP-bound state.

Microtubules are composed of dimers of the protein α - and β -tubulin, but eukaryotic cells can have numerous different tubulin subtypes, making it challenging to investigate molecular-level details of kinesin–tubulin interaction. To overcome this problem, Muto and Uchimura developed yeast strains that express only a single subtype each of α - and β -tubulin, thus enabling simple screening of the effects of individual tubulin mutations. In their most recent work, they have used this approach to extensively characterize points of interaction between kinesin and microtubules by generating 36 yeast strains with individual mutations in either tubulin subunit.

Their data suggest that α -tubulin is primarily responsible in the initial association with kinesin-ADP, with β -tubulin providing important stabilizing interactions following the release of ADP (Fig. 1). The researchers were particularly surprised to note that mutations targeting one highly conserved glutamate (E415) in α -tubulin caused a five-fold reduction in kinesin enzymatic activity,

apparently by impairing binding-induced release of ADP. “Our results indicate that kinesin binding to residue E415 in α -tubulin transmits a signal to the kinesin nucleotide pocket, triggering its conformational change and leading to release of ADP,” explains Muto. “I did not expect that residues in α -tubulin would play such an important role.”

In future studies, Muto and Uchimura hope to further dissect the amino acid network that communicates these structural changes across the kinesin protein. Since microtubules play a key role in diverse cellular functions beyond molecular transport, Muto believes that their mutational analysis strategy should also offer a powerful tool for studying processes ranging from the separation of chromosome pairs during cell division to cilia-mediated cell propulsion. ■

1. Uchimura, S., Oguchi, Y., Hachikubo, Y., Ishiwata, S. & Muto, E. Key residues on microtubule responsible for activation of kinesin ATPase. *The EMBO Journal* **29**, 1167–1175 (2010).

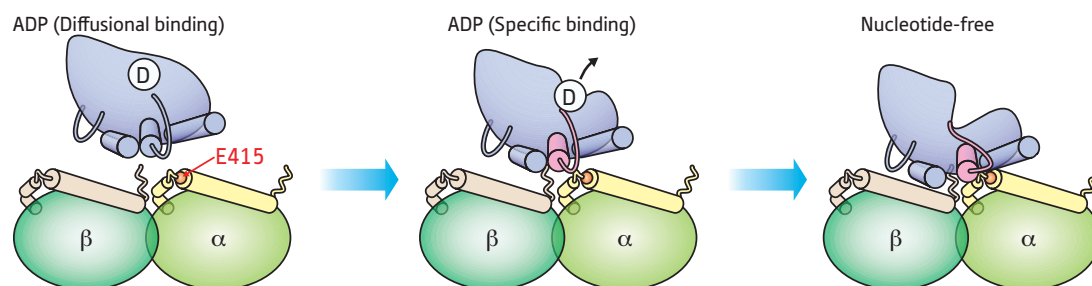


Figure 1: Schematic of the two-step association of kinesin (blue) with microtubules as revealed by mutational analysis. The initial interaction of kinesin-ADP with residue E415 of α -tubulin (left) triggers a conformational change that leads to release of ADP (middle), and the nucleotide-free kinesin subsequently participates in additional stabilizing interactions with residues on the β -tubulin subunit (right).

Wired for sight

Proper maturation of the visual cortex in mice, and possibly humans, depends on a maternal gene

The copy of the gene *Ube3a* inherited from the mother—not the father—is crucial to proper wiring of the visual cortex in mice after birth, a RIKEN researcher and his American co-worker have found¹. In human babies, a defective maternal *Ube3a* gene causes a developmental condition known as Angelman syndrome that leads to mental retardation, speech impairment and brain seizures, and affects behavior. The researchers therefore suggest that stimulating the silenced paternal gene at the right time of development might be worth exploring as therapy for this syndrome.

Nerve cell wiring of the visual cortex of the brain occurs after birth, and is then refined by experience (Fig. 1). Typically, images are constructed using signals from nerve cells that receive more input from one eye than the other, an occurrence known as ocular dominance (OD). The balance of OD displays plasticity; it can be altered, for instance, by temporarily blinding one eye during the critical period of development when the interconnections of nerve cells in the brain are susceptible to experience.

Masaaki Sato from the RIKEN Brain Science Institute, Wako, and Michael Stryker from the University of California, San Francisco, confirmed in mice that by about four weeks of age—their critical period of development—the presence of the *Ube3a* protein is mainly restricted to the nucleus of nerve cells in the visual cortex of the brain; and it is dependent on the maternal gene.

The researchers then investigated how the interconnections of the nerve cells



Figure 1: Visual experience is needed to refine the nerve connections of the visual system.

could be changed before, during and after the critical period. They tested these changes using optical imaging, a technique that can provide information on activity in the brain. They found that both maternal and paternal copies of the gene contributed to normal development until the critical period. From this stage on, however, the maternal copy alone was active and required for maturation of the cortical circuits. Without the maternal gene the visual system did not rapidly adjust OD to the experience of having one eye briefly blinded during the critical period. And afterwards, there was impairment of the ability to make the minor wiring adjustments as the system matured.

“We now want to investigate how *Ube3a* participates in the maturation process of cortical neuronal circuits,” Sato says. “The other direction [of our work] will be to screen genes or compounds which have potential to restore the impaired plasticity of *Ube3a* maternal-deficient mice.” ■

1. Sato, M. & Stryker, M.P. Genomic imprinting of experience-dependent cortical plasticity by the ubiquitin ligase gene *Ube3a*. *Proceedings of the National Academy of Sciences USA* **107**, 5611–5616 (2010).

Time to grow

Fresh insights into the workings of the ‘internal timetable’ of plants could ultimately guide the engineering of hardier, faster-growing crops

Plants may not have any appointments to keep, but they still need to know what time it is, and proper growth and survival depend on the existence of a circadian ‘clock’ that modulates gene expression patterns over the course of the day and night (Fig. 1).

A team led by Norihito Nakamichi of the RIKEN Plant Science Center in Yokohama has now made important progress in exposing the ‘gears’ that power this clock in the thale cress, *Arabidopsis thaliana*, by clarifying the regulatory mechanism of three genes—*PRR9*, *PRR7* and *PRR5*—that play a key role in regulating other circadian factors¹. “The function of the proteins encoded by the ‘clock genes’ was undetermined,” says Nakamichi. “We focused on the molecular function of these proteins because triple knockouts of these *PRR* genes result in disruption of the circadian clock.”

Previous studies have suggested that these proteins overlap functionally, and Nakamichi and his colleagues demonstrated that all three are equally effective at inhibiting expression of the *Circadian Clock-Associated1* (*CCA1*) and *Late Elongated Hypocotyl* (*LHY*) clock genes. They subsequently identified two evolutionarily conserved stretches of amino acids that contribute to this gene repression, and determined that this activity appears to depend on physical recruitment of *PRR9*, *PRR7* and *PRR5* to DNA sequences that regulate *CCA1* and *LHY* expression.

Although these three proteins appear to be functionally redundant, each is selectively produced at a specific time of day, and subsequent experiments helped

to dissect the time-table governing each protein’s activity. The investigators generated strains of *Arabidopsis* that express only *PRR9*, *PRR7* or *PRR5*—with expression peaks in the morning, from day to night, and at night, respectively—and noted the effects on *CCA1* and *LHY* expression. Their results revealed a collaborative relationship between the three repressors; for example, *PRR9* alone can inhibit *CCA1* and *LHY* in the morning, but is insufficient to enforce night-time inhibition of these genes in the absence of *PRR5* and *PRR7*.

Considering how many key processes related to plant growth and development are guided by this internal clock, Nakamichi predicts that these findings

could eventually benefit the agricultural sector. “We have created a transcriptional activator version of *PRR5*; when it is over-expressed in *Arabidopsis*, it activates target genes by binding their promoters,” he says. “Using such constructs, we can cancel the function of redundant *PRR* proteins one at a time in useful plants such as crops and trees in order to improve their stress tolerance and increase their biomass.” ■

1. Nakamichi, N., Kiba, T., Henriques, R., Mizuno, T., Chua, N.-H. & Sakakibara, H. PSEUDO-RESPONSE REGULATORS 9, 7, and 5 are transcriptional repressors in the *Arabidopsis* circadian clock. *Plant Cell* **22**, 594–605 (2010).



Figure 1: The circadian ‘clock’ of plants helps them to grow properly and survive by regulating gene expression patterns at appropriate times.

New progress from a ‘joint’ venture

The discovery of novel risk factors for osteoarthritis illuminates a probable role for the immune system in the pathology of this joint disorder

The debilitating knee, hip, wrist or back pain associated with osteoarthritis (OA) is commonplace within diverse populations around the world and represents a broadly recognized hallmark of old age (Fig. 1), yet remarkably little is known about the origins of this disease.

Most evidence suggests that OA arises from a mix of genetic and environmental factors, but researchers of this disease, including Shiro Ikegawa of the RIKEN Center for Genomic Medicine in Tokyo, have found it a considerable challenge to uncover risk factor genes. “It is our long-standing dream to know the ‘real cause’ of this disease,” says Ikegawa, “but it has proven very difficult.”

Advances in techniques for genomic analysis have now enabled his group and a team of collaborators from across Japan and Europe to achieve an important breakthrough on this front¹. They screened over 4,000 Japanese individuals—906 with OA of the knee, and 3,396 unaffected control subjects—in an effort to identify genome sequence variations that exhibit a statistically significant association with this condition. Of fifteen candidates identified in this initial search, two of these ‘single nucleotide polymorphisms’ (SNPs) warranted further close scrutiny.

Unexpectedly, the researchers found that both of these SNPs are located within a region of chromosome 6 containing numerous genes involved in the immune response: the rs7775228 variation occurs near genes that help instruct immune cells to ignore host proteins, while rs10947262 falls within a gene that controls T cell activation. “OA has long been thought of



istockphoto/PStricker

Figure 1: The knee and wrist are among the body parts most commonly affected in the tens of millions of patients suffering from osteoarthritis worldwide.

as having only limited association with immunological abnormalities,” explains Ikegawa, “but it turns out this is not the case.” These results are also in keeping with a handful of recent studies that have hinted at an inflammatory component of OA pathology.

Intriguingly, the team noted that simultaneous variations at both sites were more significantly associated with OA among Japanese subjects than either of the two SNPs individually. On the other hand, the disease association of this particular combination of SNPs—also known as a ‘haplotype’—was less notable among a European sample group.

Many more risk factors likely remain to be discovered—including some

that may be specific to OA affecting individual body parts—and Ikegawa and colleagues are now moving on to larger scale association studies to characterize additional genes. He adds that such efforts are only a beginning. “Association is just statistics,” he says. “After finding associations, we need to prove functionality of the genes and SNPs to achieve our final goal of treating OA.” ■

1. Nakajima, M., Takahashi, A., Kou, I., Rodriguez-Fontenla, C., Gomez-Reino, J.J., Furuichi, T., Dai, J., Sudo, A., Uchida, A., Fukui, N. *et al.* New sequence variants in HLA Class II/III region associated with susceptibility to knee osteoarthritis identified by genome-wide association study. *PLoS ONE* 5, e9723 (2010).

Illuminating the genetic alphabet

Fluorescent unnatural base pairs light up the structures of DNA and RNA chains with minimal disturbance

The development of fluorescent tags—small, light-emitting molecules attached to DNA strands—has revolutionized cell biology over the past two decades, allowing precise tracking of labeled segments in living systems. Because fluorescent tags project outwards from the nucleic acid chains, they can interfere with the mobility and geometry of their targets, skewing the view of critical biological actions.

Now, a research team led by Ichiro Hirao at the RIKEN Systems and Structural Biology Center in Yokohama has synthesized an unnatural fluorescent base pair system that can be incorporated directly into DNA and RNA molecules¹. Because this new addition to the genetic alphabet only minimally disturbs the delicate biochemical functions of cells, it holds great potential for advanced medical techniques such as DNA-based diagnostic testing.

Hirao and colleagues had previously discovered that a fluorescent 2-amino-6-thienylpurine molecule containing a thiophene ring, termed 's', formed a base pair with a pyrrole-aldehyde compound known as 'Pa'². The new s–Pa pair could be site-specifically incorporated into RNA chains alongside the natural nucleotides; unfortunately, DNA replication processes with the s–Pa system were not as successful.

To solve this problem, the research team altered the structure of 's', removing the amino group and a nitrogen in the purine ring, then adding an extra thiophene ring to give a new nucleotide, called 'Dss'. This highly fluorescent molecule retained the same complimentary base pairing with Pa

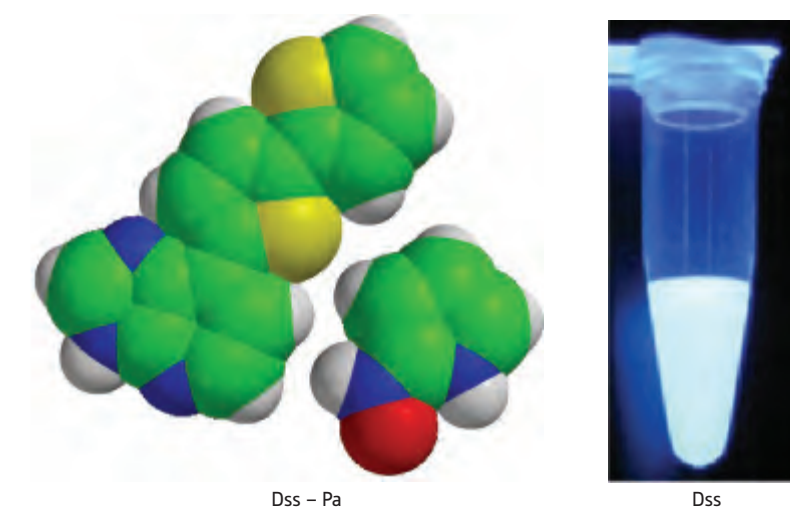


Figure 1: New, unnatural base pairs called Dss–Pa (left) can be specifically incorporated into DNA and RNA molecules while retaining bright fluorescent emissions (right).

(Fig. 1), and could be easily transcribed into specific RNA positions by cell enzymes. Furthermore, single-nucleotide insertion experiments with *Escherichia coli* DNA polymerase I revealed that the Dss–Pa pairs could be replicated with nearly the same efficiency as natural bases.

Intriguingly, Dss also acted as a universal base—forming thermodynamically stable pairs with the four natural bases of DNA. Hirao says that the mechanisms of duplex DNA formation and polymerase reactions in replication and transcription with Dss are completely different than for natural systems.

Fluorescent imaging of functional DNA and RNA is becoming increasingly important as researchers seek to understand the conformations of these biopolymers in liquid environments. “The fluorescent intensity of the unnatural

bases change greatly depending on the three-dimensional structure—providing a powerful tool to analyze the local structure of DNA and RNA molecules in solution,” says Hirao.

Hirao envisages a wide range of basic and applied technologies arising from these bright new pieces of genetic code. “We are now applying Dss to molecular beacons, realtime PCR, and structural analysis,” he says. ■

1. Kimoto, M., Mitsui, T., Yokoyama, S. & Hirao, I. A unique fluorescent base analogue for the expansion of the genetic alphabet. *Journal of the American Chemical Society* **132**, 4988–4989 (2010).
2. Kimoto, M., Mitsui, T., Harada, Y., Sato, A., Yokoyama, S. & Hirao, I. Fluorescent probing for RNA molecules by an unnatural base-pair system. *Nucleic Acids Research* **35**, 5360–5369 (2007).

Projecting pain relief

Radioactively labeled drugs can track inflammation in the brain

Widely prescribed, nonsteroidal anti-inflammatory drugs (NSAIDs), such as Ibuprofen and Ketoprofen, inhibit enzymes called cyclooxygenases (COXs) that regulate pain, fever and other inflammations in the body. Now, a specially synthesized series of NSAIDs, and their so-called '2-arylpropionic acid derivatives' labeled with carbon-11 (^{11}C) radioisotope, can be used to trace inflammations *in vivo* using the bioimaging technique positron emission tomography (PET)¹. This development results from a research team led by Masaaki Suzuki at the RIKEN Center for Molecular Imaging Science, Kobe.

Many researchers have designed radioisotope-labeled COX inhibitors to target and monitor COXs in living systems. Most efforts have focused on COX-2, a COX enzyme that can serve as a biomarker for early disease diagnosis because its concentration increases rapidly in cells during inflammation. However, no one has managed to image this enzyme using radioisotope labels to date.

Suzuki and his team created their radiotracer library through a general and efficient approach. First, they reacted each NSAID precursor with a small ^{11}C -containing molecule for two minutes to produce the NSAID methyl esters by [^{11}C]carbon-carbon bond formation. Then, they heated the esters at 50 °C under basic conditions—the pH of the solution was greater than 9—for one minute to generate the ^{11}C -labeled drugs.

According to Suzuki, the short half-life of ^{11}C expedites the screening of ^{11}C -labeled compounds and makes their biological function easier to evaluate. "In

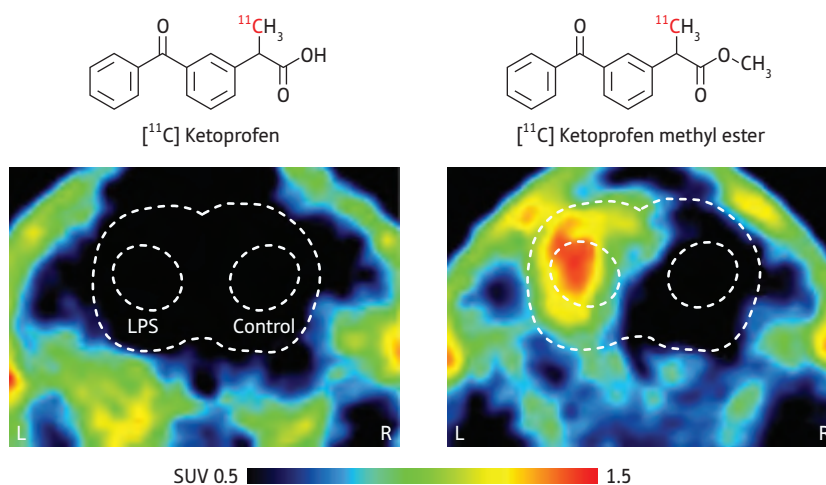


Figure 1: PET images of an inflamed rat brain summated from 5 to 45 minutes after administration of [^{11}C] Ketoprofen (left) and [^{11}C] Ketoprofen methyl ester (right). The inflammation results from the injection of lipopolysaccharide (LPS) into the left, inner part of the brain.

addition, the [^{11}C]carbon-carbon bond is metabolically very strong, providing highly reliable PET images and ideal molecular probes for investigations of dynamic behavior in vital *in vivo* systems," he says.

After injecting a toxic bacteria-derived substance known as lipopolysaccharide into the left, inner-part of rat brains to induce inflammation, the researchers administered intravenously the ^{11}C -labeled compounds for evaluation. They discovered that the radioactivity of [^{11}C]Ketoprofen methyl ester was highly concentrated in the inflamed area, but that of [^{11}C]Ketoprofen was not (Fig. 1). These PET experiments indicated that the methyl ester readily penetrated the blood-brain barrier—a thin layer of blood vessel cells that separates circulating blood from cerebrospinal fluid and prevents particular molecules

from entering the brain—and delivered the drug to the inflammation upon hydrolysis.

The researchers are planning to label NSAIDs with fluorine-18 that has a longer half-life. "The information obtained from longer times will give a more accurate and detailed analysis of dynamic behaviours *in vivo*," says Suzuki. "We also hope to extend this research on brain inflammation to other important diseases by developing novel optimized PET probes." ■

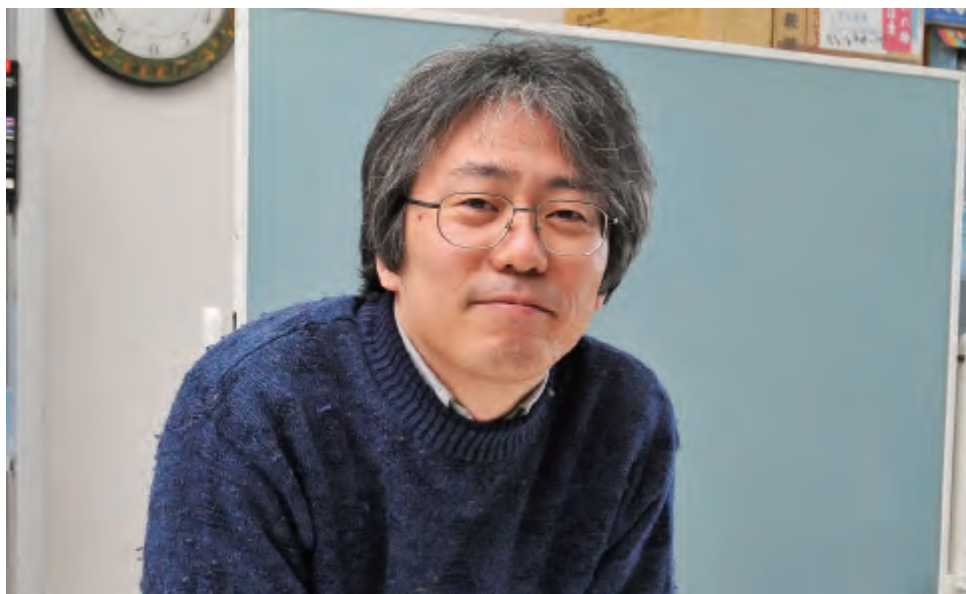
1. Takashima-Hirano, M., Shukuri, M., Takashima, T., Goto, M., Wada, Y., Watanabe, Y., Onoe, H., Doi, H. & Suzuki, M. General method for the ^{11}C -labeling of 2-arylpropionic acids and their esters: construction of a PET tracer library for a study of biological events involved in COXs expression. *Chemistry — A European Journal* **16**, 4250–4258 (2010).

Studying cell signaling using single-molecule imaging

Yasushi Sako

Chief Scientist
Cellular Informatics Laboratory
RIKEN Advanced Science Institute

Single-molecule imaging (SMI) is a powerful scientific tool for visualizing individual molecules. Among its wide range of potential uses, SMI is particularly promising for its ability to provide new insights into the molecular processes in living cells. With this technique, it is possible to count the number of proteins in a cell and observe their distribution, movement, reaction rates and even changes in conformation. Yasushi Sako, chief scientist in the Cellular Informatics Laboratory of the RIKEN Advanced Science Institute (ASI), is investigating the complex signaling mechanism in cells by studying the proteins involved, one by one, using SMI. His research has revealed that the reactions of individual proteins are more complex than previously thought, and that signaling is controlled by dynamic conformational changes and reaction memory.



First encounters with single-molecule imaging

Sako remembers vividly the excitement he felt when he succeeded in using SMI to observe the molecules in a cell for the first time. “I saw many small twinkling dots that moved randomly back and forth. Their movements were so mesmerizing and fascinating that I couldn’t stop looking at them.” Single-molecule imaging is performed using a fluorescence microscope to observe the trace fluorescence produced by individual proteins that have been labeled with green fluorescent protein (GFP) by gene transfer or chemical reaction. The method is so effective that it allows the number, distribution and movement of proteins to be observed—it can even be used to trace which protein binds to which, and the time required for a certain reaction (Fig. 1).

Single-molecule imaging was developed in 1995 by a group of researchers from Osaka University and other institutions. They succeeded in viewing the movement and chemical reaction of a molecule in aqueous solution in a test tube, for example,

using myosin isolated from a cell. Myosin is a motor protein responsible for muscle movement. What happens in a test tube, however, may not necessarily happen in a living cell. Researchers around the world therefore started to compete to develop an SMI technique that could be used to observe the behavior of proteins within a living cell. Sako was not one of those researchers. “My research interest back then was cell membrane proteins. The labels I used were several tens to several hundreds of nanometers in size, much larger than the fluorescent proteins used for SMI. As we already had a technique to view the movement of a labeled molecule in a cell membrane protein in living cells, we did not need SMI.”

In 1997, Sako moved to Osaka University. “In order to continue the research I’d been doing at my previous laboratory, where I had used a two-photon excitation fluorescence microscope, I assembled a new device. But I could not see what I wanted, no matter what adjustments I made. It was then that I wondered if SMI might work—the idea just sprang to mind.”

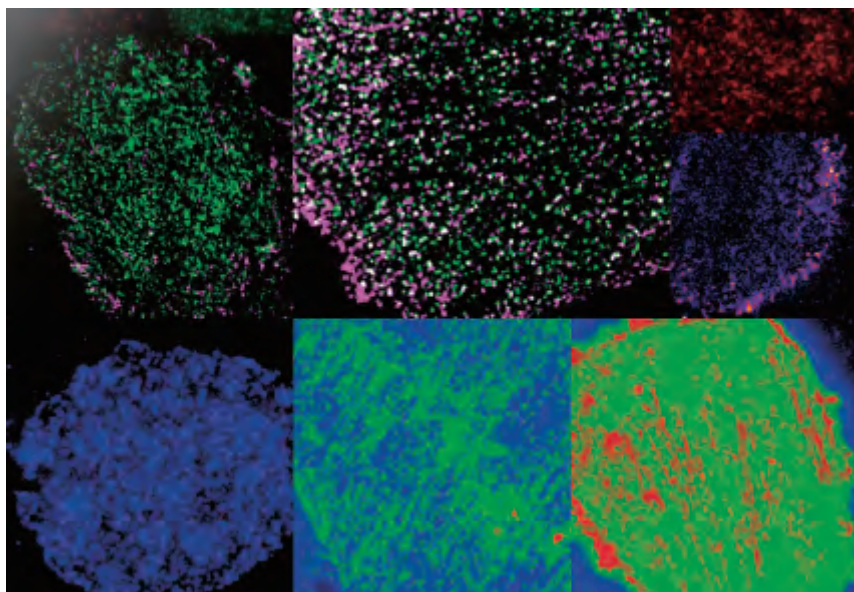


Figure 1: An example of visualization using single-molecule imaging.

A protein labeled with a fluorescent protein is observed under a fluorescence microscope. Each point of fluorescence represents one molecule. Methods for labeling proteins with a fluorescent protein include using a chemical reaction and introducing the gene of the fluorescent protein into the protein of interest.

At the time, Sako was working in the laboratory of Toshio Yanagida, one of the leading researchers involved in the original development of SMI. “There were researchers at the laboratory who were developing a new device for SMI, but none were successful. I attempted to make a device by copying a conventional instrument, and using that microscope I was lucky enough to be able to see molecules in a living cell.” Looking back at his unexpected success in 1998, he says, “I used what I had at that time. I had no preconceived ideas.” In 2000, he published a paper on the technique, and that is when his relationship with SMI began.

Understanding the response network of signaling proteins

Sako set up his Cellular Informatics Laboratory at the RIKEN ASI in 2006. “I wanted to know how the fate of cells is determined.” Cells may have a variety of fates: they may divide and increase in number, differentiate into different kinds of cells, or die

(apoptosis). “Determination of the fate of a cell starts when a protein called a signaling molecule binds to a receptor embedded in the cell membrane,” says Sako. When bound by a signaling molecule, the receptor is activated and information is transmitted into the cell. The information is then conveyed from one protein to another within the cell through repeated binding, dissociation and migration until it eventually reaches the cell nucleus, where it induces the expression of a specific gene. This gene triggers various cellular responses, including proliferation, growth inhibition, differentiation, apoptosis, and oncogenic transformation.

There are many kinds of signaling molecules that can determine the fate of a cell. Among them, Sako is particularly interested in epidermal growth factor (EGF), which stimulates cell proliferation and movement. The response network of signaling proteins induced by EGF has been studied in detail and more than 100 proteins associated with the network have

been isolated. “Based on the results of previous studies, we can draw a rough schematic of the network to show what elements are involved and how they are interconnected. But merely drawing a schematic does not mean we understand the response network. We need to observe the individual proteins that make up the response network in living cells directly to obtain quantitative information such as the number of proteins that elicit a response, their concentration and migration speed. Single-molecule imaging allows us to do this.”

Epidermal growth factor receptor undergoes dynamic conformational change

A receptor is thought to be activated when bound by a signaling molecule, but the epidermal growth factor receptor (EGFR) is not activated merely by the binding of EGF. The receptors are activated only when two EGFRs join together to form a dimer, and then only after EGF binds to both sides of the dimer. The mechanism of activation had not been fully elucidated until Sako decided to use SMI to study it in detail. There are about 50,000 EGFRs on the surface of a cell (Fig. 2). The first thing he studied was the number of EGFs that need to be bound to EGFRs to induce a response. “I found that a response occurred when about 300 EGFs had bound to about 50,000 EGFRs. I counted the fluorescent dots one by one. Single-molecule imaging requires considerable patience!”

Those 300 EGFs on 50,000 EGFRs represent less than one percent. It is known that one to two percent of EGFRs form dimers, so how do so few EGFs find the dimers to bind to? “I found that EGFR uses a very sophisticated mechanism. A conformational change occurs in EGFR after dimerization, which makes it 100

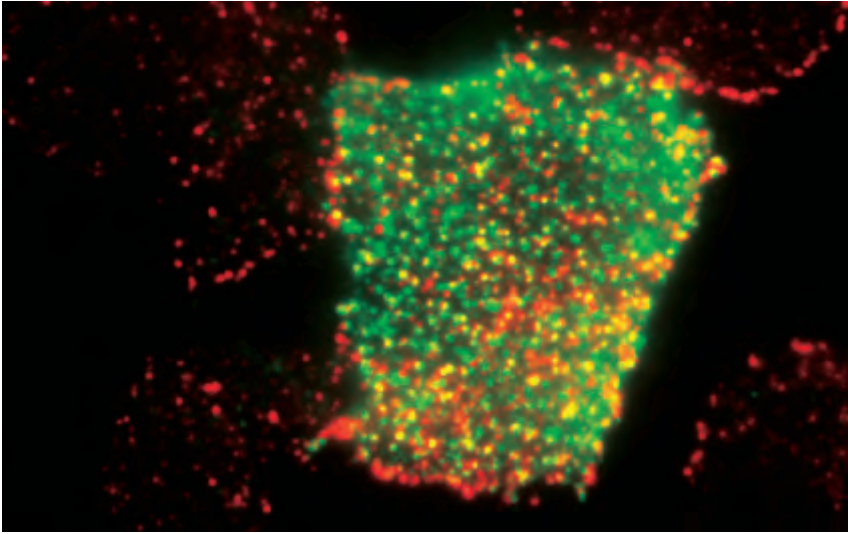


Figure 2: Single-molecule imaging of EGFR.

Green dots represent GFP-labeled EGFRs. Red dots represent phosphorylated membrane proteins.

times easier for EGF to bind to the dimerized EGFR than to the monomer EGFR. When EGF binds to one side of the dimer, the conformational change occurs again, which makes it another 10 times easier for EGF to bind to the other side of the dimer (Fig. 3). EGFR undergoes conformational change upon dimerization, which enables it to bind to EGF effectively and start the signaling process.”

However, Sako stresses that the conformational change in EGFR is still speculative. “We need to investigate if such conformational change actually occurs. As it is possible to study

conformational change through detailed observation of the fluorescence emitted from fluorescent proteins, we are now working on this study.”

Reaction memory in proteins

When EGFRs in a cell membrane are activated, phosphate binds to the intracellular part of the receptors. A protein that recognizes a phosphorylation site is then attracted to the site. There are more than ten different kinds of such proteins. Sako’s Cellular Informatics Laboratory is particularly interested in a protein called Grb2.

Upon recognition of the phosphorylation site of EGFR, Grb2 is attracted to the cell membrane. After a certain time, the Grb2 bound to the site will dissociate. The process up to this point is already well understood. In a study of the reaction rates of EGFR and Grb2 using SMI, however, researchers in Sako’s laboratory made a surprising discovery. “As the concentration of the binding protein increases, the reaction rate generally increases proportionally. However, even after increasing the concentration of Grb2 by ten times, we found that the reaction rate only increased three times. We consider that reaction memory may be involved in the reaction between EGFR and Grb2,” says Sako.

What is reaction memory? Upon binding to Grb2, EGFR undergoes conformational change (Fig. 4). However, even after Grb2 has dissociated from EGFR, the receptor retains its bound conformation, returning to its original state only after a certain period of time. “EGFR, after completing the reaction, keeps a memory of the Grb2 bound to it. This is called reaction memory. Until the EGFR returns to its original unbound conformation, it is difficult for Grb2 to bind to it.” This explains why the reaction rate only increased three times despite the concentration of Grb2

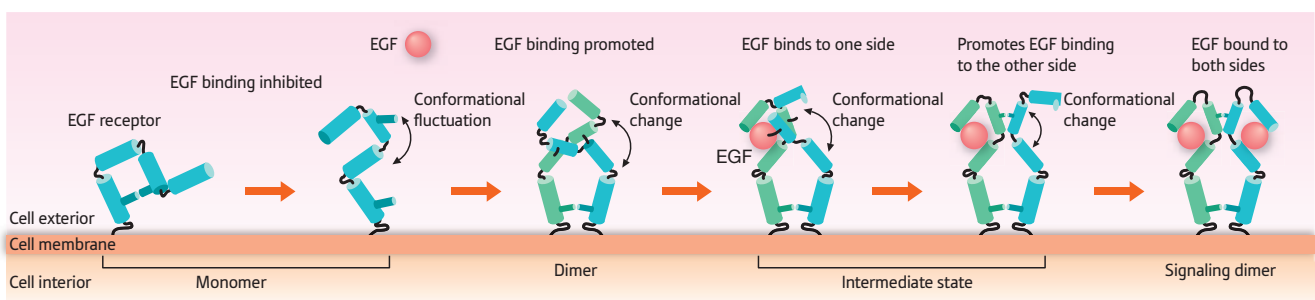


Figure 3: Binding between EGF and EGFR and conformational change.

EGFR is embedded in a cell membrane. When EGF binds to both sides of an EGFR dimer, information is transmitted into the cell. EGFR undergoes conformational change upon dimerization, making it easier for EGF to bind to the dimer than to the monomer. When EGF binds to one side of the dimer, another conformational change occurs, resulting in a transition to an intermediate state, which makes it even easier for EGF to bind to the remaining side of the dimer.

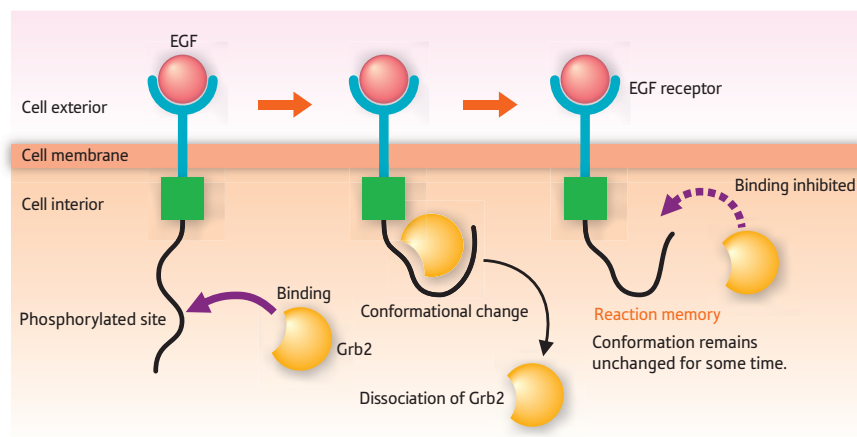


Figure 4: Conformational change and reaction memory of EGFR.

When EGF binds to EGFR, the intracellular part of the receptor is phosphorylated. A protein called Grb2, which recognizes phosphorylation, then binds to EGFR, inducing conformational change in the receptor. Although Grb2 eventually dissociates from EGFR, the conformation of the EGFR does not return immediately to the original state (reaction memory). This makes it difficult for Grb2 to bind to the receptor (figure shows only one side of the dimer for clarity).

being increased ten times. “It is possible in theory to use reaction memory to control the reaction rate in such a way that it remains unchanged even when the intracellular concentration of Grb2 changes. We are not sure yet if reaction memory is used for this purpose, and we will look at this issue in future research.”

Elucidating the complexity of protein reactions

The cellular responses induced as a result of signaling vary widely. For example, the binding of EGF to the receptor does not necessarily lead to cell proliferation; it may sometimes result in differentiation and, at other times, cell death. It has generally been understood that the diversity in cellular responses stems from the complexity of the response network of signaling proteins, whereas the reactions of individual proteins have been assumed to be very simple, involving just binding and unbinding. However, researchers are having to correct this long-held belief because of the discoveries Sako and his laboratory researchers have made using SMI. “It is true that the network is complex, but the reactions of individual proteins are also very complex. We have

revealed that proteins control signaling by dynamic conformational change and reaction memory.”

The reactions of EGF, EGFR and Grb2 are just a small part of the beginning of the response network of signaling proteins. Is it possible to understand the entire network, which involves as many as 100 different kinds of proteins? “It may be difficult to observe all of the proteins that are associated with signaling using SMI. But I think, with the help of mathematical science, we can understand much of the network,” says Sako. The key reactions will be observed in detail and, based on the results, a model will be developed through computer simulations to better understand the entire network. “In our research, we collaborate with groups of researchers in mathematical biology and computational biology both within RIKEN and with other institutions.”

What does it mean to be alive?

Japanese researchers are pioneers of SMI, and yet Japan lags far behind the US in terms of the number of researchers and budget allocated to research in this field. “But we’re still far ahead in producing creative ideas

and performing quality research.” For example, Sako is particularly interested in fluctuation. “This is a subject that Japanese researchers are good at. Researchers in Europe and the US prefer subjects that can be explained in a more clear-cut manner, so they pay little attention to fluctuation.” Cells are always exposed to fluctuation. The conformation of a protein, for example, is variously affected by thermal fluctuations, and there are also number fluctuations. “While cells have a mechanism to correct fluctuations, they also make good use of them. I want to demonstrate specific examples of how they use fluctuation in signaling and in determining their fate.”

Sako is philosophical about his research. “A protein is not defined as being alive, but a cell is alive. Something that is alive is generated from something that is not alive. Where is the boundary between them?” We still have no answer to this question. “When the border between the protein layer and the cell layer is removed, we may find the answer to what it means to ‘be alive.’” ■

About the researcher

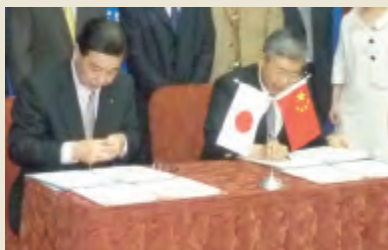
Yasushi Sako was born in Oita Prefecture, Japan, in 1961. He graduated from the Faculty of Science at Kyoto University in 1984, and obtained his PhD in 1990 from the same university. He worked at The University of Tokyo from 1989 to 1997, spent a period at Nagoya University in 1997, and then joined the Osaka University Medical School as assistant professor. He was promoted to associate professor of the Medical School and the Graduate School of Frontier Bioscience at Osaka University in 1998. He moved to RIKEN as chief scientist in 2006. His research interests focus on the biological intracellular protein network of biosignaling, the structure and function of biomembranes, and optical microscopy.

Cementing the partnership with Xi'an Jiaotong University in China

On 23 May 2010, RIKEN Executive Director Yoshiharu Doi and Xi'an Jiaotong University (XJTU) President Nanning Zheng formalized a General Collaborative Agreement and an Agreement for International Joint Graduate School Program. The new agreements will broaden cooperation between the two institutions across a wide range of research fields while also encouraging the exchange of personnel.

RIKEN and XJTU already have strong ties. In March 2007, XJTU conferred an honorary professorship to RIKEN President Ryoji Noyori, who travelled to XJTU to accept the honor and give a special lecture. In June of that same year, RIKEN's volume computer-aided design (VCAD) program entered into a joint agreement with XJTU's School of Human Settlements and Civil Engineering on the establishment of an international joint graduate school.

In October 2009 and January 2010, Nobuaki Teraoka, director of the RIKEN China office, and three RIKEN researchers, Kiwamu Kase, Kangbin Lei and Kuniaki Kawabata, visited XJTU to exchange ideas and prepare the way for general research collaboration. The trip included a visit to the XJTU Institute of Artificial Intelligence and Robotics and an introduction to research at XJTU in



Doi and Zheng sign the agreements for general collaboration and an international joint graduate program.



RIKEN Executive Director Yoshiharu Doi (left) and Xi'an Jiaotong University President Nanning Zheng (right) at the signing ceremony.

areas such as physics, chemistry and the life sciences.

The agreements signed on May 23 this year present new possibilities in a wide range of areas. The General Collaborative Agreement sets out to promote the exchange of researchers, staff and students, as well as information sharing through the organization of lectures, joint seminars and symposiums. It also covers the sharing of research material, the initiation of joint research projects, and cooperation toward the establishment of associated laboratories and a joint research center in the future.

The Agreement for International Joint Graduate School Program, on the other hand, seeks to foster closer ties in education by making it possible for RIKEN researchers to be appointed to positions of visiting professor and associate professor at XJTU, and for students at XJTU to conduct up to three years of research at RIKEN under the

supervision of appointed RIKEN and XJTU research staff. Future PhD students graduating from the new program would be awarded, in addition to their degree from XJTU, a certificate attesting to their completion of the joint program.

RIKEN hopes that these agreements will lead to further collaborative research and will stimulate an increase in the exchange of young researchers, students and other personnel between XJTU and RIKEN. Xi'an Jiaotong University is particularly eager to expand cooperation with RIKEN in the areas of robotics, artificial intelligence, and brain science.



Delegates at the signing ceremony held at Xi'an Jiaotong University in Shaanxi, China on 23 May 2010.

Prof. Kazuo Makishima
Group Director
Coordinated Space Observation and Experiment Research Group
RIKEN Advanced Science Institute
Wako, Saitama, Japan

Dear Prof. Makishima,

I happen to live only about a 15 minute walk from RIKEN's Wako campus, and can gaze upon the buildings almost every day from my balcony. Sometimes, after the rains clear the air, Mt Fuji becomes visible in the distance, only adding to my desire to return to the beautiful campus with its wide open spaces, gorgeous sakura trees and excellent facilities (including a nice on-site barber shop!), which I left a few months ago.

But it is the people that I miss most of all. I joined the Cosmic Radiation Laboratory at the end of 2006 upon the recommendation of several colleagues in the UK, where I had completed my PhD. Not being able to speak a single word of Japanese, I was well supported by all members of the group, and was able to start several scientific collaborations that continue to this day. I recall bothering the group members with endless questions on Japanese language, culture and most importantly, food (RIKEN's canteen really is excellent value for money!). Everyone's patient answers and kindness are the reasons that I am able to speak the language now, and why I can 'feel at home' here.

The ample research budget provided to me, first as a JSPS Fellow and then as a RIKEN Foreign Postdoctoral Researcher, allowed me to expand my research on black holes, and to publish widely. Among other things, our very first collaboration, on rapid timing observations of Galactic black holes, showed the presence of very fast and intriguing visible-light fluctuations, resulting in a world-wide science press release. This research opened up my own horizons, and made me ask questions that I had never thought to ask before.

I learnt many things during my three years at RIKEN, and not just about science. I am still amazed by your energy and efforts in educating many students, and in managing two labs: one at RIKEN, and one at the University of Tokyo. During the funding review in 2009, your leadership in drawing the entire Japanese astronomy community together and petitioning the ruling government was an inspiration. In the end, very few cuts were made, which was a great victory.

In early 2010, I was fortunate enough to secure an International Top Young Fellowship at the Japan Aerospace Exploration Agency in Kanagawa. This is undoubtedly a result of the growth and training that I went through during my time at RIKEN. It is an exciting time to be part of Japanese space science because of the many successful satellites and telescopes that are now in operation. I hope that space science here will continue to soar higher in the future, just like Mt Fuji's summit.

Very best regards,

Poshak Gandhi
Institute of Space and Astronautical Science (ISAS)
Japan Aerospace Exploration Agency (JAXA)
3-1-1 Yoshinodai, Chuo-ku,
Sagamihara, Kanagawa 252-5210, Japan





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