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Mapping the cloud

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Evolutionary profits and losses

The disruption of melatonin production in laboratory mouse strains represents an apparent evolutionary advantage in terms of reproductive development

Animal models can yield valuable insights into the biology of human disorders, although they can also introduce additional levels of complexity that may make it a challenge to experimentally untangle the bases for specific phenotypes.

Tadafumi Kato and Takaoki Kasahara of the RIKEN Brain Science Institute in Wako ran into such a challenge in their attempts to characterize abnormalities in the activity of melatonin, a hormone that fine-tunes the circadian rhythms that establish an organism's day–night cycle, in their mouse model of bipolar disorder. "Since early times, researchers and psychiatrists have believed that melatonin has something to do with mood disorders, because many patients experience sleep disturbance and light therapy is used effectively in the treatment of seasonal affective disorder," says Kasahara. However, they quickly found their efforts thwarted by the utter absence of melatonin from their laboratory mice.

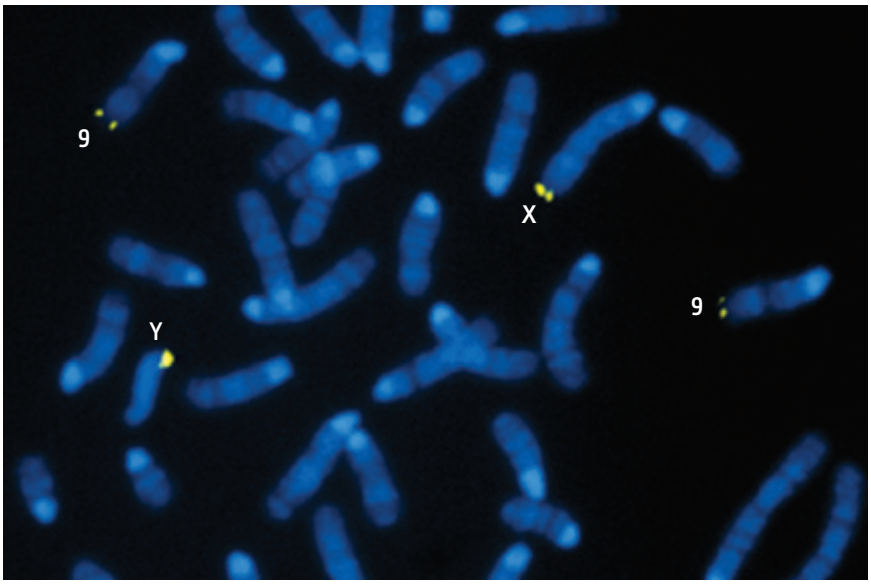


Figure 1: A fluorescent probe reveals the chromosomal location of the *Hiomt* gene. The gene itself is located in the pseudoautosomal region (PAR) of the two sex chromosomes (X and Y); a weak signal is also detected from a DNA stretch homologous to the PAR that resides within chromosome 9.

Filling in the blanks

Indeed, a growing body of evidence suggests that several strains of laboratory mice—including the C57BL/6J (B6J) line used by Kato and Kasahara—are deficient in the production of melatonin, a process that depends on the sequential action of two enzymes: arylalkylamine *N*-acetyltransferase (AANAT) and hydroxyindole *O*-methyltransferase (HIOMT).

Scientists have successfully identified the mouse *Aanat* gene, and have even uncovered an inactivating mutation within this gene in B6J mice. However, even with high-quality mouse genome sequence data available, nobody has yet succeeded in tracking down its partner, *Hiomt*. "I studied the mechanism of circadian clocks when I was a PhD student, and I heard that mouse *Hiomt* was really enigmatic," recalls Kasahara, "and even after being away from the field for about six years, I became aware that mouse *Hiomt* still had not been identified."

This is no longer the case, thanks to an extensive analysis of the mouse genome by Kato, Kasahara and colleagues[1]. Using the rat HIOMT protein sequence as a basis for comparison they have finally managed to uncover this mysterious gene and have thereby revealed why it has remained hidden from scientists for so long.

Notably, mouse HIOMT bears only limited resemblance to its counterparts in other species, with an amino acid sequence that is less than 70% identical to that of the rat protein. Furthermore, this gene was likely masked by its residence within the pseudoautosomal region (PAR), a poorly characterized stretch of DNA within the sex chromosomes that enables them to efficiently 'pair up' and undergo recombination during meiosis (Fig. 1). "The PAR contains extremely repetitive sequences and high guanine-cytosine content, both of which make it difficult to sequence using either traditional or next-generation sequencing methods," says Kasahara.

Developmental consequences

Closer analysis of the sequence of this gene revealed two notable sequence variations in B6J mice relative to MSM animals—a strain derived more recently from wild mice that exhibits normal melatonin production. Both of these changes affect the amino acid sequence of the encoded protein, and the investigators showed that each mutation leads to a strong reduction in HIOMT levels. These

Figure 2: Melatonin productivity has a marked effect on male reproductive development in mice. Relative to specimens collected from mice that produce melatonin (left), those collected from melatonin-deficient males with the B6J version of the *Hiomt* gene (right) show significantly accelerated testicular growth by eight weeks of age.

mutations also proved to be widespread among a variety of other inbred mouse strains, including several lines commonly employed in laboratory research.

Kato, Kasahara and colleagues also noted that although this HIOMT deficiency appears to have a limited impact on circadian behaviors, it has a clear effect on gonadal development; melatonin-deficient animals with the B6J versions of the *Hiomt* and/or *Aanat* genes exhibited significantly greater testicular growth than their melatonin-producing counterparts (Fig. 2). Conversely, in experiments with ICR mice, another melatonin-deficient strain, the researchers showed that treatment with melatonin was associated with a reduction in testicular weight.

These findings are in keeping with other data showing a vital link between melatonin and reproductive development—including observations in human patients. "Children with little or no melatonin due to pineal tumors often show premature sexual maturation," says Kasahara.

Evolution in a cage

Because these defects appear to be specifically prevalent among cultivated strains of laboratory mice, it appears likely that there is some manner of selection taking place that favors the emergence of strains with reduced melatonin levels and accelerated reproductive development—even if this evolution was unintentional and, until now, invisible. This finding is supported by similar research in domesticated chickens[2], which has spotlighted the emergence of other gene variations that may potentially influence the same developmental pathway. "One of the most intriguing [variants] is found in the gene encoding the receptor for thyroid-stimulating hormone, because TSH and melatonin are closely related in seasonal breeding," says Kasahara.

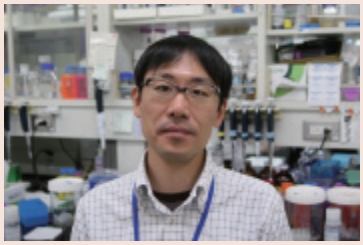
These findings could also have potential implications for previous animal studies that have investigated circadian rhythms, given that much of this research has been conducted in B6J and other inbred strains. For example, one recent study has shown that the circadian rhythm defects observed in the widely used B6J-derived *Clock* mutant mice are markedly diminished in the presence of normal levels of melatonin[3].

These findings will closely inform future work from Kasahara and Kato, who are in the process of engineering a melatonin-producing B6J strain for use in their future investigations of mood disorders. However, Kasahara also suggests that conventional laboratory strains in general may be too interbred for their own good. "Our B6J mouse model for mood disorder has many phenotypes similar to bipolar disorder, but they don't get manic spontaneously," he says. "I hypothesize that laboratory mice have lost their potential to develop manic or aggressive episodes, and we are consequently using wild-derived mice, which are very aggressive, alert and agile in order to study these disorders." ■

1. Kasahara, T., Abe, K., Mekada, K., Yoshiki, A. & Kato, T. Genetic variation of melatonin productivity in laboratory mice under domestication. *Proceedings of the National Academy of Sciences USA* **107**, 6412–6417 (2010).
2. Rubin, C.-J., Zody, M.C., Eriksson, J., Meadows, J.R.S., Sherwood, E., Webster, M.T., *et al.* Whole-genome resequencing reveals loci under selection during chicken domestication. *Nature* **464**, 587-91 (2010).
3. Shimomura, K., Lowrey, P.L., Vitaterna, M.H., Buhr, E.D., Kumar, V., Hanna, P., Omura, C., Izumo, M., Low, S.S., Barrett, R.K. *et al.* Genetic suppression of the circadian *Clock* mutation by the melatonin biosynthesis pathway. *Proceedings of the National Academy of Sciences USA* **107**, 8399-8403 (2010).

About the researchers

Takaoki Kasahara was born in Fukui, Japan, in 1972. He graduated from the College of Arts and Sciences, The University of Tokyo, in 1996. He studied the molecular mechanism underlying the phase shifting of circadian rhythms by light when he was a PhD student. After he obtained his PhD in 2001 from The University of Tokyo, he joined the Laboratory for Molecular Dynamics of Mental Disorders, RIKEN Brain Science Institute. He has been studying the pathophysiology of mood disorders and developing mouse models for these disorders. His mouse model for bipolar disorder was reviewed in the premier issue of *RIKEN RESEARCH* **1**, 10 (2006). He was a Special Postdoctoral Researcher (RIKEN) from 2003 to 2006 and is now a Deputy Team Leader.



Artificial atoms light up

A superconducting circuit that strongly interacts with light paves the way for optical computing schemes

Before a quantum effect such as resonance fluorescence—resulting from the interaction of light with atoms—can be applied to quantum computing schemes, scientists need to replicate it in the laboratory. Thus far, however, efforts using artificial atoms made from superconducting circuits have been unsuccessful. Now, resonance fluorescence of a single artificial atom has been demonstrated by researchers from the NEC Nano Electronics Laboratory in Tsukuba and the RIKEN Advanced Science Institute in Wako[1].

Resonance fluorescence occurs when a light beam with an energy that matches an atom's resonance energy gets absorbed by the atom and then re-emitted in random directions. As resonance fluorescence can be used to couple two photons, or light particles, scientists are keen to exploit this effect in quantum computing operations. However, this effect in atoms is too small to be useful for practical applications since photons and atoms interact very weakly due to their small size, according to Jaw-Shen Tsai, who led the research team.

To circumvent this problem, researchers created artificial atoms on computer chips (Fig. 1), where the interaction between light and the artificial atom can be optimized. "With a solid-state device such as ours, made from superconducting circuits, the coupling can be very strong," says Tsai.

Earlier attempts by researchers in the field to observe resonant fluorescence in artificial atoms resulted in low efficiencies of around 12%, owing to poor re-emission of the absorbed light by these atoms.

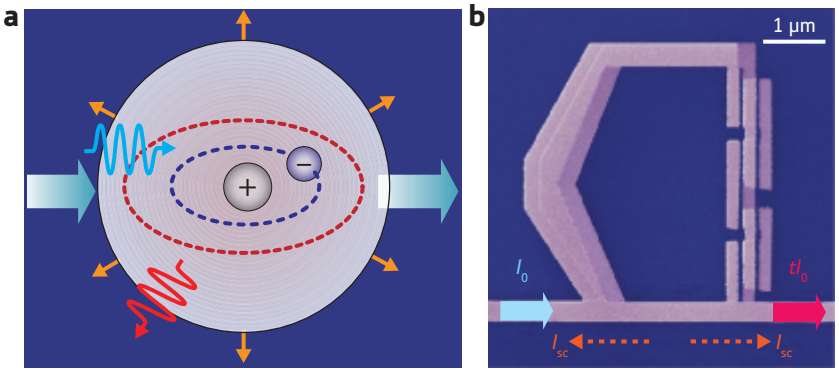


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Figure 1: A schematic representation of resonance fluorescence. (a) In a natural atom, an incoming light beam (left arrow) is absorbed and light is re-emitted in all spatial directions. (b) An artificial atom made from a superconducting circuit can achieve the same function. The light coming in along a one-dimensional waveguide (I_0) couples to the circuit. Light is then scattered (Is_c) in both directions of the wire, so that the original transmitted light (tI_0) is suppressed.

To enhance the re-emission process, the researchers used a one-dimensional waveguide coupled to the artificial atom. This resulted in an efficient re-emission of light from the artificial atom because in the waveguide the light is channelled in only two directions. Tsai and colleagues demonstrated that about 94% of the incoming light at the resonance frequency of the superconducting circuit was absorbed and re-emitted.

By building on this strong interaction between incoming light and the artificial atom a number of potential applications are now possible, according to Tsai. "There are a whole series of experiments one can do, for example towards photon-based quantum computing," he says. The absorption of a photon by an artificial atom, for example, could be used to control the propagation of a second photon along the waveguide, owing to the non-linear nature of the interaction of light with the artificial atom, Tsai explains. ■

1. Astafiev, O., Zagoskin, A.M., Abdumalikov, A.A., Pashkin, Yu.A., Yamamoto, T., Inomata, K., Nakamura, Y. & Tsai, J.S. Resonance fluorescence of a single artificial atom. *Science* **327**, 840–843 (2010).

The 'Hall' mark of a quantum magnet

The presence of exotic particles, called spinons, might now be detectable in a magnetic field, providing insight into quantum magnet properties

An important model to explain high-temperature superconductivity is the so-called 'quantum spin liquid'. Scientists are therefore interested in understanding the low-energy excitations of this magnetic state. Now, a theoretical study by a research team from RIKEN and the Massachusetts Institute of Technology USA, has explained how the properties of spin liquids could be revealed by a simple heat-transfer experiment[1].

In an insulating magnetic crystal, the electronic spins are localized to the atoms that form the crystal lattice. For most such magnets, or antiferromagnets, the chemical bonds favor an arrangement where, at low temperatures, each spin points in a direction opposite to that of its neighbor. However, on a triangular lattice, such as the 'Kagome lattice' (Fig. 1), a spin cannot simultaneously be opposite to all of its neighbors. The spins in these magnets never order, even at very low temperatures—giving rise to the name quantum spin liquid.

"Spin liquids have an exotic electronic state because [their] electrons can effectively dissociate into distinguishable spin- and charge-carrying particles," explains team-member Naoto Nagaosa from the RIKEN Advanced Science Institute, Wako. "The spin-carrying particle is called a spinon and determines the low-energy properties of the magnet."

To date, however, few experiments have found spinons. Nagaosa and his collaborators explain how a method similar to the so-called 'Hall measurement'—an indispensible technique for studying the properties of semiconductors—could be used to detect spinons.

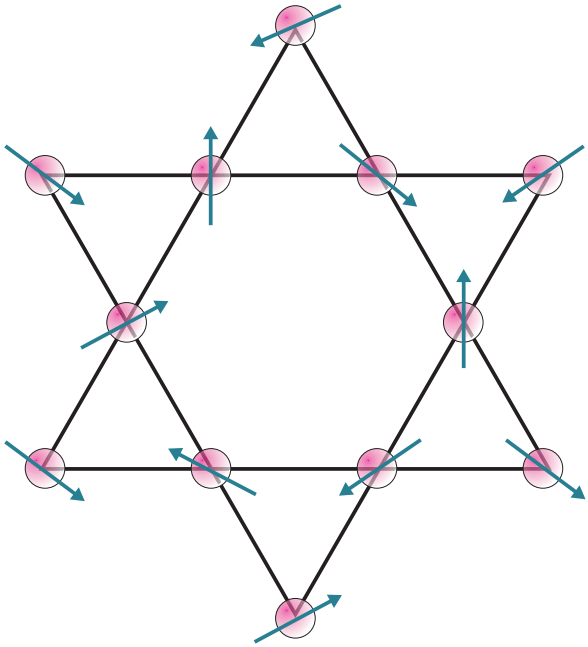

Figure 1: A schematic of electron spins on the Kagome crystal lattice, named for its similarity to a form of Japanese basket weaving (the spheres denote atoms). The Kagome spin lattice is believed to be an example of a spin liquid.

In the classic version of the Hall measurement, a magnetic field is applied perpendicular to a charge-carrying current, causing positive charges to curve one way and negative charges the other. The deflection of the charges provides information about their properties, including their sign.

In the 'thermal Hall effect' considered by Nagaosa and his colleagues, temperature serves as the driving force to create a current—not of charges, but of magnetic excitations—that flow in a magnetic field. For a spin liquid, these excitations are the spinons. As in the classic Hall effect, a magnetic field will deflect these excitations, which will change the direction of the heat flow—an effect that experimentalists should be able to measure.

Nagaosa and his colleagues showed that while there is no thermal Hall effect in most conventional antiferromagnets, the presence of spinons in a spin liquid would result in a clear effect. This experimental probe could therefore become an important way to identify and study excitations of quantum magnets. ■

1. Katsura, H., Nagaosa, N. & Lee, P.A. Theory of the thermal Hall effect in quantum magnets. *Physical Review Letters* **104**, 066403 (2010).

Unveiling spiral magnetism

Photoemission experiments shed light on the origin of an enigmatic type of magnetism in iron

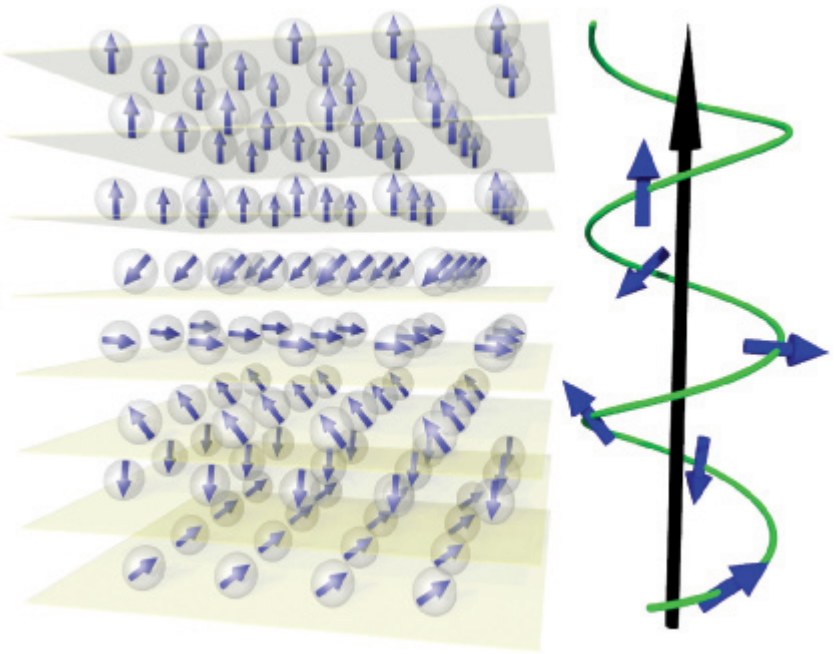

Figure 1: Schematic representation of an iron thin film consisting of eight monolayers with a spin-spiral magnetic order (left). The angle between the spins associated with localized spins on the iron atoms varies periodically in a spin spiral (right).

Iron is the most useful magnet in our daily life. It is a 'ferromagnet' at room temperature and ambient pressure, but displays a variety of other magnetic properties that depend on its crystallographic structure. Now, using a technique called angle-resolved photoemission spectroscopy (ARPES) on iron thin films, Jun Miyawaki from the RIKEN SPring-8 Center, Harima, and colleagues have uncovered the origins of a particular type of magnetic order[1], called the spin spiral (SS), which has eluded understanding despite extensive experimental and theoretical efforts.

Magnetism in iron is associated with the spin, or quantum angular momentum, of the valence electrons of its atoms. In a ferromagnet the spins are parallel to each other; however, a structural change in the position of atoms in the crystal matrix of iron can order the spins into a spin spiral, such that the angle between the spins varies periodically (Fig. 1).

Miyawaki says he was shocked when he realized that the electronic structure of SS-ordered iron was still an open question; it is fundamental to understanding the material's behavior.

To elucidate the origin of this magneto-structural behavior, Miyawaki and colleagues studied ultrathin iron films consisting of eight monolayers with a ferromagnetic bilayer at the top and six SS-ordered monolayers below. The researchers used the ARPES technique to bombard the SS layers with soft x-ray photons and knock out electrons. Then they measured the intensities and angles of the emitted electrons. This yielded information about the electrons' energy and momenta from which they constructed Fermi surfaces to characterize and predict various properties of iron.

Crucially, the researchers successfully mapped the energy-momentum relationship for electrons moving in- and out-of-plane of the iron film separately. Miyawaki notes that this required a concerted effort to develop the necessary instrumentation by his team at RIKEN and a team at the Japan Synchrotron Radiation Research Institute (JASRI).

The experimental results showed stark differences with respect to the symmetries of the in-plane and out-of-plane Fermi surfaces. A detailed analysis revealed that the SS magnetic order is directly linked to electrons 'inhabiting' specific regions of the out-of-plane Fermi surface, thus providing direct information about its origin on a microscopic level.

These findings not only provide vital clues to theoretical studies, but also suggest that iron thin films may be used in spintronics devices based on the spin-transfer torque phenomenon, Miyawaki notes. "Because right- and left-handed spin spirals exert a different spin-transfer torque on spin-polarized electrons, iron thin films could serve as memory devices," he says. ■

1. Miyawaki, J., Chainani, A., Takata, Y., Mulazzi, M., Oura, M., Senba, Y., Ohashi, H. & Shin, S. Out-of-plane nesting driven spin spiral in ultrathin Fe/Cu(001) films. *Physical Review Letters* **104**, 066407 (2010).

In the electron cloud

Mapping the shape and dynamics of a molecule's outer electron cloud is now possible using a novel experimental technique

The chemistry between atoms and molecules is strongly determined by their outer electron orbitals, or clouds, which participate in chemical processes. A team from three Japanese research institutes has now developed a method that can measure the three-dimensional shape and dynamics of an electron cloud[1]. "The shape of an electron cloud is at the heart of intermolecular interactions that lead to beautiful chemistry," comments Toshinori Suzuki from the RIKEN Advanced Science Institute in Wako, who led the research team.

Measuring the dynamics of an electron cloud is challenging because molecules in gases and liquids always move randomly; this makes it difficult to take a 'snapshot' of movement averaged over many molecules at a specific moment in time. However, the excitation of nitric oxide (NO) by a polarized laser beam can align those molecules along one axis, so that the measurement of their outer electron cloud becomes possible.

To detect the shape of the outer electron cloud of an NO molecule aligned by the first laser pulse, Suzuki and colleagues released the electrons from the molecule using a second laser pulse. They then applied an electric field to accelerate and project the expanding electron cloud onto a fluorescent screen where it was visualized as a direct representation of the original electron distribution (Fig. 1). The researchers then used computer algorithms, similar to those from computer tomography, to construct a three-dimensional picture from the two-dimensional representation.

Fundamental quantum mechanical principles limit the degree to which the molecules can be aligned by the laser pulse, Suzuki notes. This means that there is always unavoidable blurring in the reconstructed three-dimensional image. Removing this blurring in the final images was the most difficult part of the process, he says.

Suzuki and colleagues therefore analyzed how a three-dimensional image changes when the molecules rotate out of alignment. By correcting these misalignment effects, they eventually succeeded in perfectly sharpening the image.

The team's algorithm can visualize the outer electron cloud of a molecule at rest, but the challenge now is to map the rapid changes that occur during chemical reactions. "The NO molecule was just a testing ground," explains Suzuki. "Our main target is more complex molecules and their chemical reactions in response to light of different color." Outlining his future vision, Suzuki says he would like to study the mechanism of photodamage to DNA starting with real-time observations of electron motions in their constituent base molecules. ■

1. Tang, Y., Suzuki, Y.-I., Horio, T. & Suzuki, T. Molecular frame image restoration and partial wave analysis of photoionization dynamics of NO by time-energy mapping of photoelectron angular distribution. *Physical Review Letters* **104**, 073002 (2010).

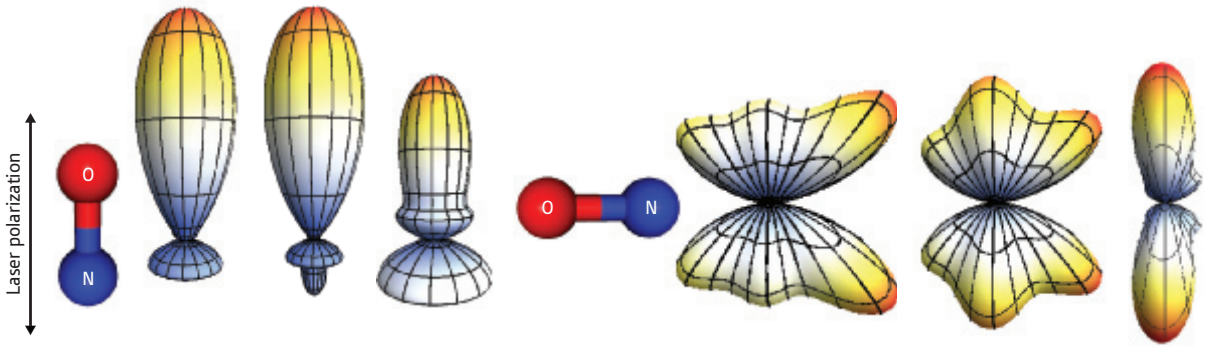

Figure 1: Experimental measurement of the temporal evolution cycle (left to right) of the outer electron cloud of an NO molecule. The arrows show the polarization of the laser beam that aligns the molecules during the experiment.

Unfolding the genesis of 'bad' seeds

Specific characteristics of small clumps of prion proteins dictate the conformation of larger aggregates that could influence disease symptoms

When the prion protein misfolds and aggregates in humans, it can cause fatal neurodegenerative diseases such as Creutzfeldt-Jakob disease and Gerstmann–Sträussler–Scheinker syndrome. These diseases have different symptoms, partly because the prion protein can misfold into different shapes. Just how a single protein can misfold into different aggregate conformations, however, has confounded scientists.

Now, Motomasa Tanaka and colleagues at the RIKEN Brain Science Institute in Wako have reported that small clusters of prion proteins called oligomers, which develop from monomer proteins, determine the eventual shape of the larger prion aggregate[1]. The findings were published in the journal *Nature Chemical Biology* in collaboration with researchers from the United States and from the RIKEN SPring-8 Center in Harima.

The research team used a yeast model system to study prion misfolding and aggregation, because yeast contain a prion-like protein called Sup35. This yeast protein misfolds into different aggregate conformations that cause the yeast to turn various colors—from white to pink—when they are grown on nutrient plates. A synthetic version of Sup35 can also form these distinct conformations when grown at different temperatures.

Using various biophysical techniques, the researchers observed that the synthetic Sup35 formed oligomers when they were grown at a low temperature, but not at a high temperature. The Sup35 grown at a low temperature made the yeast turn white, while Sup35 grown at a high temperature made the yeast turn pink. This suggests that the oligomers, formed at only the low temperature, may be an intermediate step in the formation of the larger aggregates that cause the 'white' phenotype (Fig. 1).

The team then investigated which amino acid region of Sup35 is involved in the formation of the oligomer. By mutating various amino acids of the Sup35 protein, the researchers found that the parts of the protein required for oligomer formation were different to those required for creation of the larger aggregate. In addition, while oligomer formation was involved in acquisition of the 'white' phenotype, it was not required for driving the growth of the larger prion aggregate. These findings suggest that oligomers serve as an initial scaffold to determine the eventual shape—and therefore the physiological characteristics—of the larger prion aggregate. Tanaka proposes that "inhibiting these interactions between prion proteins could become a therapeutic strategy for the neurodegenerative prion diseases." ■

1. Ohhashi, Y., Ito, K., Toyama, B.H., Weissman, J.S. & Tanaka, M. Difference in prion strain conformations result from non-native interactions in a nucleus. *Nature Chemical Biology* **6**, 225–230 (2010).

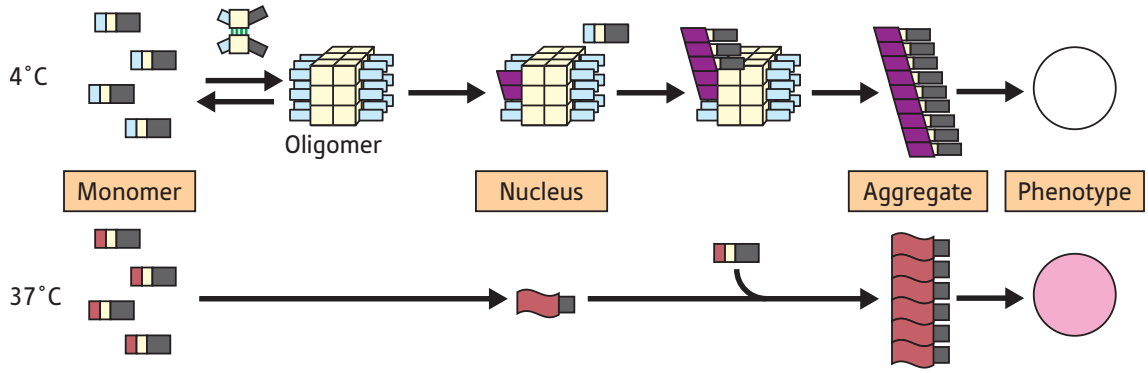

Figure 1: Schematic diagram depicting how prion oligomers determine the shape of the larger aggregates at different temperatures. The oligomer was not observed at higher temperatures (bottom) for reasons yet to be determined.

Twisting in the right direction

Spontaneous rotating movements cause growing nerve fibers to turn to the right

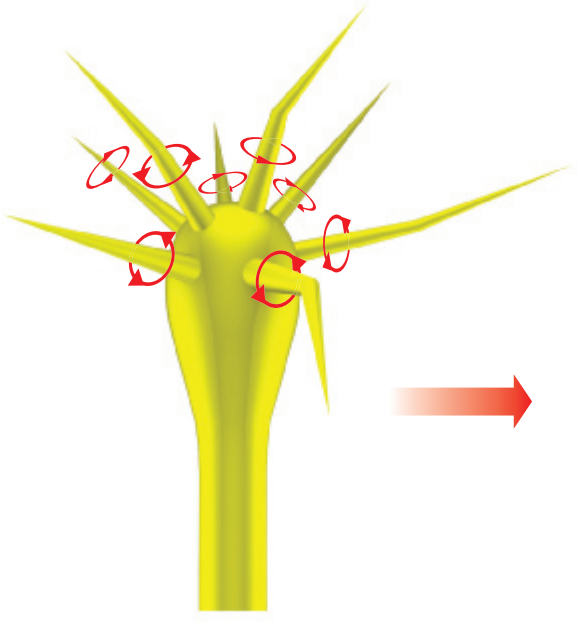

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Figure 1: Spontaneous rotations of the filopodia cause inherent turning behavior in growth cones.

During neural development, immature nerve cells extend axons and dendrites toward their targets then form connections with other cells. At the tip of these extending fibers is the growth cone, a structure with finger-like protrusions called filopodia. As the growth cone moves like an amoeba through the environment, the filopodia detect chemical guidance cues that steer it in the right direction. These processes are dependent on rearrangements of the actin cytoskeleton, a protein scaffold inside the cell.

Now, a team of researchers led by Hiroyuki Kamiguchi of the RIKEN Brain Science Institute has shown that nerve fibers turn clockwise in the absence of external clues, when growing on flat two-dimensional surfaces, because the filopodia rotate of their own accord[1].

The researchers first confirmed that nerve fibers from the hippocampus of embryonic rats turn rightwards when grown on a two-dimensional substrate, but grow straight when embedded in a three-dimensional gel. Addition of the fungal toxin cytochalasin D, which stops elongation of actin filaments, prevented the turning of fibers growing on a flat surface, showing that the turning is dependent on the cytoskeleton.

Hypothesizing that filopodia rotate autonomously, the researchers developed a new technique to directly observe the movements in three dimensions. They embedded hippocampal neurons in a gel, so the nerve fibers grew vertically towards the lens of an upright microscope. This revealed that individual filopodia tended to rotate counter-clockwise. This rotation generates a leftward force on the surface, causing the growth cone to turn to the right (Fig. 1).

The researchers then tested whether or not this turning is powered by myosins, the motor proteins responsible for actin-based cellular movements. They transfected hippocampal neurons with three different full-length myosins (Va, Vb and Vc), as well as shortened forms of them that prevent endogenous myosin molecules from binding actin filaments. All were fused to, or co-expressed with, a fluorescent protein to allow easy visualization.

As expected, filopodial rotation was blocked in neurons expressing the shortened myosins, but could be rescued by transfecting the cells with myosins Va and Vb, but not myosin Vc. The rightwards rotation was also observed in neurons from the cerebral cortex, thalamus and cerebellum, suggesting that this is a general mechanism.

Commenting on the findings, Kamiguchi says that: "Rotating filopodia would probe a larger volume of the environment and contribute to the precise perception of cues by the growth cone." Alternatively, the rotations could promote nerve bundle formation, by enabling new fibers to twine around older ones. ■

1. Tamada, A., Kawase, S., Murakami, F., & Kamiguchi, H. Autonomous right-screw rotation of growth cone filopodia drives neurite turning. *Journal of Cell Biology* **188**, 429–441 (2010).

Cleaning up cellular trash

Inducing cells to destroy a misfolded protein alleviates the symptoms of Huntington's disease in mice

Huntington's disease (HD) is a progressive neurodegenerative disease characterized primarily by involuntary movements. Inherited mutations in the huntingtin gene cause a stretch of glutamine residues in its associated protein, huntingtin, to increase in length, so that the mutant protein misfolds and accumulates within neurons. Neurologists believe that failure to clear aggregates of this misfolded protein is an underlying mechanism involved in the onset of HD.

Nerve cells can now be induced to destroy mutant huntingtin protein and reduce aggregate formation, according to the results of a study led by Nobuyuki Nukina of the RIKEN Brain Science Institute in Wako[1]. The researchers suggest that their approach could be used to effectively treat HD.

Nukina and colleagues used genetic engineering to construct a fusion protein consisting of two copies of polyglutamine binding peptide 1, which is known to bind mutant huntingtin and suppress its aggregation, and the binding regions of heat shock cognate protein 70 (HSC70), which is a 'chaperone' protein that targets the mutant huntingtin for destruction.

The researchers found that their construct inhibited the aggregation of mutant huntingtin in cultured cells by inducing a process called chaperone-mediated autophagy, which does not normally break down the misfolded proteins. They also observed that the fusion molecule bound to the mutant huntingtin, forming complexes with HSC70 that were recognized as abnormal and sent to a structure called the lysosome for degradation (Fig. 1).

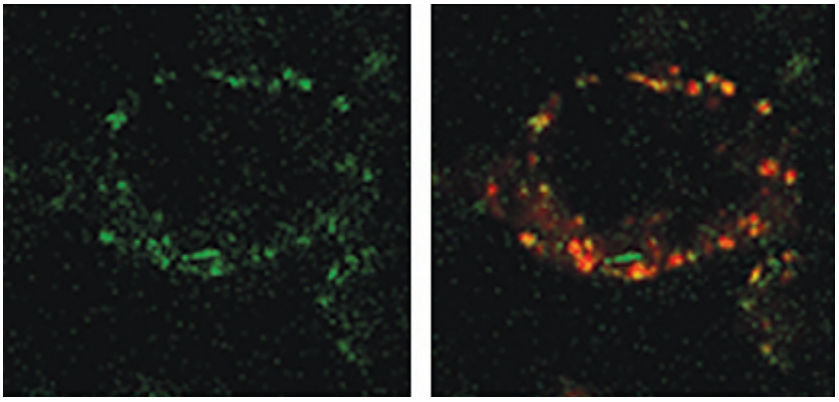

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Figure 1: The fusion molecule binds to the misfolded Huntingtin protein (green, left) and recruits the chaperone-mediated autophagy machinery, which sends them to the lysosome (red, right) for degradation.

The construct was also effective in clearing aggregates of several other misfolded proteins, including ataxin1, which causes a neurodegenerative disease called spinocerebellar ataxia.

The researchers tested their construct in two strains of mice with HD symptoms. They injected viral vectors containing the construct into the striatum, a brain region that is involved in the control of movement and degenerates in HD patients.

When they examined the animals' brains four weeks later, they found the construct widely distributed throughout the striatum in both mouse strains. Importantly, the fusion protein had significantly inhibited huntingtin aggregation compared to control mice. As well as reducing the number of aggregates, the construct reduced the average size of the remaining aggregates. The treatment also alleviated HD symptoms in the animals: their movements improved in a behavioral task, they lost less weight, and their survival rate increased.

"Genetic therapy for Huntington's is currently not possible because of the difficulties involved in delivering genes to the brain," says Nukina, "so we would like to develop or find a compound that can bind to expanded glutamine tracts and HSC70." ■

1. Bauer, P.O., Goswami, A., Wong, H.K., Okuno, M., Kurosawa, M., Yamada, M., Miyazaki, H., Matsumoto, G., Kino, Y., Nagai, Y. & Nukina, N. Harnessing chaperone-mediated autophagy for the selective degradation of mutant huntingtin protein. *Nature Biotechnology* **28**, 256–263 (2010).

Ever alert for inflammation

Regulatory T cells can travel to and from the skin while controlling immune responses in that organ

By showing that anti-inflammatory regulatory T cells (Tregs) move to and from the skin whilst regulating an immune response[1], an international research team involving RIKEN researchers has provided insight into how immune cells behave during inflammation.

The team, including Michio Tomura, Shohei Hori and Osami Kanagawa from the RIKEN Research Center for Allergy and Immunology in Yokohama and Kenji Kabashima from the Kyoto University Graduate School of Medicine, used a specially engineered line of mice to track immune cells in a living animal model. The mice—developed previously by Tomura, Kanagawa and colleagues—express a protein called Kaede that usually causes their cells to glow green, but glow red once exposed to violet light (Fig. 1). This color switching allowed the researchers to tag cells from one part of the body and track them as they moved elsewhere. "This kind of approach is only possible in our original Kaede mouse system and by collaboration among research centers within RIKEN," says Tomura.

Tracking the tagged cells revealed that T cells traveled from the skin to a nearby lymph node in the absence of any immune stimulus, suggesting to the researchers that immune cells migrate through non-inflamed tissues as part of their surveillance function in the body.

When the researchers painted an antigen on the skin of these mice to induce an immune response, they observed an increase in the proportion of T cells in the nearby lymph node that had come from the skin. In mice with depleted immunosuppressive Tregs, they recorded an increase in skin swelling after antigen exposure. The team therefore believes that Tregs are required to reduce inflammation within the skin.

In tissue culture experiments, Tomura, Kabashima and colleagues found that the Tregs sourced from inflamed skin suppressed the proliferation of immune cells from the lymph node, better than Tregs that had not come from skin. The researchers suggest that was probably because skin Tregs expressed higher levels of anti-inflammatory molecules.

When they injected Tregs from inflamed skin of one mouse into inflamed skin of other mice, those Tregs reduced swelling better than cells from non-inflamed skin. The researchers also observed Tregs moving to newly inflamed areas of skin from other areas.

Since Tregs can travel to and from the skin while controlling immune responses in that organ, the researchers suggest that enhancing Treg migration or function could therefore be a promising therapeutic approach to dampen inflammation in various organs. ■

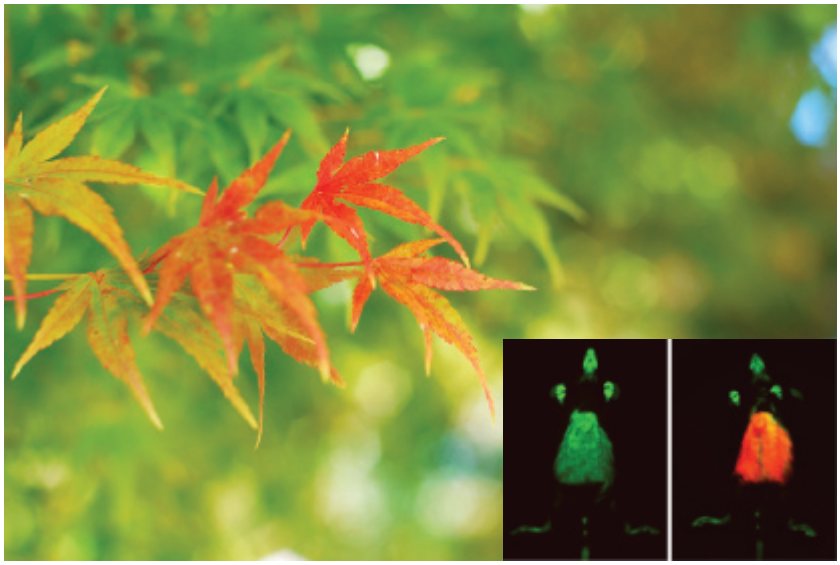

Figure 1: *Kaede*, Japanese maple, leaves turn from green to red in the autumn. Cells in Kaede mice (inset) have the same color-switching property after being exposed to violet light, and can be tracked as they move to and from the skin during an immune response.

Credit (background image): istockphoto/jennybonner Inset reproduced, with permission, from Ref. 1 © 2010 American Society for Clinical Investigation

1. Tomura, M., Honda, T., Tanizaki, H., Otsuka, A., Egawa, G., Tokura, Y. Waldmann, H., Hori, S., Cyster, J.G., Watanabe, T., Miyachi, Y., Kanagawa, O. & Kabashima, K. Activated regulatory T cells are the major T cell type emigrating from the skin during a cutaneous immune response in mice. *The Journal of Clinical Investigation* **120**, 883–893 (2010).

Kidneys at risk

A large-scale genetic study reveals a novel risk factor for a potentially fatal kidney disorder associated with diabetes

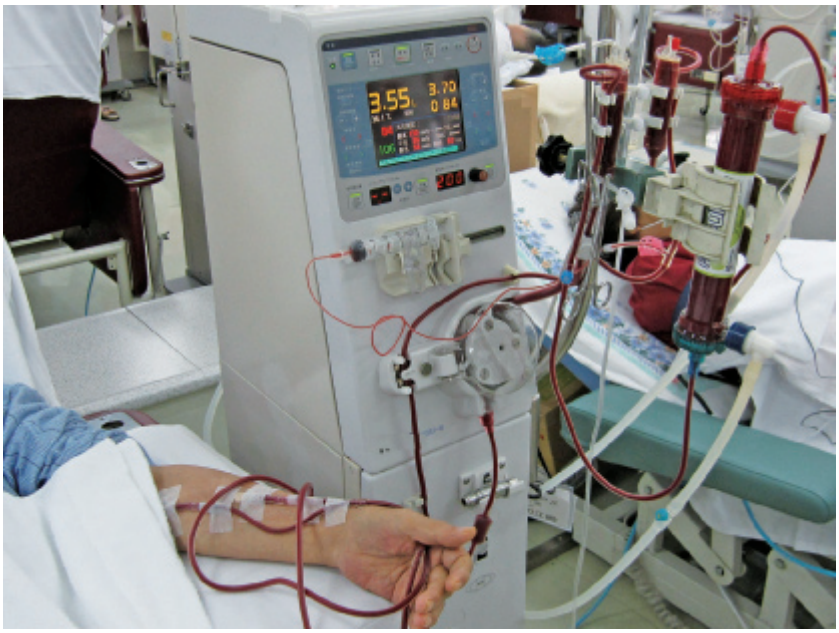

Figure 1: Patients with end-stage renal disease resulting from diabetic nephropathy require extreme therapeutic measures, such as hemodialysis. Individuals receiving dialysis typically need three four-hour treatments every week.

It is estimated that nearly a third of all diabetic patients may be at risk for diabetic nephropathy, a renal disorder that progresses slowly but can inflict severe and irreversible kidney damage (Fig. 1). "In Japan, more than 16,000 patients with diabetic nephropathy enter dialysis therapy per year, and these individuals account for 43% of all new [Japanese] cases requiring renal replacement therapy," explains Shiro Maeda of the RIKEN Center for Genomic Medicine in Yokohama.

There is considerable evidence suggesting the existence of genetic risk factors for diabetic nephropathy, but efforts to directly identify candidate genes have been undermined by limited understanding of disease pathology. In such situations, genome-wide association studies, in which large populations are subjected to genetic analysis in order to identify single-nucleotide genomic variants potentially 'linked' to a condition of interest, offer a powerful alternative for disease gene discovery.

Maeda's group recently launched such a study in partnership with a team of researchers from around the world, and their data have fingered a single nucleotide change in the gene encoding the enzyme acetyl-coenzyme A carboxylase beta (*ACACB*) as a significant risk factor for diabetic nephropathy among both Japanese and European populations[1]. This genetic variation was located within a non-protein-coding, regulatory segment of the gene, and appears to boost expression levels relative to the standard *ACACB* variant.

Strikingly, although type I and type II diabetes patients are both at risk for diabetic nephropathy, the polymorphism identified by Maeda and colleagues only showed significant association for patients with type II diabetes, which arises when individuals acquire resistance to the hormone insulin. "I think our report is the first to provide evidence suggesting the existence of diabetic nephropathy genes specific to patients with type 2 diabetes," Maeda says. "However, this finding should be evaluated further."

The gene *ACACB* is involved in the metabolism of fatty acids, which is in keeping with previous findings that have linked defects in this process with kidney disease. Nevertheless, as the first genetic factor to be explicitly linked to diabetic nephropathy, Maeda points out that considerably more research will be required to clarify the pathological role of *ACACB* and to uncover other potential accomplices.

"We will examine possible mechanisms by which *ACACB* contributes to development and progression of diabetic nephropathy using cultured human kidney cells or genetically engineered mice," he says, "and we are also performing a larger-scale genome-wide association study to identify additional susceptibility genes." ■

1. Maeda, S., Kobayashi, M., Araki, S., Babazono, T., Freedman, B.I., Bostrom, M.A., Cooke, J.N., Toyoda, M., Umezono, T., Tarnow, L. *et al.* A single nucleotide polymorphism within the acetyl-coenzyme A carboxylase beta gene is associated with proteinuria in patients with type 2 diabetes. *PLoS Genetics* **6**, e1000842 (2010).

Mapping the power of networking

An atlas of protein–protein interactions reveals the collaborative efforts underlying gene regulation in mice and men

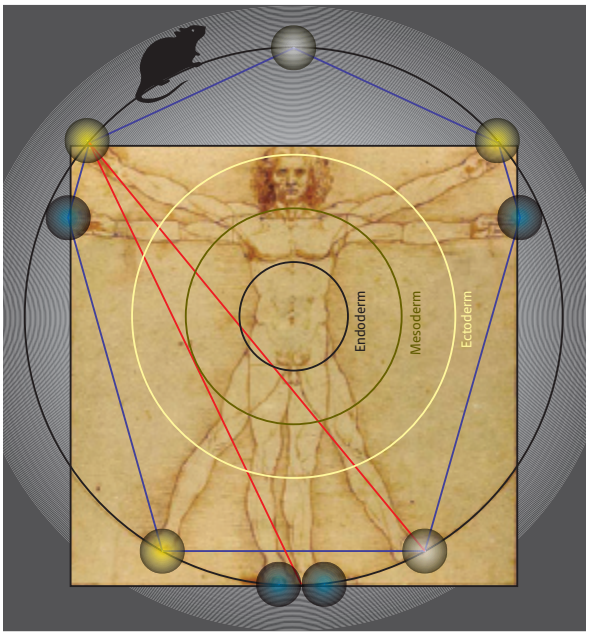

Figure 1: An atlas of TF–TF interaction networks in humans and mice may offer powerful new insights into the principles of gene regulation and reveal new strategies for treating disease.

Transcription factor (TF) proteins act as switches that turn genes on and off, and the timing and localization of their activity ensures that genes are activated only when and where they are needed—an essential consideration in processes like embryonic development.

However, the TF–gene relationship is seldom simple. "In many cases, TFs work as complexes in which two or more proteins physically interact," explains Harukazu Suzuki, project director at the RIKEN Omics Science Center, Yokohama, and scientific organizer of the international FANTOM4 Consortium. "Depending on the combination, expression of different sets of genes is regulated; thus, these protein–protein interactions are essential information for analysis of transcriptional network regulation."

Both research organizations have made it their business to untangle these networks, and new work from Suzuki and collaborators provides a useful foundation for mapping functional TF associations[1]. The team generated protein-producing clones for a majority of the known transcription factors from humans and mice and used these to perform 'two-hybrid' experiments that reveal physical interactions between pairs of proteins in both species (Fig. 1). An exhaustive screen of both pools of clones enabled the assembly of an 'atlas' of 762 and 877 likely TF–TF interactions in human and mouse, respectively, with subsequent experiments suggesting that these data potentially represent approximately one-quarter of all such interactions.

They then determined where each TF is produced in an effort to classify individual factors as tissue-localized 'specifiers' or broadly expressed 'facilitators'. Further analysis enabled them to identify clusters of interactions associated with different subsets of tissues, revealing a fraction of TF–TF associations that help coordinate the development of embryonic tissue into the diverse range of cell types seen in mature organisms. "We identified a small protein–protein interaction sub-network consisting of only 15 TFs, which plays a crucial role in the regulation of cell fate," says Suzuki. Strikingly, this network contained mostly promiscuously expressed 'facilitators', suggesting that the localization of multi-factor interactions is as important as the restricted expression of individual factors in governing tissue-specific gene expression.

Suzuki and colleagues hope to expand this 'first draft' atlas soon, and to explore the clinical implications of disruptions within these interaction networks. "We would like to expand the information to include diseased tissues and cells, and especially cancer," he says. "[By comparing] these TF interaction networks to normal ones, we may be able to identify TFs involved in these diseases … and the [associated] interactions may offer novel targets for therapy." ■

1. Ravasi, T., Suzuki, H., Cannistraci, C.V., Katayama, S., Bajic, V.B., Tan, K., Akalin, A., Schmeier, S., Kanamori-Katayama, M., Bertin, N. *et al.* An atlas of combinatorial transcriptional regulation in mouse and man. *Cell* **140**, 744–752 (2010).

Exploring technology for the preservation of genetic lines

Atsuo Ogura

Head
Bioresource Engineering Division
RIKEN BioResource Center

The BioResource Engineering Division headed by Atsuo Ogura is developing a technique that allows life to be propagated more efficiently with the aim of preserving about 4,000 strains of mice that have been collected by the RIKEN BioResource Center (BRC). In 2006, Ogura and his team successfully generated mice from sperm cells retrieved from a mouse cadaver that had been frozen at −20 °C for 15 years. The BioResource Engineering Division is known as one of the few laboratories in the world where cloned mice can be reliably generated from somatic cells, and is at the forefront of some rapidly advancing techniques for propagating life.

Development of new cryopreservation techniques

Mice and men have much in common: 99% of their genes are identical. Mice are therefore indispensable as experimental subjects for research in life science and medicine. "For example, we can modify genes to create a strain of disease-model mice that display symptoms of disease similar to those in humans. This genetic modification has been contributing to our understanding of disease mechanisms and drug discovery. Research in our laboratory has been advancing toward preserving more than 4,000 strains of mice, including disease-model mice developed by researchers around the world and in particular by Japanese researchers. These model mice are made available to the international scientific community by the BRC, which ranks second after the Jackson Laboratory in terms of the number of strains of mice it maintains, and the number of model mice is increasing every year," says Ogura.

Germ cells, including sperm and eggs, and fertile eggs of most strains of mice, can be frozen in liquid nitrogen and stored at −196 °C. This method, however, is not suitable for certain strains that fail to develop after thawing due to freezing-induced damage or inactivation by the drugs used to prevent damage. In 2000, Ogura and his colleagues successfully froze a wider range of mouse strains using an ethylene glycol solution instead of the conventionally used drugs. "Even now, 20% of all strains stored at the BRC are not frozen. Unless these strains can be frozen, we have to continue to keep, breed and maintain mice to prevent the strains from becoming extinct, at considerable cost. There is thus a strong need to continue our research on cryopreservation techniques."

Generational propagation in half the time

Ogura's team is developing a new technique that allows a mouse to

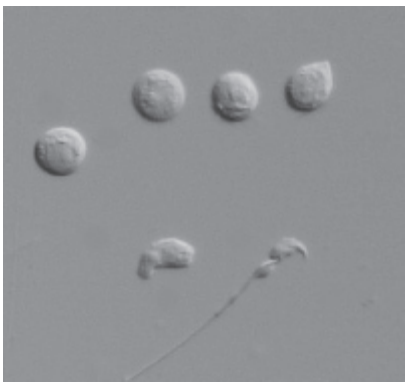

Figure 1: Round spermatids and a mature sperm. Round spermatids, immature germ cells that have the same set of genomes as normal sperm cells, are incapable of fertilizing an egg without artificial assistance.

be generated without relying on the normal fertilization process of a sperm 'swimming' to meet an egg. Instead, thawed sperm, which have lost the ability to swim, are injected into an oocyte under the microscope in a process called 'microinsemination'.

The first microinsemination technique for humans was established in 1992. However, the application of the technique to mice was unsuccessful for a long time because mouse eggs are much more prone to breakage than human eggs. In 1991, Ogura studied abroad in the Ryuzo Yanagimachi Laboratory at the University of Hawaii. Yanagimachi was a world authority on mammalian reproduction. There, Ogura worked on developing a new microinsemination technique for mice under the leadership of Yanagimachi. After returning to Japan in 1994, Ogura succeeded in generating mice based on the microinsemination technique for the first time (Fig. 1). "In the experiment, I used round spermatids that do not have the ability to swim. These cells are immature germ cells in a state before differentiation into mature sperm. I used a micromanipulator to place a round spermatid and an egg close to each other, and gave them an electric shock to fuse them together. As a result, microinsemination took place successfully."

Later, the Yanagimachi Laboratory attempted to incorporate a piezoelectric element into a micromanipulator for faster pipette movement, and with the aid of the new manipulator, the laboratory successfully established a microinsemination technique to quickly drill a hole in an egg through which a sperm cell could be injected. The operation requires some skill, but there are three specialists in microinsemination including Ogura.

In 2009, utilizing the microinsemination technique, Ogura's team developed a new technique that enables a mouse line to be transmitted in half the time normally required. An adult male mouse needs two or three months after birth before it begins to produce mature sperm and become ready for mating. Since the gestation period of a mouse is 20 days, it took at least 80 days for the generations to alternate. Ogura used round spermatids from a 22-day-old male mouse for microinsemination. They successfully generated a mouse in just 42 days, which is the shortest period achieved for propagation in mammals.

Techniques that double the speed required for propagation can contribute significantly to accelerating research using mice. "For example, when disease-model mice are generated through the process of genetic modification, we can compare them with healthy, standard mice through various experiments to explore the relationship between the modified genes and diseases. However, we should note that we will not be able to explore the relationship unless the genetic information of the disease-model mice and the standard mice is the same except for the selected modified genes. Thus, genetic information other than the modified genes should be 'homogenized' between the model and standard mice. Back-cross-mating target model mice with standard mice for homogenization is repeated up to more than ten times. This homogenization process, called congenics, generally takes up to three years." Ogura shortened the congenic process to just three to six months by doubling the speed of generational propagation. It is anticipated that this congenic technique can also be applied to livestock improvement.

The X-chromosome holds the key to successful generation of cloned mice from somatic cells

In 2006, Ogura succeeded in generating mice by microinsemination from sperm cells retrieved from a mouse cadaver preserved at –20 °C for 15 years (Fig. 2). "Being alive as a living organism or individual cells is one thing, but being alive as a genome is another."

This research was developed further. "The next year, Dr Wakayama, a team leader, tipped me off that somatic cells from frozen mice could be used to generate a cloned mouse, and so I sent a frozen mouse preserved for 16 years to him." Teruhiko Wakayama, who generated the world's first cloned mouse from somatic cells in 1998 at the Yanagimachi Laboratory, is currently heading the Laboratory for Genomic Reprogramming at the RIKEN Center for Developmental Biology. In 2008, Wakayama and his

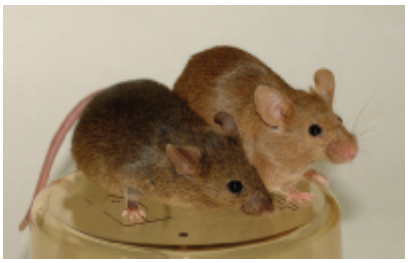

Figure 2: Mice generated from a sperm cell retrieved from a 15-year-frozen mouse cadaver. The success of this experiment demonstrated the possibility of developing a new sperm preservation method by freezing mouse cadavers or spermaries at –20 °C.

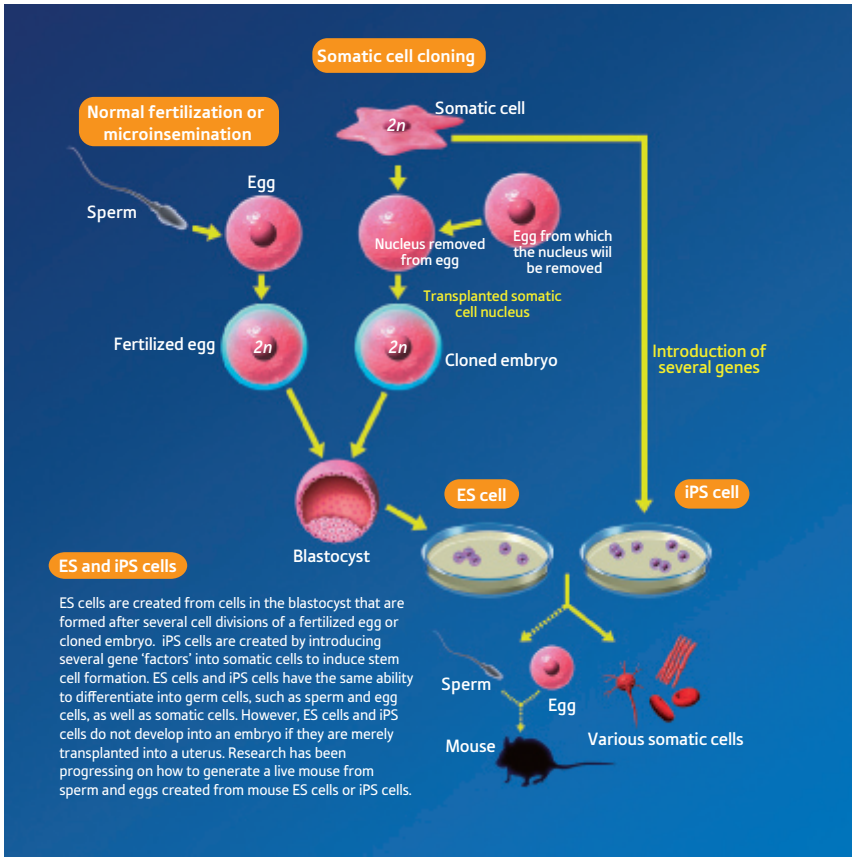

Figure 3: Normal fertilization vs. microinsemination and somatic cell cloning.
In normal fertilization or microinsemination, fertilized eggs have two sets of genomes: one set derived from the sperm cell and the other from the egg. In somatic cell cloning, the nucleus of an egg is replaced with a nucleus that already contains two sets of genomes. The cloned embryo has the same genome as that of the mouse that provided the somatic cell nucleus.

team succeeded in generating cloned mice from the somatic cells of the 16-year-frozen mouse.

The cells of multicellular organisms are classified into germ cells, such as sperm and egg, and somatic cells, which build the other bodily cells (Fig. 3). The nucleus of a somatic cell has two sets of genomes: paternal and maternal, whereas a sperm or egg produced by meiotic division has just one set of genomes. In normal fertilization, an egg and sperm fuse together to form a fertilized egg that has one set of sperm-derived genomes and one set of egg-derived genomes. The fertilized egg undergoes repeated cell division and develops into a mouse embryo. The process of microinsemination is essentially the same, except that the sperm is injected directly into the egg.

In somatic cell cloning, the nucleus of the egg is removed and replaced with the nucleus of another somatic cell having two sets of genomes to generate a cloned embryo. The embryo develops into a cloned mouse fetus with genetic information identical to that of the mouse that provided the original somatic cell nucleus.

"The number of somatic cells in an individual mouse is much greater than the number of germ cells, and they can also be cultured outside the body. If mice can be generated from somatic cells, it will significantly benefit the maintenance of mouse strains." The BioResource Engineering Division is also one of the few laboratories in the world where cloned mice are generated reliably from somatic cells. Many laboratories in the world have reported low success rates for somatic cell cloning. "More than ten years have passed since the birth of the world's first mouse cloned from somatic cells, but the success rate still remains at about one to two percent."

One reason for the poor success rate of somatic cell cloning is related to the genome state. Every gene in the genome of a fertilized egg is ready to express itself. Once expression begins, the necessary genes begin expressing in a cascade, inducing repeated cell division and proliferation. The fertilized egg develops into an embryo, and some embryonic cells differentiate into specific somatic cells such as skin and nerves. During the differentiation process, only the necessary genes express themselves in each cell, and the other genes are inhibited; the unnecessary genes are blocked so that they are not expressed.

To make somatic cell cloning a success, the genome of a somatic cell injected into an egg must be unblocked, and as with the genome of a fertilized egg, all the genes in the genome must be initialized to the state in which they can be expressed at any time. "It is considered that certain proteins in an egg affect the nucleus of an injected somatic cell, causing initialization of the nucleus. However, the mechanism remains a mystery."

Strangely, in some cases the success rate for cloning with fully differentiated cells is better than for stem cells, although fully differentiated cells are considered to have more blocked factors than stem cells, which still have the ability to differentiate into various types of cells. "We confirmed that the success rate is generally higher

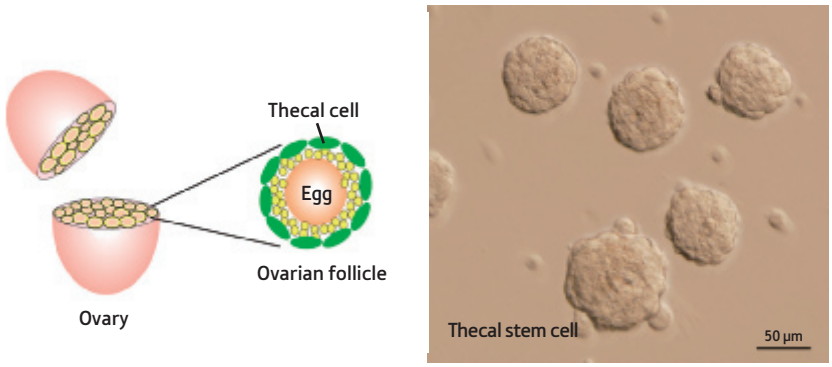

Figure 4: Thecal cell and thecal stem cell.
Thecal cells release hormones to help the development of eggs. Ogura and his team discovered thecal stem cells that can produce thecal cells in a mouse ovary.

with NKT lymphocytes (a kind of white blood cell) than with hemopoietic stem cells (the source of blood cells), even though NKT lymphocytes are differentiated from hemopoietic stem cells."

The low success rate for somatic cell cloning suggests that there is some difference between the genome state of normal fertilized eggs and that of cloned embryos. "We compared the expression state of genes in fertilized eggs with that in cloned embryos. The comparison showed that in the cloned embryos, the expression of only particular genes in the X-chromosome is strong, and other genes were inhibited. This may be the reason why the cloned embryos were inhibited from developing." Ogura has initiated an experiment to generate cloned mice while inhibiting the strong expression of particular genes in the X-chromosome before injecting the nucleus of a somatic cell into an egg. "This method can increase the success rate of somatic cell cloning by up to ten times."

Ogura, however, points out the limitations of cloning. "The success rate will be less than 20% because I think the success rate is significantly dependent on factors other than genomic reprogramming, including damage to the eggs caused on extraction of the nucleus."

Generating mice from iPS-derived germ cells

For the generation of mice by both microinsemination and somatic cell cloning, eggs are indispensable. Conventional theory dictates that all eggs are produced through the process of development, and a female has only a limited number of eggs at birth. There are no stem cells that can produce eggs in the ovary of a postnatal mouse.

In 2004, however, American researchers reported that they had discovered stem cells that differentiate into eggs in a mouse ovary. When these stem cells are separated and cultured, many eggs can be produced outside the body. "This discovery brought about a firestorm in the academic community. To confirm the finding, we investigated mouse ovaries in detail, but we did not find any such stem cells. At present, many scientists remain skeptical about the existence of these stem cells. However, we came across another type of stem cell in the ovaries in our search."

What they discovered were the stem cells that differentiate into 'thecal cells'. Thecal cells are somatic cells that wrap around an ovarian follicle and release the sex hormones necessary for the growth of eggs (Fig. 4). "We succeeded in separating and culturing thecal cells, and we intend to use these thecal cells to recreate the process for a mouse egg to grow and mature outside the body, thus clarifying the mechanism. Our research results will also be able to help maintain extracted eggs in good condition."

Separated and cultured thecal cells will contribute significantly to research on the generation of egg cells *in vitro*. Ogura and his team began their research in this are using embryonic stem (ES) cells from mice. ES cells have the ability to differentiate into various cells, whereas the alternative—induced pluripotent stem (iPS) cells—are created by introducing several kinds of genes into somatic cells. Both iPS and ES cells have similar abilities. There are many research groups around the world working on how to generate sperm or egg cells from mouse ES cells or iPS cells. "Research in this field is very competitive. In ten years, germ cells might be generated *in vitro* from iPS cells derived from mouse somatic cells, and mice might be generated from these germ cells," says Ogura. ■

About the researcher

Atsuo Ogura was born in Tokyo, Japan, in 1960. He graduated from the Faculty of Agriculture at The University of Tokyo in 1982, and obtained his PhD in 1987 from the same university. After working at the National Institute of Infectious Diseases (NIID) in Japan as a researcher for four years, he joined the University of Hawaii, USA, where he started his career in reproductive biology under the supervision of Ryuzo Yanagimachi. He returned to NIID in 1992 and was promoted to laboratory head in 1999. In 2002, he moved to the RIKEN BioResource Center and has since acted as head of the BioResource Engineering Division. His research focuses on the biology of reproduction and development including nuclear transfer cloning, fertilization, stem cells and cryopreservation of gametes/embryos.

Delegation of representatives from five leading Chinese universities visits RIKEN

On April 21, a delegation of 12 dignitaries from China visited the RIKEN Wako campus to meet with RIKEN President Ryoji Noyori and a number of RIKEN directors. The dignitaries included Nanning Zheng, president of Xi'an Jiaotong University, Chen Xiao Man, vice president of Fudan University, Li Xiaohong, president of Chongqing University, Jian Chu, vice president of Zhejiang University, and Jianguo Hou, president of the University of Science and Technology of China. Issues discussed included the management of research institutes, support for young research personnel and the status of ongoing internationalization efforts.

The delegation's visit to RIKEN stems from cooperative efforts in joint graduate school programs coordinated by RIKEN. Fifteen Chinese universities are involved in the joint programs, including Peking University, Zhejiang University, Shanghai Jiao Tong University, Nanjing University, the University of Science and Technology Beijing (USTB) and Xi'an Jiaotong University. The overall aim of the visit to Japan, which also included visits to The University of Tokyo, the Tokyo Institute of Technology, Keio University, Waseda University and Kyoto University, was to discuss with Japanese universities and research institutes various issues surrounding education and personnel support.

In discussions with the Chinese dignitaries, questions focused on RIKEN's flexible management style, its fluid employment system and its systems for supporting young research personnel, as well its internationalization strategy in particular in relation to China. Discussions also touched on technology transfer efforts, support policies for RIKEN venture businesses, thoughts on how to assess individual researchers, and RIKEN's strategy for attracting talented young researchers.

RIKEN executives with the delegation of presidents and vice presidents from major universities in China.

Nanning Zheng, president of Xi'an Jiaotong University (left), and Ryoji Noyori, president of RIKEN (right) hold a scroll describing a famous Chinese historical event.

For their part, members of RIKEN focused their attention on the current state of science and technology in China. Questions to the Chinese visitors touched on the increasing level of Chinese state investment in science and technology, on measures for attracting Chinese researchers based in Europe and the US back to China, and on the growth in the number of Chinese foreign exchange students in the US.

Following the discussion period, the group of university presidents visited RIKEN's world-class Radioactive Isotope Beam Factory (RIBF), where they learned about ambitious research to uncover new chemical elements and explore the origins of the universe. A two-week school for undergraduate students that hosts 15 students every year, organized jointly by the RIKEN Nishina Center for Accelerator-Based Science (the center that operates RIBF) and Peking University, also attracted a great deal of interest.

Following the tour of RIBF, the group visited the research laboratory of Chief Scientist Hou Zhaomin, who is himself originally from China. Hou explained specifically how research funds are used in laboratory management and the rules that come into play, and also delved into the issues of how topics are selected for research and how lab members are recruited.

With 25% of its international researchers arriving from China and more than 500 exchange students visiting from China over the past 30 years, RIKEN's connection with China remains strong and will only grow stronger in the future. The visit of dignitaries from five leading Chinese universities further supports this connection, contributing to deeper collaboration in research while also strengthening ties at the national level between Japan and China. ■

Dr Kimitoshi Kono
Low Temperature Laboratory
2-1 Hirosawa, Wako
Saitama 351-0198, Japan

Dear Dr Kono,

It's already been more than two years now since my return to France and I still have some very vivid memories of my stay at RIKEN, probably due to the fact that I spent such a great time there.

I remember very well our first contact when I came to give a seminar at the Low Temperature Laboratory looking for a postdoc position. I was struck both by the impressive experimental apparatus your lab has gathered and built over the years and by the casual and friendly atmosphere that seemed to reign amongst your group.

When I arrived in Wako, almost one year later, to start my work on the detection of single electrons floating on liquid helium, I was not disappointed. On a professional level, my 18-month stay at RIKEN was an important step for me. While benefitting from your advice and experience in low-temperature physics, I learned to move on from my PhD student mindset to work more independently and gather new techniques. I really enjoyed discovering new solid-state physics fields I scarcely knew beforehand, be it nanoscience or helium-related physics. The scientific interactions with people in the group as well as with the numerous visitors you welcomed each year were very important for me to discuss, learn and ultimately build up a larger physics culture.

The research environment at RIKEN is also one I am grateful to have experienced. The administrative staff were so helpful and nice that they almost made paperwork look easy! The research facilities were fantastic to work in: everything you needed was within arm's reach: cryostats, low-temperature equipment or fabrication facilities, and the very nice Nanoscience Joint Laboratory. The campus itself was so pleasant: every season had its highlights—cherry blossoms, crickets chirping, the beautiful autumn leaves, you name it—and each morning I had the impression of going to work in a garden.

On a more personal level, I think you already know how much I enjoyed the company of all the people I worked with. Gladly, we were able to talk about more than just physics at the different nomikai, badminton or softball matches, or at the parties we had. I've made some friends who I always look forward to meeting at conferences or whenever I come back to Japan. This, of course, has been an invaluable benefit from my stay at your laboratory.

I would like to thank you again for giving me the opportunity to work in such a pleasant and stimulating environment. I think I will cherish the memories from this experience for a very long time.

All the best,

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

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