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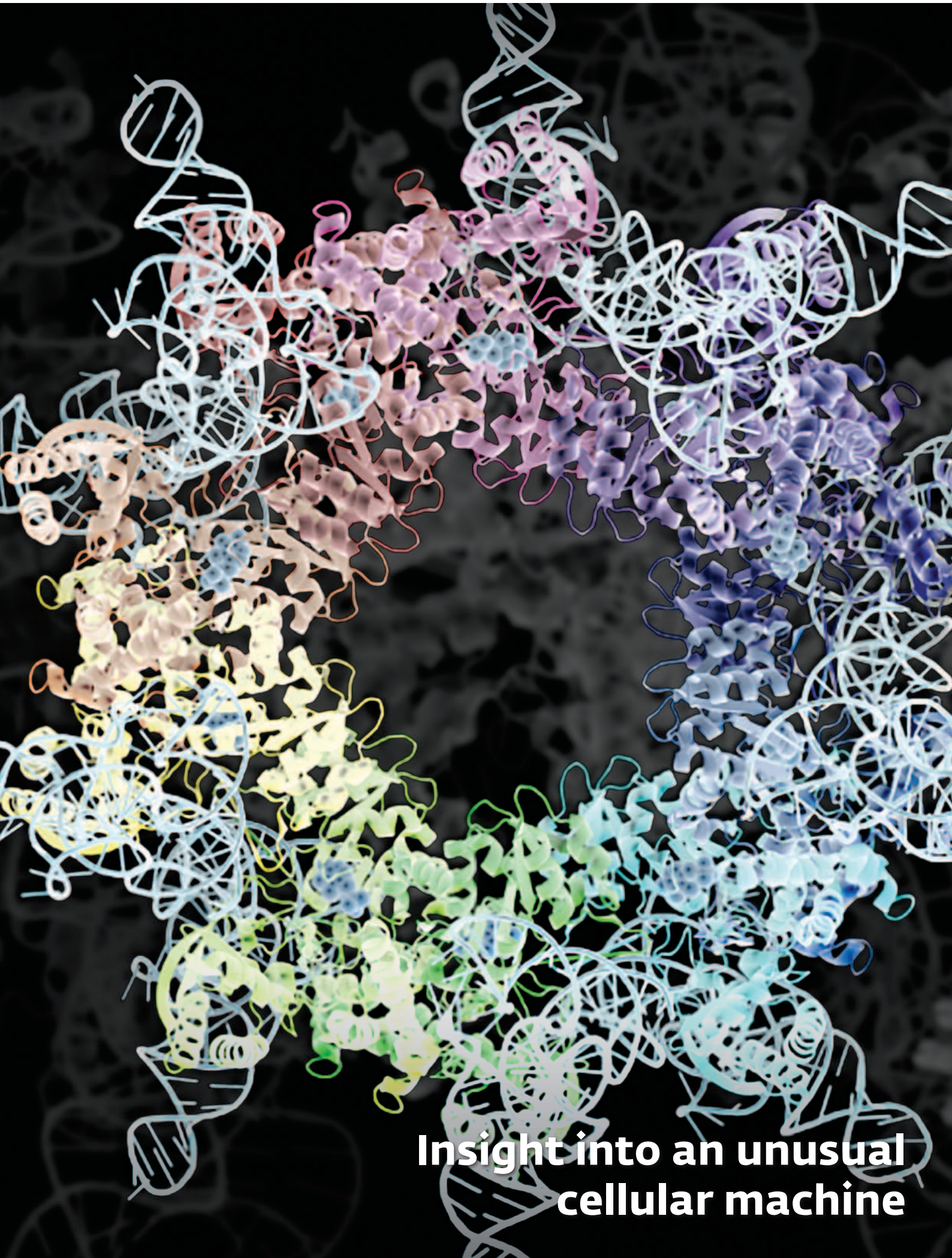

Insight into an unusual cellular machine

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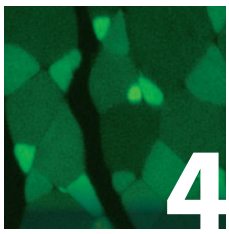

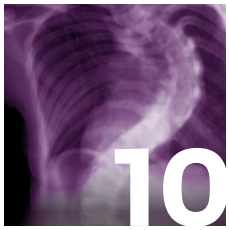

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RIKEN RESEARCH

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Medicine

An eel protein illuminates human health

The unusual properties of a fluorescent protein produced in eel muscle could prove useful for both clinical diagnostics and specialized biomedical imaging applications

Visualizing individual molecules within the crowded cellular interior is a daunting task. Thanks to the discovery of naturally occurring fluorescent proteins in the 1990s, however, such experiments are now routine. Since the first successful cloning of the gene encoding green fluorescent protein (GFP) from a species of jellyfish, hundreds of other fluorescent proteins have been identified, giving biologists a remarkable array of tools with which to label and image specific molecules, cells or even entire tissues within living organisms.

Atsushi Miyawaki, head of the Laboratory for Cell Function Dynamics at the RIKEN Brain Science Institute in Wako, has spent much of his career searching for and studying fluorescent molecules. His team has now cloned an unusual fluorescent protein with particularly promising clinical utility[1].

Most natural fluorescent proteins isolated to date have been purified from marine invertebrates such as jellyfish and coral. Until quite recently, no such proteins had been identified in vertebrate species. Miyawaki was therefore surprised to learn that Seiichi Hayashi, a food chemist at Kagoshima University, had isolated a GFP from the muscle of the Japanese freshwater eel, commonly known as unagi (Fig. 1).

After learning of Hayashi's discovery, Miyawaki immediately purchased two eels at a local fish market. "I tried just shining light on the muscle, and I was astonished by the bright green fluorescence," he says. "After that, I was very curious about the fluorescence in the eel muscle and set about cloning this gene."

Figure 1: A green fluorescent protein produced in the Japanese freshwater eel is the first of its kind to be discovered in a vertebrate species.

Providing long-range protection for muscles

GFP and related proteins exhibit chemical properties that make them inherently fluorescent. This novel 'UnaG' protein, however, needs additional assistance from a chemical known as bilirubin in order to fluoresce. Bilirubin is an intermediate molecule in the degradation of heme—the iron-binding component of hemoglobin that gives red blood cells their distinctive color and enables them to transport oxygen throughout the body. UnaG recognizes and binds to bilirubin astonishingly fast, and the two molecules generate bright green fluorescence almost immediately after they combine.

Upon analyzing its sequence and structure, the researchers determined that UnaG belongs to the family of fatty-acid-binding proteins (FABPs), which typically store or transport lipid molecules. Compared to its relatives, however, UnaG is extremely choosy. "Members of the FABP family generally have low affinity for their ligand, and some are 'broad-spectrum' and bind to more than one fatty acid ligand," says Miyawaki. "But UnaG only binds to bilirubin, and with very high specificity and high affinity."

UnaG is produced in a subset of thin muscle fibers (Fig. 2), where it specifically recognizes what is known as 'unconjugated' bilirubin. This heme metabolite is subsequently transported by the blood protein albumin to the liver, where it is converted enzymatically to 'conjugated' bilirubin prior to its eventual excretion

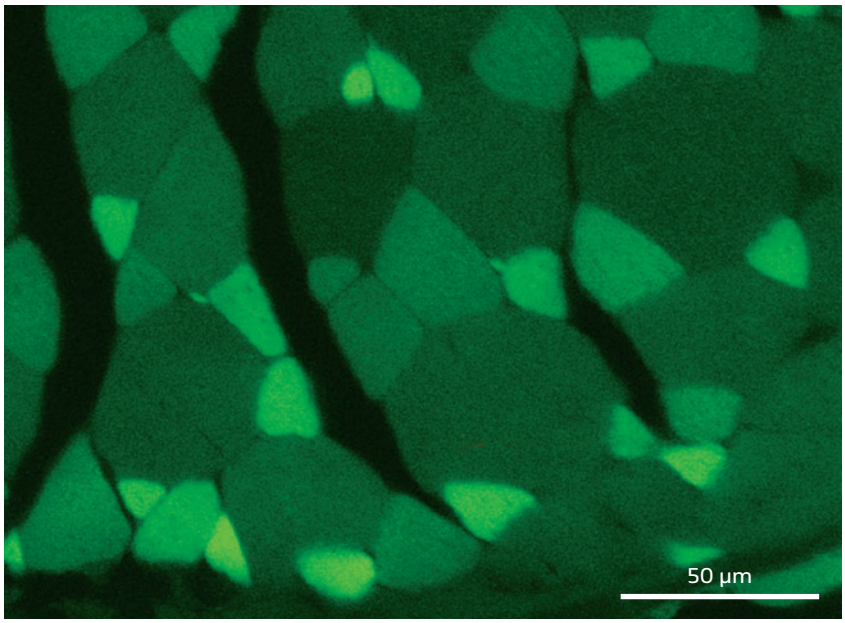


Figure 2: The UnaG fluorescent protein is produced primarily in a subset of thin muscle fibers, as seen in this cross-section.

from the body. However, mounting evidence suggests that the body deliberately maintains a pool of unconjugated bilirubin, which acts as a potent antioxidant that protects tissues against damage from oxidative stress.

Unagi eels migrate for several months at a time, requiring extended muscle exertion that can generate the sort of chemicals that promote oxidative stress. Miyawaki hypothesizes that UnaG may offer a countermeasure to protect muscle tissue in such circumstances. "If you look at locusts or birds, which travel over long distances, their muscles show greatly increased expression of a type of FABP that probably works as a store for some kind of fatty acid for fuel," he says. "In analogy, we think that the UnaG protein works as storage for this powerful antioxidant."

A visible improvement in diagnostics

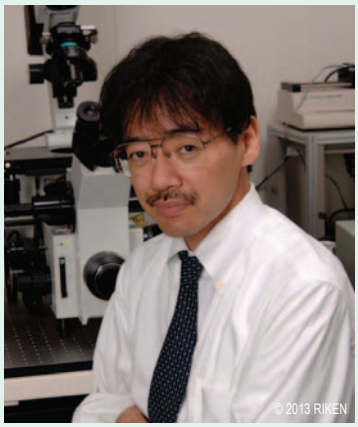
Several studies have suggested that antioxidant properties protect humans against disorders associated with oxidative stress, such as cardiovascular disease and metabolic syndrome. "A mild increase in bilirubin in our bodies is quite good," says Miyawaki. On the other hand, excessive unconjugated bilirubin can be toxic, and infants in particular are vulnerable to bilirubin-induced brain damage—a potentially fatal condition known as kernicterus.

Doctors have been performing blood tests to detect elevated bilirubin for almost a century but continue to rely on the same slow and imprecise methodology of days past. "Technology for measuring bilirubin has shown very little evolution," says Miyawaki, who previously trained as a physician. Since the speed with which UnaG generates a visible readout in the presence of unconjugated bilirubin could make it a superior alternative to these older methods, the researchers performed a head-to-head comparison. "With our kit, we were able to obtain reproducible results within 10 minutes," says Miyawaki. UnaG exhibited at least a thousandfold greater bilirubin sensitivity than existing methods, and the researchers also found that the protein could be stored as a dried powder and subsequently reconstituted, making it suitable for integration into a ready-to-use diagnostic kit.

UnaG is in fact overly sensitive to bilirubin, and Miyawaki's group is now using protein-engineering techniques to generate variants with finely tuned affinities that are better suited for use in clinical diagnostics and physiological research. These derivatives could also prove useful in research efforts to dissect the broader contributions of bilirubin to human health and disease. Among its other unique and potentially useful features, UnaG does not require oxygen to generate its glow, unlike other fluorescent proteins. This means UnaG could be used to illuminate anaerobic physiological and experimental environments where GFP would be snuffed out, such as the interior of a solid tumor—although Miyawaki points out that it remains to be seen whether bilirubin can effectively penetrate these dark corners. "We are going to find out the limitations of UnaG and how much it can contribute," he says.

1. Kumagai, A., Ando, R., Miyatake, H., Greimel, P., Kobayashi, T., Hirabayashi, Y., Shimogori, T. & Miyawaki, A. A bilirubin-inducible fluorescent protein from eel muscle. *Cell* **153**, 1602–1611 (2013).

ABOUT THE RESEARCHER



Atsushi Miyawaki obtained his PhD from Osaka University in 1991, after which he carried out postdoctoral research on intracellular signal transduction at the University of Tokyo. In 1995, he began to study fluorescent proteins and their application under Roger Y. Tsien at the University of California, San Diego, in the United States. Since 1999, his group at the Laboratory for Cell Function Dynamics, part of the RIKEN Brain Science Institute, has been interested in the development of new bioimaging technologies, principally using fluorescent proteins.

Visualizing a memory trace

Whole brain imaging of zebrafish reveals neuronal networks involved in retrieving long-term memories during decision making

In mammals, a neural pathway called the cortico-basal ganglia circuit is thought to play an important role in the choice of behaviors. However, where and how behavioral programs are written, stored and read out as a memory within this circuit remains unclear. A research team led by Hitoshi Okamoto and Tazu Aoki of the RIKEN Brain Science Institute has for the first time visualized in zebrafish the neuronal activity associated with the retrieval of long-term memories during decision making[1].

The team performed experiments on genetically engineered zebrafish expressing a fluorescent protein that changes its intensity when it binds to calcium ions in neurons and thereby acts as an indicator of neuronal activity. "Neurons in the fish cortical region form a neural circuit similar to the mammalian cortico-basal ganglia circuit," says Okamoto.

The fish were trained in an avoidance task by placing individual fish into a two-compartment tank and shining a red light for several seconds into the compartment containing the fish. If the fish did not move into the other compartment in response to the light, it was 'punished' with a mild electric shock. After several repetitions, the fish learned to avoid the shock by switching compartments as soon as the light came on.

The researchers then examined the neuronal activity of the fish under the microscope in response to exposure to red light. One day after the learning task, the fish showed specific activity in a discrete region of the telencephalon, which corresponds to the cerebral cortex in mammals, when presented with the red light. However, just 30 minutes after the learning task, no activity was observed in this part of the brain (Fig. 1). The results suggest that this telencephalonic area encodes the long-term memory for the learned avoidance behavior. Confirming this, removing this part of the telencephalon abolished the long-term memory but did not affect learning or short-term storage of the memory.

In humans, the ability to choose the correct behavioral programs in response to environmental changes is indispensable for everyday life, and the ability to do so is thought to be impaired in various psychiatric conditions such as depression and schizophrenia.

"Combining the neural imaging technique with genetics, we will be able to investigate how neurons in the cortico-basal ganglia circuit choose the most suitable behavior in any given situation," says Okamoto. "Our findings open the way to investigate and understand how these symptoms appear in human psychiatric disorders."

1. Aoki, T., Kinoshita, M., Aoki, R., Agetsuma, M., Aizawa, H., Yamazaki, M., Takahoko, M., Amo, R., Arata, A., Higashijima, S.-I. *et al.* Imaging of neural ensemble for the retrieval of a learned behavioral program. *Neuron* **78**, 1–14 (2013).

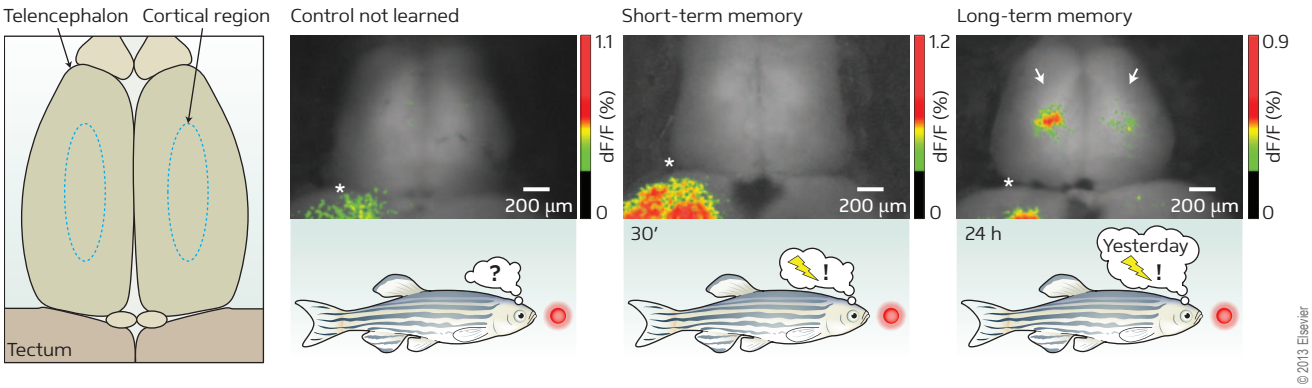


Figure 1: Neuronal activity in the zebrafish telencephalon is associated with long-term memory of learned avoidance behavior.

The rhythm of sleep

Measurement of brain activity reveals a sleep regulation mechanism that could be disturbed in depression

When sleeping, the brain cycles through various stages of sleep characterized by different neural activity. The way in which these stages are regulated, however, remains poorly understood. Hitoshi Okamoto and Hidenori Aizawa from the RIKEN Brain Science Institute have led research that reveals a previously unknown mechanism of sleep regulation, which may provide insight into the disruption of sleep associated with disorders such as depression[1].

Okamoto and his colleagues studied the rapid eye movement (REM) stage of sleep. REM sleep is usually associated with dreaming and is characterized by what is known as theta activity in the hippocampus region of the brain. "Theta activity is a rhythm of neural activity that shows oscillation, with four to ten cycles per second," explains Aizawa. "This specific rhythm appears when animals are in REM sleep."

REM sleep is abnormal in patients with depression; it starts sooner than normal after going to sleep and eye movement is more rapid. However, the biological basis for this has been unclear. Previous studies indicated the involvement of the brain chemical serotonin, the metabolism of which is also abnormal in patients with depression. A region of the brain called the lateral habenula (LHb) has also been implicated. The LHb is connected to the raphe nuclei where serotonin is produced and shows higher than normal activity in patients with depression.

To determine the exact role of the LHb in REM sleep, the researchers measured brain activity in sleeping rats. They discovered that when the LHb was damaged, theta activity in the hippocampus—and therefore REM sleep—lasted for a shorter period of time. For this effect to be seen though, the serotonin-producing raphe nuclei had to be intact. The team concluded that the LHb maintains theta activity in the hippocampus during REM sleep and that this effect is mediated by serotonin (Fig. 1).

"This reveals a novel role of the LHb in linking serotonin with REM sleep," explains Aizawa. "It suggests that a hyperactive habenula in patients with depression causes altered REM sleep."

Although these findings go some way to explaining the disrupted sleep in depression, Okamoto says that there is more to be done to confirm the link. "Now we've shown that inhibition of lateral habenular activity leads to shortening of REM sleep, the next step is to check whether animals with a hyperactive LHb show depressive behaviors and sleep disturbance."

1. Aizawa, H., Yanagihara, S., Kobayashi, M., Niisato, K., Takekawa, T., Harukuni, R., McHugh, T. J., Fukai, T., Isomura, Y. & Okamoto, H. The synchronous activity of lateral habenular neurons is essential for regulating hippocampal theta oscillation. *The Journal of Neuroscience* **33**, 8909–8921 (2013).

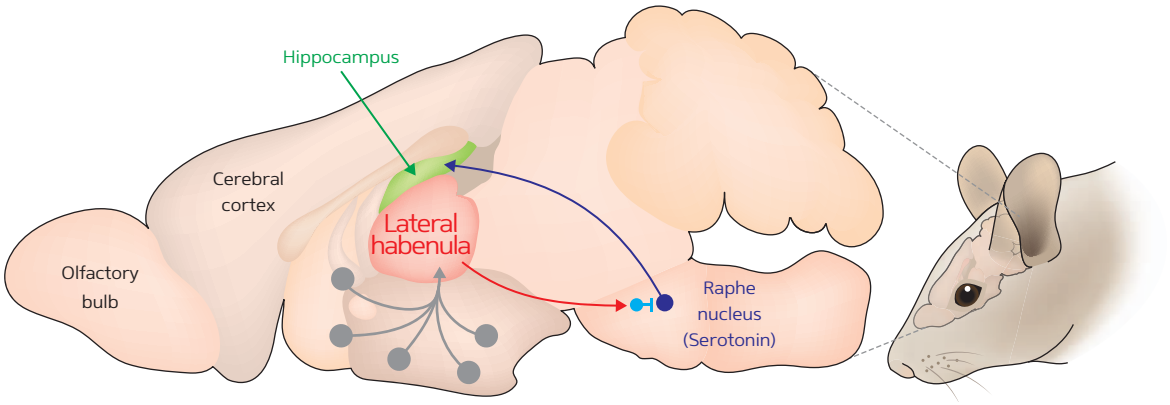


Figure 1: The lateral habenula (red) maintains rhythmic theta activity in the hippocampus (green) during REM sleep by modulating serotonergic activity (blue).

A new link between eye and ear

A gene responsible for building neural circuitry contributes to proper development of both the eye and ear

Myopia is a visual defect arising from abnormalities in the length of the eyeball and is widespread throughout the world. Hearing loss is also relatively common, affecting nearly 1 in 500 individuals by the age of 9. Although rarely occurring together, a study of a small number of individuals affected by both deficits by Jun Aruga of the RIKEN Brain Science Institute, in collaboration with colleagues from around the world, has now revealed a gene with a prominent role in the development of both the eye and ear[1].

Almost a decade ago, Aruga's group discovered that a particular gene, *SLITRK6*, is active only in sensory tissues including the eye, ear and the thalamus region of the brain. "The thalamus is often described as a 'relay station' for mediating sensory signal processing," he explains. His work drew notice from geneticists in the United States and United Kingdom who had uncovered striking evidence linking *SLITRK6* to human myopia and hearing loss, and Aruga proposed a collaboration between the three teams to explore this connection.

The UK team identified an Amish family with three sibling children manifesting severe myopia and hearing loss, while the US team studied a Turkish family with four similarly affected adult siblings. Using two different genomic analysis strategies, each team identified inherited mutations in the *SLITRK6* gene that appeared to be associated with these sensory problems. Follow-up work with a third family from Greece identified an additional *SLITRK6* mutation, further supporting this hypothesis.

Aruga's team subsequently performed a series of experiments to determine the functional impact of these mutations. The SLITRK6 protein normally localizes to cell surfaces, where it helps to coordinate synapse formation and neuron growth. However, the mutant versions of the protein fail to reach the membrane (Fig. 1) and exhibit impaired function. Previous experiments showed that mice lacking *SLITRK6* suffer hearing loss, and Aruga's team proceeded to examine eye development in these animals. They were surprised to find abnormalities that had been previously overlooked. "This gene deficiency resulted in increased axial length of the eye, as seen in myopia," he says.

Although this link between eye and ear development is unexpected, both the retina and hair cells of the inner ear feature the same subtype of synapse, whose formation may be governed by *SLITRK6*. In future studies, Aruga hopes to explore the details of *SLITRK6* function and how it helps determine the structure of the mature eye.

1. Tekin, M., Chioza, B. A., Matsumoto, Y., Diaz-Horta, O., Cross, H. E., Duman, D., Kokotas, H., Moore-Barton, H. L., Sakoori, K., Ota, M. *et al*. *SLITRK6* mutations cause myopia and deafness in humans and mice. *The Journal of Clinical Investigation* **123**, 2094–2102 (2013).

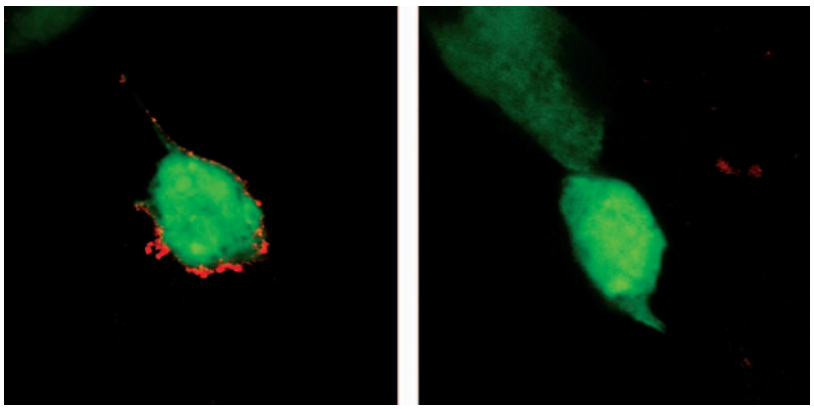


Figure 1: *SLITRK6* **mutations in myopia patients yield proteins (red) defective in cell surface localization. Proteins from the wild-type gene localize to the cell surface (left) in mammalian cells, while mutant proteins do not (right).**

Connecting the genetic dots on connective tissue disorders

Scientists identify a mutated gene responsible for a spectrum of skeletal and connective tissue disorders

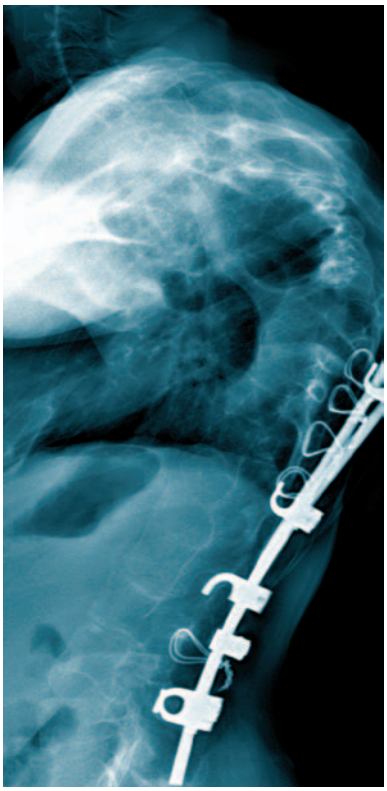


Figure 1: Spinal x-ray of an SEMD-JL1 patient.

The disorder known as spondyloepimetaphyseal dysplasia with joint laxity type 1, or SEMD-JL1, is characterized by skeletal abnormalities and loose ligaments that result in spinal misalignment and respiratory problems. The genetic basis of SEMD-JL type 2, a related disorder, was recently determined, but the genetic underpinnings of the type 1 form of the disease remain unknown.

By studying the genomes of seven people with SEMD-JL1, a large international research team led by Shiro Ikegawa from the Laboratory for Bone and Joint Diseases at the RIKEN Center for Integrative Medical Sciences has now identified a gene that, when mutated, is responsible not only for SEMD-JL1 but also a range of other bone and connective tissue defects[1].

Noriko Miyake, who joined Ikegawa's team from Yokohama City University, discovered the gene by sequencing the entire protein-coding region of the genomes of seven Japanese people with SEMD-JL1 from six unrelated families. Using targeted sequencing to confirm initial 'hits', Masahiro Nakajima, a member of Ikegawa's lab, showed that all of the subjects, in addition to an eighth Vietnamese individual with SEMD-JL1, had mutations in a gene called *B3GALT6* on the short arm of chromosome 1. This gene codes for a type of enzyme known as a galactosyltransferase II, which is involved in the synthesis of the proteoglycan linker region that helps form the structural cement of connective tissue. The researchers found that mutations in *B3GALT6* caused disease in a recessive fashion, meaning that all of the affected individuals had inherited two faulty copies of the gene.

The researchers then noticed that some of the individuals with SEMD-JL1 in the study exhibited many of the same clinical characteristics as those found in people with another connective tissue disorder—the progeroid form of Ehlers-Danlos syndrome, which is characterized by a defect in the synthesis of collagen. To investigate this further, they sequenced the *B3GALT6* gene in four people with progeroid-form EDS of unknown genetic cause. All four subjects carried mutations in the *B3GALT6* gene.

"B3GALT6 enzyme deficiency results in a wide variety of disorders," says Ikegawa. "These diseases have been considered to belong to totally different categories of disease, but actually are a spectrum of disorders affecting bone, cartilage, muscle, tendon, ligament and skin. Our findings will enable genetic diagnosis of these diseases." The researchers anticipate that the findings could open the door to future therapies for disorders related to *B3GALT6*.

1. Nakajima, M., Mizumoto, S., Miyake, N., Kogawa, R., Iida, A., Ito, H., Kitoh, H., Hirayama, A., Mitsubuchi, H., Miyazaki, O. *et al.* Mutations in *B3GALT6*, which encodes a glycosaminoglycan linker region enzyme, cause a spectrum of skeletal and connective tissue disorders. *The American Journal of Human Genetics* **92**, 927–934 (2013).

Getting ahead of the curve

A gene involved in spinal development may contribute to a common childhood disease responsible for spinal curvature

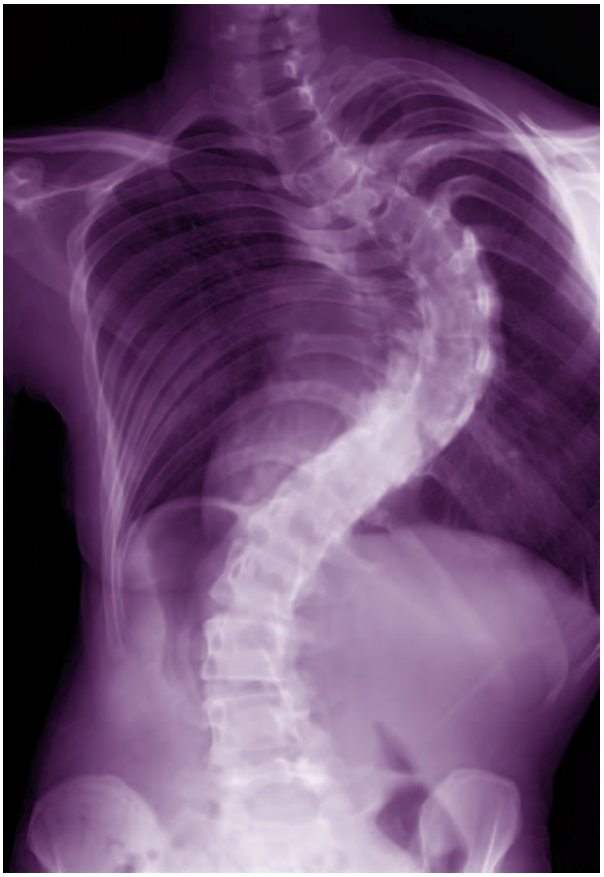


Figure 1: Spinal x-ray of a child with adolescent idiopathic scoliosis.

Adolescent idiopathic scoliosis (AIS) is the most common pediatric skeletal disease, causing complex rotational deformity of the spine in approximately 2% of school-age children worldwide. Recent studies have implied that AIS may, at least in part, be caused by genetics, but the pathogenesis of AIS still remains poorly understood. An international research team led by Shiro Ikegawa from the Laboratory for Bone and Joint Diseases at the RIKEN Center for Integrative Medical Sciences has now identified another gene that may contribute to AIS by altering spinal development[1].

Ikegawa's group previously identified a gene associated with AIS in Japanese populations. To further reveal the genetics underlying the disease, Ikuyo Kou from Ikegawa's lab conducted a step-wise association study including over 27,000 Japanese individuals. In this study, Kou identified another single nucleotide polymorphism (SNP) that is significantly associated with AIS in Japanese subjects. Additional testing showed that the association was replicated in Han Chinese and European populations, marking the first time a SNP associated with AIS has been identified in distinct populations.

Kou also determined that the SNP is located in the gene *GPR126*, which encodes a receptor protein. *GPR126* is known to be associated with the development of the sheaths that insulate nerve fibers, but its other functions are largely unknown. By examining the tissue-specific expression of *GPR126*, the researchers found that it is expressed in spinal cartilage, implying a role in the development of the spine.

Using zebrafish, Long Guo and Chisa Shukunami who joined Ikegawa's team from Kyoto University knocked down *GPR126* expression to investigate the role of the gene in vertebrate development. They found that zebrafish with lower levels of the GPR126 protein had shorter bodies and their vertebrae showed delayed bone formation, or osteogenesis. "Our zebrafish knockdown studies indicate the importance of GPR126 in bone tissue growth and formation, and raise the possibility that abnormal spinal development and growth induce AIS," explains Kou.

GPR126 is also known to be associated with shorter trunk length and reduced height in European populations, which coincides with the new findings. "Their associations are in the same direction; the susceptibility allele is the same for both AIS and shortened trunk," says Kou.

Although the identification of *GPR126* sheds new light on the pathogenesis of AIS, the two genes implicated so far explain only ~1% of the variation in AIS traits. Further studies therefore remain necessary to determine precisely how mutations of *GPR126* lead to AIS in humans.

1. Kou, I., Takahashi, Y., Johnson, T. A., Takahashi, A., Guo, L., Dai, J., Qiu, X., Sharma, S., Takimoto, A., Ogura, Y. *et al.* Genetic variants in *GPR126* are associated with adolescent idiopathic scoliosis. *Nature Genetics* **45**, 676–679 (2013).

Patience reaps rewards

Brain imaging shows how prolonged treatment of a behavioral disorder restores a normal response to rewards

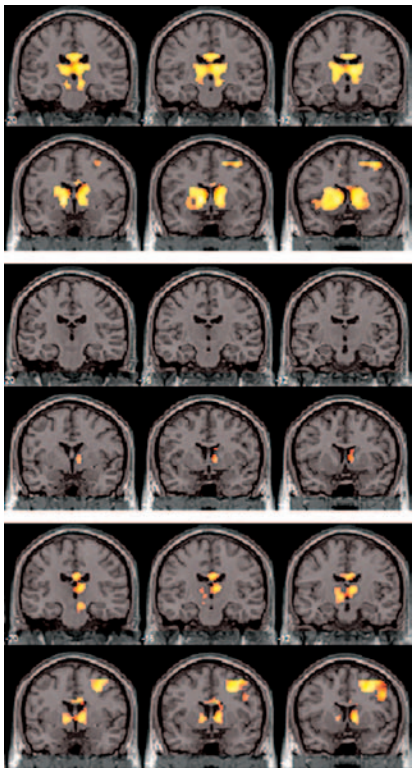


Figure 1: Brain imaging shows that the brain activity in healthy individuals (top) during testing under low monetary reward conditions was missing in ADHD patients before treatment (center), but returned after treatment with OROS-MPH for three months (bottom).

Attention-deficit/hyperactivity disorder (ADHD) is characterized by abnormal behavioral traits such as inattention, impulsivity and hyperactivity. It is also associated with impaired processing of reward in the brain, meaning that patients need much greater rewards to become motivated. One of the common treatments for ADHD, methylphenidate (MPH), is known to improve reward processing in the short term, but the long-term effects have remained unclear.

Kei Mizuno from the RIKEN Center for Life Science Technologies, in collaboration with colleagues from several other Japanese research institutions, has now demonstrated that prolonged treatment with MPH brings about stable changes in brain activity that improve reward processing with a commensurate improvement in ADHD symptoms[1].

ADHD is thought to affect up to 5% of children worldwide, and about half of those will go on to experience symptoms of the disorder into adulthood. MPH treats the disorder by increasing the levels of the brain chemical dopamine, which is involved in reward processing.

To understand the effect of MPH on ADHD symptoms and specifically reward processing over the longer term, the researchers studied the reward response behavior of ADHD and healthy patients—all children or adolescents—before and after treatment with osmotic release oral system (OROS) MPH. They used functional magnetic resonance imaging (fMRI) to measure brain activity during a task that saw participants rewarded with payment, but in two different scenarios: a high and a low monetary reward condition.

"In the high monetary reward condition, participants earned higher than the expected reward; whereas in the low monetary condition, participants earned an average reward that was consistently lower than expected," says Mizuno.

The brain images showed that before treatment with OROS-MPH, ADHD patients had lower than normal sensitivity to reward, as demonstrated by their abnormally low brain activity in two parts of the brain associated with reward processing—the nucleus accumbens and the thalamus—during testing under the low monetary reward scenario (Fig. 1).

However, after three months of treatment with OROS-MPH, there was no difference in the activity of these brain areas in ADHD patients compared with the healthy controls under any of the reward conditions. Their sensitivity to reward had returned to normal, and the patients' other ADHD symptoms also showed improvement.

Mizuno says that this study goes further than previous work. "We knew that acute MPH treatment improves reward processing in ADHD," he explains. "Now, we've revealed that decreased reward sensitivity and ADHD symptoms are improved by treatment for three months."

1. Mizuno, K., Yoneda, T., Komi, M., Hirai, T., Watanabe, Y. & Tomoda, A. Osmotic release oral system-methylphenidate improves neural activity during low reward processing in children and adolescents with attention-deficit/hyperactivity disorder. *NeuroImage: Clinical* **2**, 366–376 (2013).

Genomic analysis solves the turtle mystery

One of the greatest evolutionary puzzles succumbs to molecular genetics

Figure 1: The turtle, although unique in many ways, follows the standard 'hourglass' model of vertebrate embryonic development before developing its distinct features.

The turtle has always been considered somewhat odd in evolutionary terms. In addition to lacking the hole in the skull—the temporal fenestra—that is characteristic of the egg-laying amniotes, the structure of its shell differs from that of other armored tetrapods such as the armadillo, and its shoulder blades are inside rather than outside the rib cage. An international research team led by Naoki Irie of the RIKEN Center for Developmental Biology has now shown through genomic analysis that turtles are most closely related to crocodiles and birds, and that their embryonic development follows the latest 'hourglass' model of vertebrate embryology[1].

Evolutionary relationships have traditionally been established by comparing differences in anatomical and structural features. However, with features inconsistent with the traditional lineages, the turtle has proved difficult to place, resulting in at least three different hypotheses for its evolution.

The international team, which included researchers from the Beijing Genomics Insitute and the Ensembl genome database project, instead sought to establish the evolutionary position of the turtle based on its genome, which conserves information that can be traced back to its evolutionary ancestors. To do this they sequenced and analyzed the genomes of two very different turtle species—a Chinese soft-shell turtle and the green sea turtle—and then compared their findings with other vertebrates.

The result was clear verification that the closest evolutionary relatives of turtles are crocodiles and birds, from which they diverged about 250 million years ago. In their analysis of the turtle genome, the researchers identified a large number of olfactory receptors but few taste receptors, suggesting that turtles potentially have a sophisticated sense of smell.

The research team also investigated the activity of genes during turtle embryonic development and found that shell formation, which in turtles represents a transformation of vertebrae and ribs, was accompanied by evidence of gene activity normally associated with the formation of limbs.

Despite their unique features, however, turtles appear to follow the same basic 'hourglass' model of development as many other vertebrates. Although their early and late embryonic development stages are distinctive, in between turtle embryos go through a bottleneck, or phylotypic stage, in which a body plan typical of vertebrates is laid down. This suggests that rather than diverging into specialized animals from the outset, turtles develop in the same way as basic vertebrates and only later enter a turtle-specific developmental phase.

"We do not yet know the diversity of animals which follow this hourglass model or why they ended up with it," Irie says. "These are the questions I want to tackle next."

1. Wang, Z., Pascual-Anaya, J., Zadissa, A., Li, W., Niimura, Y., Huang, Z., Li, C., White, S., Xiong, Z., Fang, D. *et al*. The draft genomes of soft-shell turtle and green sea turtle yield insights into the development and evolution of the turtle-specific body plan. *Nature Genetics* **45**, 701–706 (2013).

Automating sleep analysis

Software that analyzes sleep patterns without human input could help improve sleep research

Figure 1: Measurements of brain activity in sleeping mice aid in understanding sleep patterns. Automated analysis using the FASTER program promises to improve the speed and accuracy of sleep analysis.

Sleep research typically involves recording electroencephalography (EEG) and electromyography (EMG) signals of brain activity over long periods of time, then painstakingly analyzing these records in a process called sleep staging to determine how much time the subject spent in each stage of sleep. Genshiro Sunagawa from the Laboratory for Systems Biology at the RIKEN Center for Developmental Biology and colleagues have now developed a fully automated analysis process that promises to improve the speed and reliability of sleep staging analysis[1].

Sleep consists of several different stages, and each has a characteristic pattern of brain activity. Sleep staging is commonly performed by visual inspection of EEG and EMG recordings to identify these characteristic patterns—a process that is both slow and susceptible to variations among even well-trained observers. Both of these problems could be solved by the introduction of an automated process, but as Sunagawa explains, this is not as straightforward as it seems.

"Even though there are some rules that define sleep stages, the definitions are not clear enough to allow sleep staging to be performed by computers. In addition, if there is a lot of noise in the recordings, then we have to extract the real data—using a computer to do this is not easy," he says.

Sleep staging programs exist, but either require human input to define sleep stages with some subjectivity, or use 'hard' rules that are not always appropriate and can fail. Sunagawa and his colleagues combined a set of recently developed algorithms to develop an unsupervised, automated sleep staging program called FASTER that eliminates these problems.

To ensure that FASTER was accurate, the researchers used brain recordings from healthy mice to optimize the hard rules used by the program. They then tested it in mice with drug-induced disrupted sleep patterns, and with mice that displayed sleep defects due to genetic modification. In both cases, FASTER proved to be accurate more than 90% of the time.

"FASTER stages sleep using animal EEG and EMG, and requires no human judgment," says Sunagawa. "While it takes a few hours to analyze mouse data from one day manually, FASTER can do the same analysis in ten minutes."

For Sunagawa, the benefits of the system's speed and accuracy are clear. "By dramatically improving the sleep staging process in both quality and throughput, FASTER will help with processes such as screening for drugs and searching for sleep mutants. It will open the door to quantitative and comprehensive animal sleep research."

1. Sunagawa, G. A., Sei, H., Shimba, S., Urade, Y. & Ueda, H. R. FASTER: an unsupervised fully automated sleep staging method for mice. *Genes to Cells* **18**, 502–518 (2013).

Perusing the cellular library

A sensitive technique for taking the RNA inventory of individual cells offers researchers a powerful tool for exploring cellular biology and function

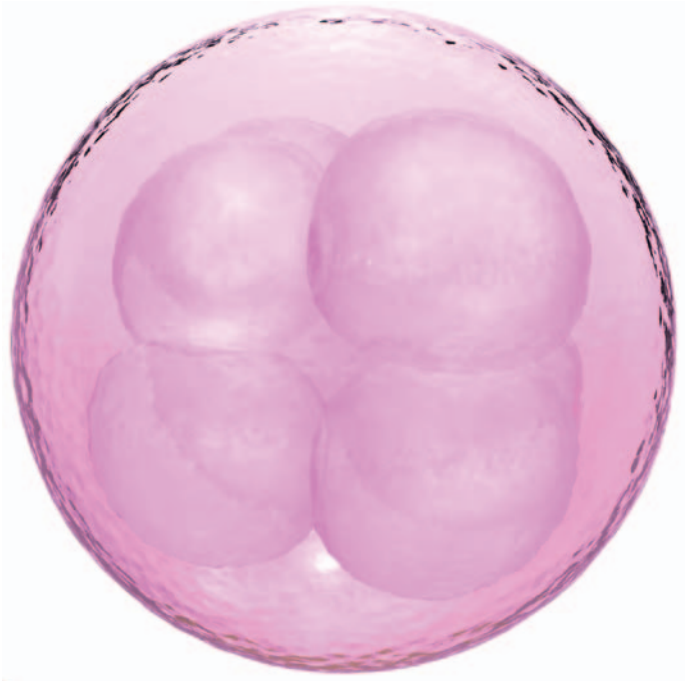


Figure 1: Sensitive single-cell RNA detection techniques could offer insight into how gene expression changes as embryonic stem cells (pictured) begin to develop into more specialized mature tissues.

Every cell is a hectic messaging center, with thousands of genes churning out messenger RNA (mRNA) transcripts for translation into functional proteins. Accordingly, sequencing the mRNA content of an individual cell can reveal critical insights into that cell's health and physiological state.

Unfortunately, as cells contain only tiny amounts of mRNA, on the order of ten trillionths of a gram, accurate quantitation is a major challenge. By introducing critical improvements to existing techniques, however, a research team led by Hiroki Ueda and Yohei Sasagawa at the RIKEN Center for Developmental Biology has now devised a robust and reproducible approach for surveying the mRNA content of individual cells[1].

Several whole-transcriptome shotgun sequencing methods are available for performing RNA analyses on large numbers of cells. These high-throughput sequencing technologies, known as RNA-Seq, can be adapted for the single-cell scale by using an enzymatic process called the polymerase chain reaction (PCR) to amplify small amounts of mRNA, but even the most efficient methods tend to be laborious and error-prone. "Multiple PCR assays are required for a single cell, and purification is needed to remove unexpected byproducts," explains Sasagawa, now based at the RIKEN Advanced Center for Computing and Communication. Such inefficiencies lead to inconsistent results, making it difficult to distinguish meaningful differences in gene expression from experimental error.

To overcome these limitations, the researchers designed a dramatically improved single-cell RNA-Seq technique with optimized reaction conditions, and introduced failsafe mechanisms to ensure that mRNA is efficiently amplified without generating confounding artifacts. Their 'Quartz-Seq' method enabled them to characterize the total mRNA content of individual embryonic stem cells (ESCs) with unprecedented reproducibility across experiments.

The Quartz-Seq method could readily distinguish between ESCs and more developmentally mature 'primitive endoderm' cells based on gene expression as with other RNA-Seq methods. However, Quartz-Seq is also able to measure clear differences in gene expression among multiple ESCs at different stages in the cell cycle. The researchers could even confidently distinguish true variations in gene activity among seemingly identical cells, demonstrating the sensitivity of the method. "Global gene expression heterogeneity may contain important biological information about cell fate, culture environment and drug response," says Sasagawa. "We showed that single-cell Quartz-Seq can detect this heterogeneity."

The Quartz-Seq method is already proving valuable for exploring genetic differences among individual cells. In the future, Ueda and Sasagawa hope to further streamline Quartz-Seq and expand their technique to detect other classes of RNA.

1. Sasagawa, Y., Nikaido, I., Hayashi, T., Danno, H., Uno, K. D., Imai, T. & Ueda, H. R. Quartz-Seq: a highly reproducible and sensitive single-cell RNA-Seq reveals non-genetic gene expression heterogeneity. *Genome Biology* **14**, R31 (2013).

A roundabout route to synthesis

In-depth analysis of an amino acid-synthesizing enzyme reveals a ring-shaped structure with numerous unusual characteristics

Every protein in every organism is composed of different combinations of the same 20 amino acids. During protein synthesis, each of these amino acids is delivered to the ribosome by a specific transfer RNA (tRNA) molecule. This list of 'ingredients', however, can be expanded to include additional 'non-canonical' amino acids. Researchers led by Shigeyuki Yokoyama of the Structural Biology Laboratory have now uncovered the sophisticated mechanism by which bacteria generate the non-canonical amino acid selenocysteine (Sec)[1].

Most amino acids are joined to their 'partner' tRNA by specialized enzymes. However, Sec lacks such an enzyme so its tRNA ($tRNA^{Sec}$) is instead linked to the amino acid serine (Ser), which is then converted to Sec by selenocysteine synthase (SelA). SelA must therefore be able to identify when Ser is linked to $tRNA^{Sec}$ rather than its normal partner tRNA, $tRNA^{Ser}$. To investigate this mechanism, Yokoyama's team performed an in-depth structural analysis of SelA from the bacterium *Aquifex aeolicus*, and collaborated with Dieter Söll's group at Yale University in the US to perform functional analysis of SelA.

The researchers' data revealed an elaborate configuration of ten identical SelA subunits, arranged into a ring of five discrete SelA pairs (Fig. 1). Each Ser-$tRNA^{Sec}$ molecule interacts with two SelA subunit pairs; one pair contributes to $tRNA^{Sec}$ recognition, while the other is responsible for catalyzing Ser-to-Sec conversion. Remarkably, each ten-subunit SelA can bind ten Ser-$tRNA^{Sec}$ molecules, indicating a complex yet efficient network of interactions.

"The arrangement of the four subunits enables each of them to do its specific task, which differs from that of the other three subunits," says Yokoyama. "This pattern of collaboration among four subunits is repeated ten times on the ring."

Eukaryotic and archaeal species also have specialized enzymes, known as 'SepSecS', that catalyze Sec synthesis, but the researchers were surprised to note that these enzymes bear virtually no resemblance to bacterial SelA. This suggests that these organisms each arrived at their own solution to a shared biochemical problem. "These two systems emerged completely independently of each other in the evolution of life," says Yokoyama. During this process, SelA acquired distinctive features that allow it to accurately differentiate between $tRNA^{Sec}$ and $tRNA^{Ser}$, and to assume the unique 'decameric' structure that sets it apart from other structurally related proteins.

"The huge structure of the SelA•$tRNA^{Sec}$ complex has provided an abundance of remarkable surprises," concludes Yokoyama. His team is now striving to obtain even more detailed structural information that might offer additional insight into how this unusual cellular machine functions.

1. Itoh, Y., Bröcker, M. J., Sekine, S., Hammond, G., Suetsugu, S., Söll, D. & Yokoyama, S. Decameric SelA•$tRNA^{Sec}$ ring structure reveals mechanism of bacterial selenocysteine formation. *Science* **340**, 75–78 (2013).

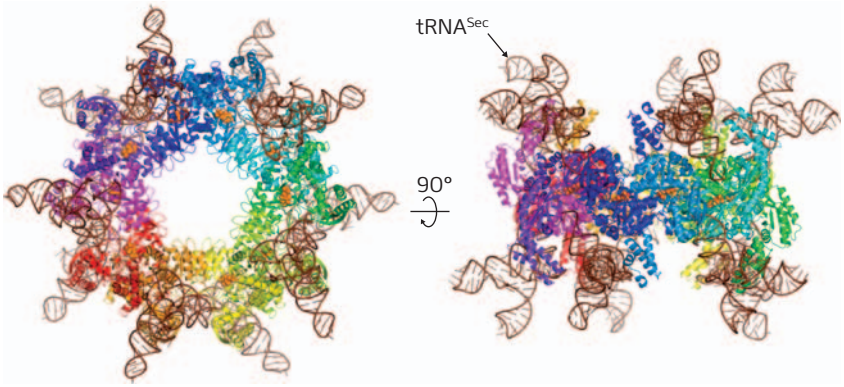


Figure 1: The SelA enzyme assembles into a complex ring-shaped structure that allows it to efficiently process up to ten Ser-$tRNA^{Sec}$ molecules at a time.

Fighting Alzheimer's disease with protein origami

The human protein prefoldin can reduce the neuronal toxicity of clumps of amyloid-β proteins that collect in the brains of Alzheimer's patients

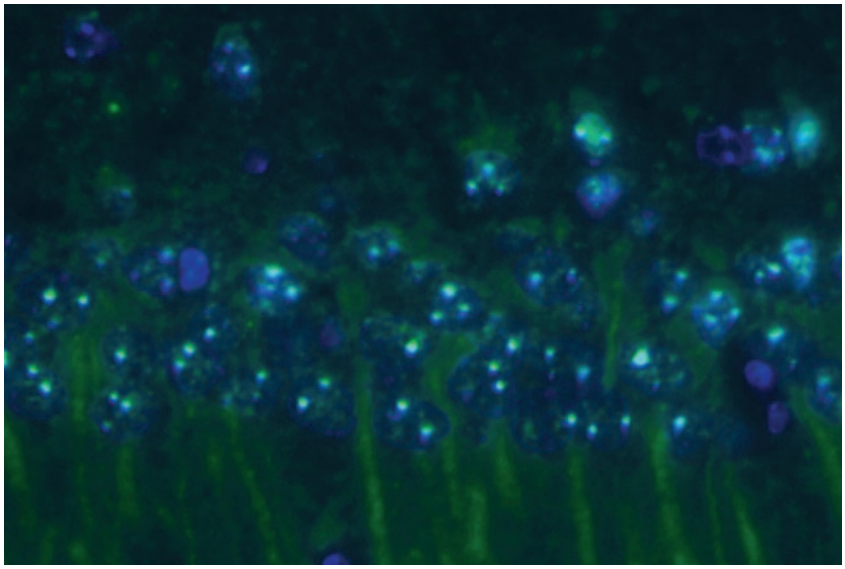


Figure 1: Prefoldin protein expression (green) in mice with high levels of amyloid-β in their brains (cell nuclei stained in blue).

Alzheimer's disease is a progressive degenerative brain disease most commonly characterized by memory deficits. Loss of memory function, in particular, is known to be caused by neuronal damage arising from the misfolding of protein fragments in the brain. Now, a group of researchers led by Mizuo Maeda of the RIKEN Bioengineering Laboratory, and including researchers from the Laboratory for Proteolytic Neuroscience at the RIKEN Brain Science Institute, has found that the human protein prefoldin can change the way these misfolded protein aggregates form and potentially reduce their toxic impact on the brains of Alzheimer's patients[1].

The formation of insoluble fibril aggregates of the protein amyloid-β has been identified as a key mechanism responsible for memory loss in Alzheimer's patients. These fibrils are toxic to neurons, and finding a means of preventing their formation represents a key strategy in the development of a therapy for the disease. Recent studies suggest methods that alter the mechanism of amyloid-β aggregates could offer a promising approach.

Prefoldin is a molecular chaperone involved in preventing the clumping of misfolded proteins and helping misfolded proteins return to their normal shape. The researchers found that amyloid-β molecules incubated with even just a small amount of human prefoldin underwent a change in aggregation behavior—they instead formed into small, soluble oligomer clumps. The observations suggest that human prefoldin interacts with amyloid-β molecules to alter their binding properties.

As in the brain, amyloid-β fibrils also kill neurons in cell culture. Using neurons from the brains of mice, the researchers showed that the amyloid-β oligomers formed in the presence of human prefoldin induced less neuron death than amyloid-β fibrils. Prefoldin expression actually increases in the brains of mice with high levels of amyloid-β (Fig. 1), suggesting that the upregulation of prefoldin expression might be a response mechanism used by the brain to protect itself from the toxic effects of amyloid-β fibrils.

Many researchers currently believe that amyloid-β oligomers are themselves a toxin that induces neuronal dysfunction. The present results, however, suggest that certain types of oligomers may in fact be less toxic than other conformations of amyloid-β aggregates. Increasing the expression of human prefoldin in the brain may therefore increase the proportion of less toxic amyloid-β aggregates, presenting a potential means of fighting the disease.

"Our findings may also apply to various other neurological diseases caused by protein misfolding, such as prion disease, Huntington's disease and Parkinson's disease," explains Tamotsu Zako from the research team.

1. Sörgjerd, K. M., Zako, T., Sakono, M., Stirling, P. C., Leroux, M. R., Saito, T., Nilsson, P., Sekimoto, M., Saido, T. C. & Maeda, M. Human prefoldin inhibits amyloid-β (Aβ) fibrillation and contributes to formation of non-toxic Aβ aggregates. *Biochemistry* **52**, 3532–3542 (2013).

RIKEN BioResource Center

Meeting researchers' needs worldwide

The BioResource Center in Tsukuba not only offers the widest selection of high-quality biological experimental materials in the world but also serves a unique role in training new experts in the field

Experimental materials such as human and plant cells, DNA and mice with tailored genetic profiles are vital for life sciences research and innovation. These materials, commonly referred to as bioresources, are used extensively and have applications in such diverse areas as cancer research, food production and environmental conservation.

Prior to 2001, there was no central facility in Japan to manage, maintain and supply bioresources. Scientists had to rely on overseas sources, resulting in an outflow of research funds and intellectual property. Furthermore, bioresources were produced in various laboratories with no common protocol. Stringent quality control was lacking, which led to poor reproducibility in results. RIKEN recognized the need for a guaranteed supply of high-quality bioresources for both academic and industrial research. In 2001, it established the BioResource Center (BRC) in Tsukuba to fill this gap.

The BRC is a centralized institute that collects, preserves, distributes and develops bioresources for researchers in Japan and around the world. Unlike other repositories that tend to specialize in one type of material, the BRC handles a variety of bioresources within five divisions: experimental animals, plants, cell lines, genetic materials and microbes. The BRC is a global distributor of cell lines, including the human induced pluripotent stem (iPS) cells established by Nobel Laureate Shinya Yamanaka.

Presently, the BRC has an annual budget of 3 billion yen and employs 400 staff members, including 87 researchers, 81 technical specialists, 80 agency workers and 109 part-time staff. The center fulfills around 16,000 bioresource requests annually, contributing to studies that are the basis of over 1,000 publications and 100 patents per year. In addition, the center offers training courses, summer schools and seminars for undergraduate and postgraduate students and early-career researchers to instruct them in the proper handling and use of bioresources. Together with the Model Animal Research Center of Nanjing University, China, the BRC co-organizes a short educational program for young scientists, which focuses on mouse genetics and related experimental techniques. The center also runs a Joint Graduate School Program with the University of Tsukuba and a similar international program with the National Yang-Ming University in Taiwan.

The BRC plans to further improve its handling of bioresource-related information and databases as well as to explore efficient ways of disseminating the materials. The center also creates novel bioresources, such as those derived from genomics research, to cater to the evolving demands of the scientific community. Guided by its founding principles of 'Trust, Sustainability and Leadership,' the center is committed to providing an even wider array of high-quality bioresources for research and development that address issues of public concern such as health, environment, energy and food. In doing so, the BRC aims to become one of the foremost centers of excellence for biological materials, improving the quality and reproducibility of research worldwide and taking RIKEN's reputation to new heights.

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Searching for transporters of plant hormones

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When a plant senses drought, abscisic acid initiates the closing of stomata—the pores present on the underside of the leaf and the surface of the stem—but the means by which abscisic acid is transported to these pores is unclear. While investigating this question, Mitsunori Seo discovered a transporter of abscisic acid using an experimental technique he developed, and his method is now attracting attention for its potential in the search for transporters of other plant hormones.

The role of abscisic acid

"When I was a child," recalls Seo, "I heard that wonderful plants could be created by genetic engineering, and I thought the power of genes was astonishing. This was the start of my research." Aiming to study genetics, he entered Tokyo Metropolitan University where, as a graduate student, he took a methodology course working with the experimental animal *Drosophila*. "The students were required to transfer *Drosophila* from one test tube to another; however, whenever I tried it, some of the *Drosophila* ran away. I thought I wasn't suited to studying animals, and so I chose plants because plants never run away," he says, laughing. His interests eventually led him to the burgeoning area of plant hormones.

When Seo established the Dormancy and Adaptation Research Unit in 2008, the goal of his work was to determine how the plant hormone abscisic acid is transported from the sites where it has been produced, the vascular bundles of the stem and leaf, to the sites where it will work, the stomata—the pores present on the underside of the leaf and the surface of the stem.

Plant hormones generically refer to chemical substances that are synthesized in the plant body and regulate growth. Abscisic acid is one of nine plant hormones discovered to date and works by closing pores on the leaf and stem to prevent transpiration when the plant body becomes dry. "A mutant plant that is unable to synthesize abscisic acid continues to transpire because its pores remain open, meaning it is likely to wilt," explains Seo; the excess transpiration will also result in a lower surface temperature (Fig. 1).

Due to the key role it plays in the response and adaptation of plants to environmental stressors such as drought, abscisic acid is studied by researchers around the world seeking to create highly drought-resistant crops. In 1996, an analysis of mutants incapable of synthesizing abscisic acid uncovered a gene encoding an enzyme essential for the hormone's biosynthesis. "When I was a graduate student at Tokyo Metropolitan University, I was conducting research to find genes related to abscisic acid biosynthesis," says Seo. "Now, nearly all of the genes encoding enzymes essential for the biosynthesis of abscisic acid have been found, and their biosynthetic processes have been clarified. New questions about abscisic acid are emerging."

Increasing evidence has been obtained indicating that abscisic acid

Figure 1: An abscisic acid-deficient mutant of *Arabidopsis thaliana*

The left panel shows *Arabidopsis thaliana* leaves cut off at the root and allowed to stand for 1 hour. Lacking abscisic acid synthase, the *aao3* mutant is more likely to wilt than the wild type. The right panel shows surface temperature observations obtained by thermography. As the mutant experiences persistent transpiration due to the inability to close its pores, its surface temperature is lower than that of the wild type.

biosynthesis enzymes are present in the cells of vascular bundles, which serve as pathways for water and nutrients. "Judging by the distribution of the biosynthesis enzymes, abscisic acid is likely to be a product of vascular bundle cells," notes Seo. "However, abscisic acid does not act there, but in the guard cells, which are a type of cell that constitutes the pores on the backs of leaves and the surfaces of stems. Hence, after production in the vascular bundle cells, abscisic acid is transported to the guard cells. But how is abscisic acid transported? I wanted to find the mechanism."

Searching for a transporter

Proteins known as transporters, located in the membranes of cells and cell organelles, are responsible for transporting plant hormones and other substances from one part of a plant to another. Seo was determined to identify a transporter of abscisic acid, something that had not previously been discovered.

After an abscisic acid biosynthesis enzyme gene was discovered in a mutated plant, it became common practice to examine mutants in search of a protein or gene with a particular function. However, mutants unable to transport abscisic acid had not been discovered. "Some researchers claimed that there was no transporter of abscisic acid because no mutants had been found. However, I hypothesized that because transportation of abscisic acid is important to the plant itself, a mutation in one abscisic acid biosynthesis gene could be compensated for by functional substitution through another gene, and that was why mutants unable to transport abscisic acid had not been found," says Seo.

"Since no one knew whether a transporter of abscisic acid actually existed, I decided to search for it. In those days, abscisic acid research was focused on discovering receptors. However, I was confident that receptors existed and would be discovered sooner or later since a great many researchers were working on this goal. It's no use doing what everyone else is doing. I encouraged myself to do things that were different."

Seo decided to take a new approach to the methodology he was using in his hunt for a transporter of abscisic acid. "If there was a sure-fire way of searching for mutants, someone would surely have tried it already. I thought that success would be unlikely without a distinct approach."

Breakthrough from the discovery of a receptor

A plant hormone cannot function unless it is bound to its receptor: the binding is essential for information to be transmitted. However, no receptor of abscisic acid had been identified and competition was fierce to discover it. In 2009, while Seo was searching for an abscisic acid transporter, there was a breakthrough in abscisic acid research: a receptor of abscisic acid was found by a group of US and European researchers.

Soon after, the signaling pathway for abscisic acid was clarified by the Gene Discovery Research Group, led by Kazuo Shinozaki, then director of the RIKEN Plant Science Center in Yokohama and currently director of the RIKEN Center for Sustainable Resource Science (Fig. 2). The receptor with abscisic acid bound to it forms a complex with protein phosphatase PP2C, inhibiting the activity of PP2C, which leads to the activation of the target factor and the induction of gene expression. As a result, various responses, including pore closure, are induced.

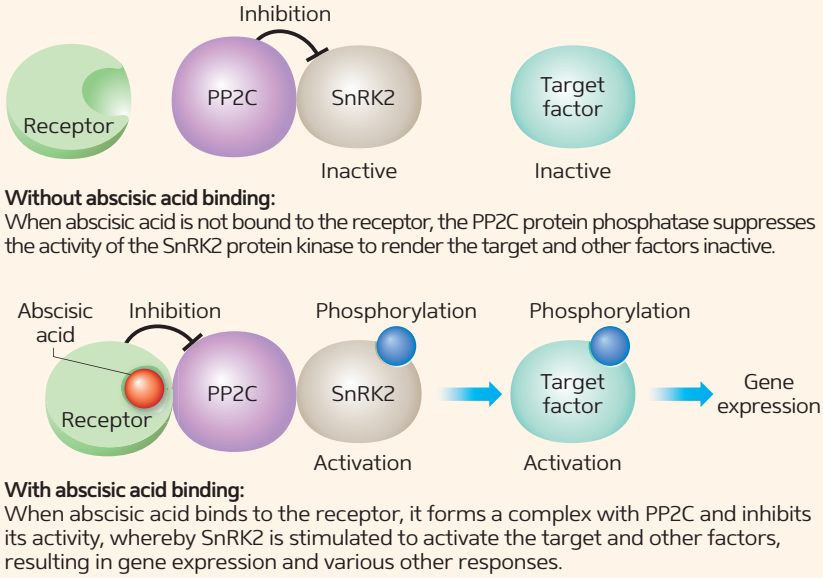


Figure 2: Abscisic acid receptor and initial signaling response

"When I read the article about the discovery of the receptor, it gave me confidence," says Seo, looking back. "Based on the formation of a complex by the abscisic acid receptor and PP2C, it would certainly be possible to discover a transporter of abscisic acid by applying the yeast two-hybrid system."

The yeast two-hybrid system is an experimental tool that has long been used to detect the interaction of two proteins (Fig. 3). A protein domain (BD) that binds to a particular site on DNA (UAS) is attached to one protein, and a transcriptional activation domain (AD) is attached to the other protein. Upon formation of a complex by the two proteins, a gene downstream of the UAS is expressed by the action of the AD. For example, a gene downstream of the UAS can be replaced with the histidine synthesis gene (*HIS*) in a yeast mutant unable to biosynthesize histidine, an amino acid essential for the growth of normal yeast. The yeast is then cultured on a histidine-free medium. If the yeast grows, the protein will be found to have bound to the complex. If the yeast does not grow, the protein will not have bound to the complex.

Finding a transporter of abscisic acid

Seo developed an experimental method for finding a transporter of abscisic acid by applying the yeast two-hybrid system. In his method, an abscisic acid receptor coupled with a domain that binds upstream of the histidine synthesis gene—forming the BD—and protein phosphatase PP2C coupled with an AD are expressed in yeast incapable of synthesizing histidine. A wide variety of genes from the model plant *Arabidopsis thaliana* are then introduced to the yeast and expressed, and the yeast is cultured on a histidine-free medium.

If the expressed *Arabidopsis* gene is not a transporter gene, abscisic acid will not be actively absorbed in the yeast and no complex will form between the receptor and PP2C. Therefore, histidine will not be synthesized and the yeast will be unable to grow on the medium (Fig. 3, left). If the expressed *Arabidopsis* gene is a transporter gene, abscisic acid will be absorbed in the yeast and bind to the receptor; the receptor and PP2C will form a complex, the histidine synthesis gene will be expressed by the action of the AD, histidine will be synthesized and thus the yeast will grow (Fig. 3, right). Hence, a transporter of abscisic acid can be found by simply confirming the growth of the yeast.

Seo and his group conducted many experiments, transferring about 20,000 *Arabidopsis* genes into yeast before finding a protein called NRT1.2. NRT1.2 serves as a transporter, allowing abscisic acid to be absorbed in plant cells. Compared to wild-type plants, mutants deprived of NRT1.2 have stem pore apertures that open about 1.4 times wider and lower surface temperatures due to continued transpiration through the more widely open pores (Fig. 4).

NRT1.2 had long been known as a transporter of nitrate ions—a source of nitrogen, an essential plant nutrient for protein biosynthesis—absorbed through roots. NRT1.2 has a low affinity for nitrate, meaning that it transports nitrate at high, but not low, concentrations. On the other hand, NRT1.2 exhibits high affinity for abscisic acid and is capable of transporting abscisic acid even when its concentration is extremely low. "I think that NRT1.2 mainly transports abscisic acid, although it also transports nitrate," says Seo.

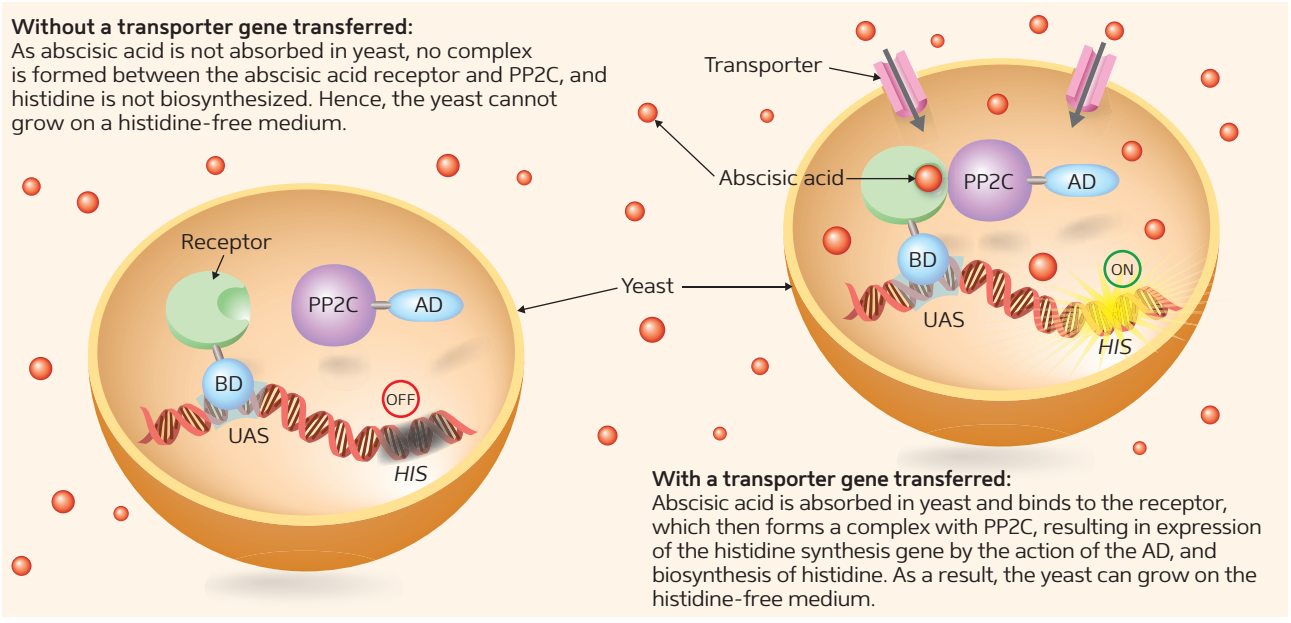


Figure 3: Discovery of the abscisic acid transporter by applying the yeast two-hybrid system

The abscisic acid transporter was discovered by applying the yeast two-hybrid system, which is widely used for detecting the interactions of two proteins. An abscisic acid receptor coupled with a domain (BD) that binds upstream of the histidine synthesis gene (*HIS*) at the UAS and the protein phosphatase PP2C coupled with a transcriptional activation domain (AD) are expressed in yeast that is unable to biosynthesize histidine, an amino acid essential for cell growth. Various *Arabidopsis thaliana* genes are introduced to and expressed in the yeast, which is then cultured in a medium supplemented with abscisic acid.

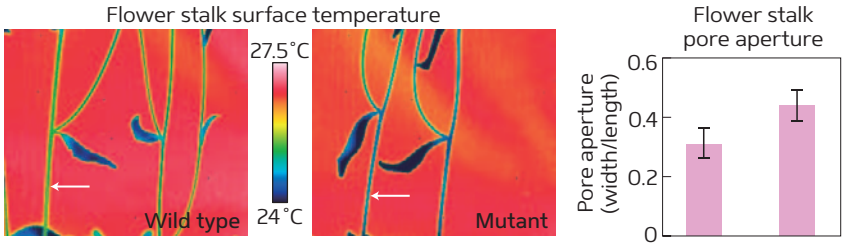


Figure 4: Temperature change and pore aperture in the wild type and NRT1.2-deficient mutant
The left panel shows photographic observations of the inflorescence stem (arrow) surface temperature obtained using an infrared camera. Because of its inability to transport abscisic acid, the NRT1.2-deficient mutant is unable to close its pores; therefore, transpiration continues, resulting in a lower surface temperature in the mutant than in the wild type. The right graphic shows pore aperture measurements on inflorescence stem surfaces. The pores of the mutant open about 1.4 times wider than those of the wild type.

Searching for all plant hormone transporters

The discovery of NRT1.2 by Seo was actually the second discovery of a transporter of abscisic acid. Some 18 months earlier, in January 2010, a group headed by Shinozaki identified the protein AtABCG25 as an abscisic acid transporter. "This protein was discovered as a result of mutant analysis and was identified as a transporter of abscisic acid from the inside to the outside of the cell, a reverse of the NRT1.2 mechanism. NRT1.2 could also have been discovered using the mutant-based approach. Although I wish I had been the first to discover the transporter, I have no regrets because I wanted to do what no one else could, and I truly achieved that."

Seo's unique approach is one of the reasons his finding received great acclaim. "While the discovery of the transporter attracted a considerable response, I was praised more for developing this experimental technique," says Seo. This is because his experimental technique can also be used to search for transporters of other plant hormones that form complexes with another protein.

His group has already begun searching for a transporter of gibberellin, another plant hormone, and is now examining candidate proteins in detail. Seo's experimental technique is attractive because it is simple, but that also means that other researchers can easily mimic it. "Therefore, I want to exhaustively search for plant hormone transporters before anyone else begins to do it," he says.

Watching the distribution of plant hormones

The discovery of abscisic acid transporter NRT1.2 uncovered another riddle. "I examined the expression of the *NRT1.2* gene. If the gene is expressed in the guard cells where abscisic acid works, the mechanism could be explained consistently. However, the gene was found to be expressed most unexpectedly in the vascular bundle cells," says Seo, with a wry smile. Abscisic acid is produced in the vascular bundle cells, whereas NRT1.2 is a transporter that allows abscisic acid to be absorbed into the cells from outside. "When I submitted my article on the discovery of the transporter to a journal, one of the reviewers denied that abscisic acid is transported into the vascular bundle cells, where it is produced. But I still thought my hypothesis was correct.

"The transportation of a plant hormone may not be as simple as exiting the cells where it is produced and entering the cells where it works. A plant hormone that has been transported out of cells may be transported back into the same cells and stored there. The behavior of plant hormones is quite complex and fascinating."

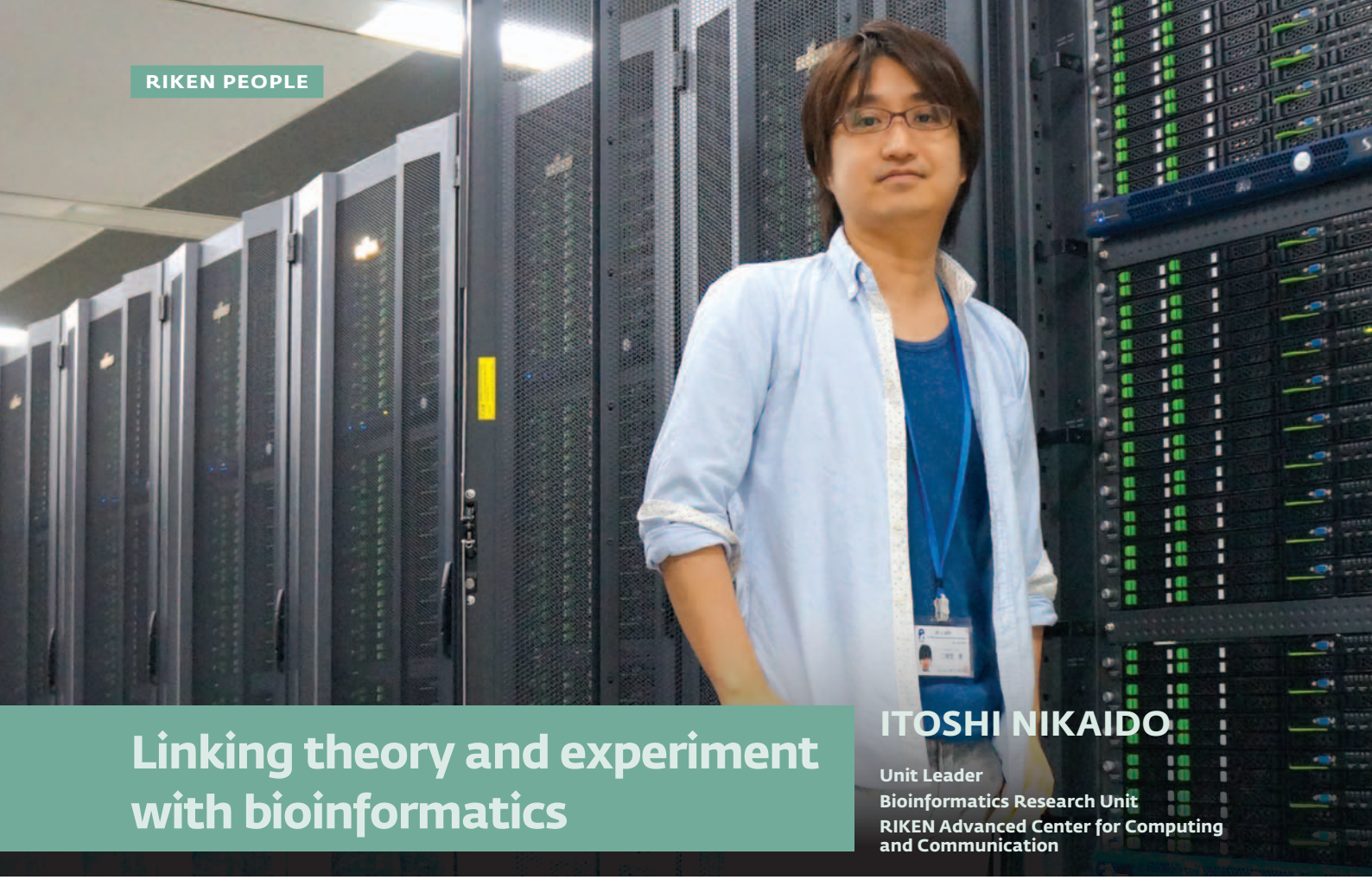
Seo is now working to determine where plant hormones are distributed in the plant body. Current approaches either estimate the distribution of a plant hormone based on the location of an enzyme involved in its biosynthesis or squash a tissue specimen and examine the mixture for plant hormone content. "In fact," says Seo, "no one knows where in the plant body a plant hormone is present."

Seo is interested in applying a single-cell analysis technique being developed by Tsutomu Masujima, team leader of the Laboratory for Single Cell Mass Spectrometry in the RIKEN Quantitative Biology Center, to answer this question. In Masujima's technique, components of a single cell are aspirated using a fine needle and analyzed both qualitatively and quantitatively using a mass spectrometer. "If the content of a plant hormone in each cell can be measured," says Seo, "it will be possible to determine its distribution in the plant body. I am very keen to do joint research with Masujima."

In the field of abscisic acid research, competition is now intense, but Seo is not discouraged. "My strong point is that I do seemingly unwise things that have never been tried in the ordinary way. I am determined to continue to do what no one else does."

ABOUT THE RESEARCHER

Mitsunori Seo was born in Gunma, Japan, in 1974. He graduated from the Department of Biological Sciences at Tokyo Metropolitan University in 1997. In 2002, he obtained his PhD from Tokyo Metropolitan University. From 1999 to 2002 he was a recipient of a Japan Society for the Promotion of Science (JSPS) Research Fellowship for Young Scientists, after which he joined RIKEN as a Special Postdoctoral Researcher. From late 2005 onwards, he served as a JSPS Postdoctoral Fellow for Research Abroad at the French National Institute for Agricultural Research (INRA). In 2008, he was appointed as a unit leader in the Dormancy and Adaptation Research Unit at the RIKEN Plant Science Center, which in 2013 was reorganized into the RIKEN Center for Sustainable Resource Science. Seo's research focuses on the biosynthesis and transport of plant hormones.



Linking theory and experiment with bioinformatics

ITOSHI NIKAIDO

Unit Leader
Bioinformatics Research Unit
RIKEN Advanced Center for Computing and Communication

How did you join RIKEN, and what kind of support did RIKEN provide?

During my master's degree, I participated in a project to decipher complementary DNA, and at the same time I studied bioinformatics by myself. Then, I met Hidemasa Bono from Yoshihide Hayashizaki's Genome Exploration Research Group, which had launched the Mouse Encyclopedia Project to sequence mouse genes. Since their project was deeply related to my analysis technology, I was invited to join FANTOM1—an international research consortium—and we wrote a paper together.

When Hayashizaki moved to the RIKEN Yokohama Institute, I embarked on a PhD at a partnering graduate school and worked on microarray data analysis as well as sequence analysis. Although I was only a student at the time, RIKEN took my research proposals seriously and allowed me to use their world-leading sequencing facility. I appreciate RIKEN's great support for my research.

Please tell us about your recent work at RIKEN.

I carried out postdoctoral research in Hiroki Ueda's lab in Kobe, engaging in analysis of next-generation sequencing data. At the same time, I worked with Yohei Sasagawa to successfully develop 'Quartz-Seq', a technique for determining the level of gene dosage per cell (see p.14).

In April 2013, I became unit leader of the Bioinformatics Research Unit at the RIKEN Advanced Center for Computing and Communication. Currently, I research and develop novel data analysis and experimental techniques for next-generation DNA sequencing and am working on a technique to support the analysis of bioinformatics data.

What else have you learned during your time at RIKEN?

The study of bioinformatics mainly revolves around developing methods and software for data analysis. However, it is important for bioinformaticians to possess strong communication skills so that they are able to carry out research projects in collaboration with experimental biologists and share problems with them. Through joint projects with many experimental biologists, I learned that bioinformaticians need to develop and submit research proposals that experimentalists are unlikely to think of.

What is the best thing about working at RIKEN?

Researchers at RIKEN are free to concentrate on their research because they have no obligation to teach, and the cost of running their labs is partially supported by their associated center. In addition, RIKEN offers excellent research support, including assistance with the planning and dissemination of research and access to external funds. Compared to those who work at other government institutions, scientists at RIKEN have more freedom to do research based on their own interests.

What would you say to other people considering joining RIKEN?

RIKEN is a fantastic research institution in terms of its facilities and work environment. The way individual labs operate varies, so it is important to carefully consider which lab you wish to work in and the kind of people you would like to work with. RIKEN can provide you with a great research environment when your goals and the atmosphere of the lab are well matched.

CONTACT INFORMATION

For details about working at RIKEN, please contact the RIKEN Global Relations and Research Coordination Office:
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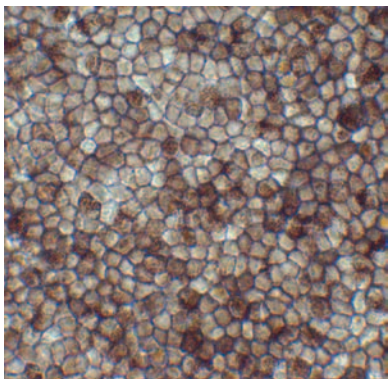
RIKEN starts pilot clinical study into iPS cell therapy for visually impaired

RIKEN recently launched a pilot study to assess the safety and feasibility of transplanting induced pluripotent stem (iPS) cells into elderly patients afflicted with a degenerative eye disease.

The study, led by Masayo Takahashi of the Laboratory for Retinal Regeneration at the RIKEN Center for Developmental Biology in Kobe, in collaboration with the Institute of Biomedical Research and Innovation (IBRI), also in Kobe, and supported by the Kobe City Medical Center General Hospital, was approved by the Japanese Ministry of Health, Labour and Welfare and opened for patient recruitment on 1 August 2013.

Age-related macular degeneration (AMD) is the most common cause of visual impairment in the elderly, affecting up to 1% of the population aged over 50 in Japan. Exudative 'wet' AMD, the focus of Takahashi's study, is characterized by progressive damage to the retinal pigment epithelium (RPE), a protective layer of non-neural cells located adjacent to the photoreceptors at the back of the eye, due to leakage caused by neovascularization.



Retinal pigment epithelium cells derived from human iPS cells.

Drug treatments currently available for this disease focus on inhibiting neovascularization but do not repair any previous damage. Earlier studies investigated the transplantation of RPE cells from various sources—including fetal tissue and unaffected parts of the RPE—but faced complications from immune rejection or involved invasive harvesting procedures.

RIKEN's clinical study plans to create autologous iPS cells from each of six research participants, which will be differentiated into RPE using a novel technology that allows transplantation of epithelial cells as one-cell thick sheets, without the need for synthetic tissue supports.

The sheets, expected to be ready in ten months, will be transplanted into the affected site of each eye after the damaged RPE and neovascular tissues are removed. The trial participants will be closely monitored for one year, followed by three years of regular observation.

To promote public awareness and understanding of iPS cell therapies, RIKEN and the IBRI are planning to launch a website describing the clinical study. ■

RIKEN and the University of Liverpool strengthen ties with joint symposium, honorary degree

RIKEN and the University of Liverpool (UoL) held their third joint symposium on 18 July 2013 at the UoL campus in the United Kingdom. The following day, Ryoji Noyori, president of RIKEN, received an honorary degree from the UoL at a graduation ceremony attended by UoL's vice-chancellor, graduating students and their families.

The University of Liverpool conferred Ryoji Noyori, president of RIKEN, with an honorary degree after the RIKEN–University of Liverpool symposium.

Ties between the two institutions date back to January 2009, when a Memorandum of Understanding was signed between the RIKEN SPring-8 Center and the UoL, covering the use of RIKEN's x-ray free electron laser for research in molecular biology and physics. In October 2010, RIKEN and the UoL signed general and strategic agreements on International Program Associates (IPAs), doctoral candidates participating in RIKEN's joint graduate school program.

President Noyori and nine researchers from various RIKEN centers participated in the joint symposium where staff from the RIKEN Global Relations and Research Coordination Office introduced RIKEN's programs for junior scientists. Researchers attending the symposium visited laboratories and held small group discussions on furthering exchange and joint research. ■

BrainTrain Conference

On 19 September 2013, the Division of Genomic Technologies at the RIKEN Center for Life Sciences in Yokohama will host a BrainTrain Neuroscience Conference organized by the BrainTrain network—a multidisciplinary neuroscience research consortium of which RIKEN is a part.

At the conference, researchers from around the world will discuss the latest developments in neuronal disorder mechanisms and diagnosis. In addition to talks on academic and industry research, a representative from the Delegation of the European Union (EU) to Japan will provide information on funding opportunities through Horizon 2020, the EU's upcoming Framework Programme for Research and Innovation.

Researchers from around the world will meet in mid-September at the BrainTrain Neuroscience Conference in Yokohama.

The BrainTrain consortium aims to establish therapeutic targets and strategies for neuronal disorders, as well as train 16 early-stage researchers. Funded by the EU's Seventh Framework Programme for Research, the consortium is composed of Japanese and European research institutes, in addition to commercial partners. ■

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