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Dividing the photosynthetic spoils

Cellular proteins assist plant cells to ensure their offspring inherit the capacity to support themselves.

A protein that plays a key role in the division of chloroplasts within nucleated or eukaryotic plant cells is derived from those involved in the separation of whole dividing cells, Japanese molecular biologists from RIKEN and the University of Tsukuba have found. They suggest the close relationship between the two allows chloroplast division to be synchronized with cell division, so that chloroplasts—the membranous organelles inside cells where photosynthesis takes place—can be allocated to both daughter cells.

**Cellular assistance**

Chloroplasts are thought to have evolved from blue-green or cyanobacteria that were engulfed by an ancient plant cell. After more than a billion years within cells, chloroplasts still multiply by division. They retain only a portion of their original genetic material, however. The rest has migrated to the nucleus of the cell, so chloroplasts cannot divide without assistance from the cell itself.

During division, protein-based ring structures form inside and outside the chloroplast's double membrane. These rings tighten and pinch the organelle into two. The structure of the inner ring is based on FtsZ protein and the outer on a member of the dynamin protein family (Fig. 1).

Earlier work by other researchers unraveled details of the evolutionary history of the FtsZ protein, which is of cyanobacterial origin. Yet little was known of the origin of the dynamin proteins involved in this process before Shin-ya Miyagishima from the RIKEN Advanced Science Institute in Wako and his colleagues published details of their work in the *Proceedings of the National Academy of Sciences*.

The role of these dynamin proteins turns out to be critical to how chloroplasts lost their independence and how they are regulated by the cell—hence, to the development and control of photosynthesis itself. And the research may also be relevant to mitochondria—the energy-production organelles of eukaryotic cells—which are thought to have a similar evolutionary history to chloroplasts.

**Determining the roles of dynamin proteins**

Dynamin proteins are widespread in eukaryotic cells, including those in plants and algae, and even in organisms which have no chloroplasts because they split off the evolutionary line before their acquisition. Each member of the dynamin family has specialized to perform fission or fusion functions in specific membranes.

Miyagishima and colleagues began by analyzing the differences in the amino acid sequences of dynamin family members to determine the evolutionary links between them. They found that the dynamins involved in chloroplast division were most closely related to those crucial to whole cell division in plants and algae, and also in amoebas and slime moulds that lack chloroplasts.

The researchers then investigated the functions of three of these closely related dynamin proteins in an organism without chloroplasts, the amoeba *Dictyostelium discoideum*. They generated non-functional mutants of each of the proteins and found these mutants led to cells with multiple nuclei (Fig. 2). This suggests the cells had duplicated their nuclei ready for division, but had never undergone cytokinesis, when the body of the cell splits into two. Knocking out each of the dynamin proteins in the amoeba had disrupted the process of cytokinesis.
Next, the researchers tracked the synthesis and localization of one of the amoeba dynamin proteins. They found the protein appeared in the cell cycle phase just before cytokinesis, and concentrated along the cleavage furrow that forms when the cells divide. The dynamin was directly involved in cytokinesis.

Again with fluorescent tagging, the team studied a pair of closely related dynamin proteins in the model organism for plant genetics, Arabidopsis thaliana. One of the proteins, DRP5B, had previously been shown to be involved in chloroplast division. Because of its similarity, the other protein, DRP5A, was also assumed to play a role in chloroplast division. An investigation of where DRP5A was synthesized in the plant, however, showed it occurred only where cells were actively dividing in the meristem tissue in the root and shoot tips. There was no necessary association with dividing chloroplasts.

Miyagishima and colleagues also grew mutant plants in which either DRP5A or DRP5B protein did not function. In the former, the size and number of chloroplasts in cells were normal, but the meristem tissues were twisted and disrupted; in the latter, the meristem tissues were normal, but there were fewer and larger chloroplasts in cells. These results, say the researchers, suggest that DRP5A is involved in whole cell division whereas the closely related DRP5B is associated with chloroplast division. Both are likely to have evolved from a common ancestor most likely involved in the cytokinesis of pre-chloroplast eukaryotic cells.

**Equal inheritance**
This close relationship between the dynamin proteins involved in chloroplast division and in whole cell cytokinesis throws up the possibility of synchronization of the two processes, according to the team. And this could lead to the establishment of permanent chloroplasts, because it provides a mechanism to ensure that where there is only one chloroplast per cell—such as in many algal species—it will divide at the same time as the whole cell, allowing one chloroplast to pass into each of the daughter cells.

Like chloroplasts, mitochondria are thought to have evolved from engulfed primitive bacteria, and like chloroplasts they divide within eukaryotic cells forming protein ring structures in the process. So, as the researchers continue to investigate chloroplast division, they now want to broaden their studies to include mitochondria.

“We want to identify more of the proteins involved in chloroplast division and its regulation,” says Miyagishima. “We are also studying how dynamin proteins are involved in mitochondrial division.”


![Figure 2: Normal amoeba (left) and multinucleate mutant amoeba (right) in which one of the dynamin proteins has been knocked out.](image-url)
A tale of two excitations

A new theory predicts an unusual excitation spectrum for a chain of ultracold gas atoms

Theoretical physicists from Argonne National Laboratory, US, and RIKEN’s Advanced Science Institute, Wako, have constructed a general theory for describing the characteristics of an unusual and newly discovered system of particles, a chain of ‘spin-1/2 bosons’.

Most particles in the Universe are either fermions or bosons. Fermions and bosons may be distinguished by a quantum mechanical property known as spin, which determines a particle’s magnetic moment. Fermions, which include electrons and protons, have a spin of 1/2. And bosons, which include photons of light and certain atoms and molecules, always have a spin of 0 or multiples of 1. However, scientists recently discovered that bosons in the form of atoms that have been cooled to a temperature of near absolute zero can behave as if they had a spin of 1/2.

“When certain ultracold bosonic atoms are held in one-dimensional trap, each can be in one of two internal states. These two states can be regarded as up and down components of an effective spin-1/2 particle. Technically, these are referred to as isospin-1/2 bosons, but theoretically there is little difference between spin and isospin,” explains RIKEN’s Akira Furusaki who, along with Konstantin Matveev from Argonne, built the theory to describe how such particles interact.

Systems of one-dimensional fermions have been studied for decades because they can be realized experimentally in solid-state systems such as quantum wires and carbon nanotubes. Consequently, their behavior is now well established and described by the so-called Tomonaga–Luttinger theory. Matveev and Furusaki’s theory now provides a framework for describing the behavior of a chain of spin-1/2 bosons.

Matveev and Furusaki’s theory begins by recognizing that the spins of a chain of bosons prefer to point in the same direction, whereas chains of fermions arrange themselves so that their spins point in alternating directions (Fig. 1). This means that a chain of spin-1/2 bosons can support both acoustic waves—formed by localized fluctuations in the density of particles along a chain—and spin waves—formed by deviations in the orientation of spins along a chain.

The spectra of acoustic waves and spin waves are markedly different, which affects the way in which a chain of ultracold spin-1/2 boson atoms absorbs different frequencies of light. The authors say this provides a relatively straightforward means to test their theory, and gain new insight into the behavior of these and other bosonic systems.

Scientists from the RIKEN Advanced Science Institute in Wako, the University of Tokyo and Sungkyunkwan University in Korea have developed a quantum theory that explains how temperature and quantum fluctuations—a direct consequence of the Heisenberg uncertainty principle—affect the properties of materials called multiferroics.

In a magnet, interactions between the 'spins' on neighboring atoms result in the spins lining up collectively; in a ferroelectric material, the crystal lattice distorts so that positive ions move away from negative ions. In a multiferroic material, electric polarization and magnetism are linked, which is particularly attractive for use in technological applications such as high-density data storage.

Quite a few multiferroic materials have been identified to date. Theory has played an important role in the search for new examples by predicting which types of crystal structures allow both ferroelectricity and magnetism. In earlier work, Hosho Katsura and Naoto Nagaosa, two of the authors of the new paper, showed that an electric polarization can occur in magnets called 'frustrated magnets', which now provides a unified view on many of the multiferroic materials.

In frustrated magnets, the spin of an atom lacks a 'preferred' direction along which to point because of competing interactions with its neighbors. Frustration can occur on a square lattice, for example, when the interaction between spins along the square edges tends to make the spins parallel, while an antiparallel interaction occurs along the diagonal (Fig. 1). As a compromise between these two interactions, the spins form a spiral arrangement—that is, the direction of the spin rotates from site to site on the lattice. This spiraling structure gives rise to ferroelectricity.

To accurately describe ferroelectric spiral magnets, which include the materials Ni$_3$V$_2$O$_8$, LiCuVO$_3$, and LiCu$_2$O$_2$, Katsura, Nagaosa and their colleagues needed to account for the effects of temperature and quantum fluctuations in an all-encompassing theory. To do this, they used what is called the 'Schwinger boson' approach, which allows them to describe each spin with both a classical and a quantum part.

Temperature and quantum fluctuations are known to disrupt magnetic order, in frustrated magnets. "Quantum fluctuations introduce new magnetic phases that are not predicted classically without magnetic anisotropy, such as a 'collinear' phase in which the spins are aligned in the same direction but have different lengths," says Katsura. As the predictions of the theory differ to those of the purely classical theory, they have important implications for interpreting experiments on particular materials, including LiCu$_2$O$_2$.

Figure 1: The source of 'frustration' in a magnet. A magnetic 'spin' (black arrow) sits on each point of the square lattice. The interaction between spins along the edge of the squares (denoted by $J_1$) tends to make the spins line up parallel to each other, but the interaction along the diagonal (denoted by $J_2$) tends to reverse the direction (red arrow). To accommodate these competing interactions, the spins form a spiral.

Nanoparticles get svelter in the heat

Scientists have discovered magnetic nanoparticles that, unlike most materials, shrink when they are heated.

Materials typically expand when they are heated, which is why the concrete slabs in sidewalks can buckle on a hot day. Now, Kenichi Kato of the RIKEN SPring-8 Center in Harima and colleagues at Saga University and Japan’s National Institute of Advanced Industrial Science and Technology have identified several materials that defy this common rule of thumb. In a paper published in Nature Nanotechnology, the team reports that nanoparticles of cupric oxide (CuO) actually shrink when they are heated.

To make the nanoparticles, Kato and colleagues started with large crystals of CuO and milled them down to particles approximately 5 nanometers in size. Using x-ray and electron diffraction (Fig. 1), they measured the average distances between the atoms that make up CuO and how these distances vary with temperature. Starting from low temperatures (-253.15 °C), they found that CuO shrinks by about 1% from its original volume when it is heated to 200 K (-73.15 °C)—an effect that is several times larger than what is found in other materials that shrink when heated. With further heating, the nanoparticles started to expand.

The reason that materials, in general, expand when they are heated is that the atoms start to vibrate around their equilibrium positions as they gain energy and move away from one another. The opposite case—negative thermal expansion—can occur if the vibrations of some atoms actually pull other atoms together. For example, if an oxygen atom that bonds two metal atoms starts to vibrate perpendicular to this bond, it will pull the two metal atoms closer together. However, Kato and his colleagues believe that the negative thermal expansion in CuO nanoparticles may be related to this material’s magnetic properties, since the crossover from normal to negative thermal expansion in the nanoparticles occurs at the same temperature that the CuO orders magnetically.

This effect is also found in nanoparticles of the magnetic material manganese (II) fluoride (MnF₂). “As the temperature is cooled and CuO orders magnetically, the magnetic Cu atoms begin to be aligned like a pair of bar magnets,” explains Xu-Guang Zheng from Saga University.

“The two bar magnets push each other so that the distance between the two magnetic atoms expands.” Conversely, as the temperature increases, the Cu atoms push against each other less, leading to negative thermal expansion. Since this is a general argument, the team expects they will be able to find other magnetic materials in nanoparticle form that will exhibit negative thermal expansion.

Selectivity at the double

A cooperative effect gives rare-earth metal complexes with two metal centers better selectivity than single metal catalysts

The discovery of new synthetic methodology is an important goal in synthetic organic chemistry. New methodology may focus on the efficient production of a particular class of small molecules, but in deciding on a target, two important factors must be taken into account. First, the target must be a useful ‘synthon’—that is, it must be easy to make into more complex molecules. Second, the new methodology needs to be able to provide a significant improvement in, for example, selectivity or yield over those already available.

Writing in the international edition of Angewandte Chemie, Zhaomin Hou and colleagues from the RIKEN Advanced Science Institute in Wako have used an yttrium catalyst to develop a new method for the synthesis of (Z)-1-aza-1,3-enynes. The methodology fulfills both objectives set out above. The products are densely functionalized—within the central four-atom structure, they contain a basic nitrogen, an imine group and a highly reactive alkyne. However, there are two possible isomers (E and Z) of the products owing to the arrangement of substituents, or stereochemistry, around the carbon-nitrogen (C=N) double bond. Previously reported synthetic methods usually yield a mixture of the two isomers.

The methodology developed by Hou and coworkers results in selective formation of only the Z isomer. The researchers first tested a series of rare-earth metal half-sandwich complexes—so called because the metal is coordinated from one side by a single planar ligand. Yttrium complexes were found to have the highest activity in this reaction. They then showed that the catalyst was effective for the cross-coupling of a wide variety of alkyl and aryl acetylenes (R’ in Fig. 1) with alkyl isocyanates (R in Fig. 1).

Further investigations showed that the active catalyst species likely contains two metal centers (Fig. 1). “We believe that the two metals work together in a cooperative way and provide products unavailable previously with a catalyst incorporating a single metal center,” says Hou. The use of a half-sandwich complex is important as it is known that the sandwich complexes tend to form monomeric active catalysts—and these produce a mixture of isomers.

The team is continuing to work on designing new catalysts based on two or more metal centers. “We hope to find catalysts for new reactions, or that can provide access to new materials not available using known catalysts,” says Hou.

Finding the sweet spot

A previously enigmatic protein has been found to play a direct role in monitoring glucose levels in the body

There is considerable evidence that the simple sugar glucose is more than just a source of sweetness and energy. "Scientists believe that glucose is not only a nutrient molecule, but also a signaling molecule that stimulates cells and tissues through receptor molecules," explains Yoshio Hirabayashi of the RIKEN Brain Science Institute in Wako.

However, although researchers have identified components from a variety of glucose-responsive pathways, it has proven difficult to identify the receptors that directly bind glucose molecules and initiate these signaling circuits.

Hirabayashi’s team was investigating an unrelated signaling pathway in the fruit fly Drosophila when they recently came across an intriguing receptor molecule known as BOSS. BOSS has been previously associated with pathways related to eye development, but very little is known about its function overall, and—tantalizingly—it contains small fragments also found in the taste receptor and a sugar transport protein.

The group decided to pursue this promising lead, and their recently published findings provide strong evidence that BOSS is a direct mediator of metabolic regulation in response to glucose levels.

BOSS is expressed primarily in the fat body, a large deposit of adipose tissue that plays an important role in sensing nutrient levels and regulating fly metabolism—in many ways, an analog of the vertebrate liver. Hirabayashi’s team also found that BOSS signaling activity was stimulated by the presence of glucose in a dose-dependent manner in cultured cells, and observed broad evidence of metabolic dysregulation in fly larvae lacking a functional boss gene. These larvae were smaller overall, and exhibited elevated levels of circulating glucose and increased lipid consumption—much like larvae with defects in insulin signaling pathways.

Together, these data suggest a direct role for BOSS in sensing and responding to glucose levels (Fig. 1). “This is the first report of a glucose-responsive receptor in multicellular organisms,” says Hirabayashi. “I believe that BOSS is one of the important factors controlling homeostasis of glucose and lipid (energy) metabolism.”

Flies offer a simple model for studying glucose metabolism, but these processes appear to also be closely conserved in higher organisms, and Hirabayashi sees these findings as a jumping point for parallel research in mammals. “In fact, mammals—including humans—have receptors homologous to BOSS,” he says. “We have knocked this gene out in mice, and I hope that these mice will tell us why the BOSS gene is so strongly conserved.”

Glycogen synthase kinase-3β (GSK-3β) is an enzyme that adds phosphate groups to the protein tau. This phosphorylated tau aggregates in the brain during Alzheimer’s disease, and seems to be involved in the memory dysfunction that characterizes this disorder.

Drugs that block GSK-3β may therefore prevent or slow the progression of Alzheimer’s disease. However, a group at the RIKEN Brain Science Institute in Wako led by Akihiko Takashima has shown that blocking GSK-3β could actually induce memory problems. These findings are reported in the journal *PLoS ONE*.

The researchers created mice that lacked one of two gene copies of GSK-3β, and exposed the mice to various memory exams. First, in the ‘Morris Water Maze’ test, the mice were trained to locate a hidden platform in a pool of water. After the first few days of training, all the mice learned the location of the hidden platform equally well. But with additional days of training, the mice lacking one copy of the GSK-3β gene seemed to forget the platform location. This may represent ‘retrograde amnesia’—or impairment to long-term memory formation.

New memories are easily forgotten, but can start to become stabilized in the brain through a process called consolidation, and become permanently fixed in the brain through reconsolidation.

To determine which of these processes is defective in mice lacking one copy of the GSK-3β gene, Takashima and colleagues examined the mice in a test known as contextual fear conditioning. The mice were initially exposed to a light shock in the foot in a novel environment. To examine consolidation, the researchers then re-exposed the mice to the novel environment a week later. To examine reconsolidation, they exposed the mice to the novel environment again both a day and a week later. For both tests, the researchers observed how well the mice seemed to remember that the novel environment was linked to the foot shock.

Based on these tests, Takashima and colleagues concluded that mice lacking one copy of the GSK-3β gene had normal consolidation but defective reconsolidation (Fig. 1). The same was true when normal mice were given a drug that blocks GSK-3β.

Takashima thinks the findings may help us understand memory dysfunction during aging. “When elderly people are confronted with a new idea, they recall related memories to help them understand this new information,” he says. “The frequent need to recall and reconsolidate memories relies on the activation of GSK-3β, which leads to tau phosphorylation and aggregation.”

Reciprocal communication

Brain cells called astrocytes secrete the S100B protein to modulate the network activity of neurons

Star-shaped brain cells known as astrocytes maintain the extracellular environment of nearby neurons. But, according to recent research, they also play an active role in neuronal activity by secreting small molecules, such as the excitatory neurotransmitter glutamate.

S100B is a calcium-binding protein found in the brain and expressed by astrocytes. In previous work, Hajime Hirase and his colleagues at the RIKEN Brain Science Institute in Wako showed that mice lacking S100B have reduced gamma oscillations, which are a type of brain wave. These oscillations represent neuronal network activity that is involved in learning and memory.

Now, as reported in a recent issue of *The Journal of Neuroscience*¹, these researchers and their collaborators at Kanazawa University have shown that S100B is also secreted by astrocytes (Fig. 1). S100B then acts on neurons via its receptor, RAGE (receptor for advanced glycation end products), to modulate gamma oscillations, which represent neuronal network activity in the brain.

Kainate, an activator of a class of glutamate receptors, induces gamma oscillations when it is injected into mice. Hirase and colleagues demonstrated that infusing S100B protein into mice lacking S100B expression could restore the gamma oscillations induced by kainate. Because the S100B protein was not taken up into the cells, it seemed to be acting extracellularly by binding to a receptor on the outside of the cells in the brain to affect neuronal network activity.

The amplitude of gamma oscillations was reduced in mice that lacked the gene for RAGE, or in mice where RAGE was blocked by a specific antibody against it. Therefore, extracellular S100B affects gamma oscillations by binding to RAGE on the surface of brain cells.

Hirase and colleagues also determined that neuronal activity plays a key role in S100B release from astrocytes, because inhibition of neuronal activity reduces its release. In addition, secretion of S100B from astrocytes could be induced by application of the neurotransmitter glutamate onto astrocytes in culture. The glutamate receptor mGluR3 (metabotropic glutamate receptor 3) is required for S100B release: administering an mGluR3 blocker onto mouse brain slices reduced secretion of S100B, and administering an mGluR3 activator induced its secretion.

S100B is a considerably larger molecule than the previously identified astrocyte signaling molecules. Therefore, it is possible that astrocytes may secrete additional proteins to modulate neuronal network activity in the brain.

As the next step in their research, Hirase says that he and his colleagues plan to determine “how neuron–glia communication is important for learning and memory”.

Figure 1: An S100B positive cell (green) labeled with an astrocyte-specific protein (red). S100B protein is secreted by astrocytes to modulate neuronal network activity.

Most Japanese people fall into one of two distinct groups genetically, biostatisticians from RIKEN’s Center for Genomic Medicine in Yokohama have shown. The finding is significant because such population structure can bias genome-wide association studies (GWASs) aimed at determining genetic links to disease, leading to spurious associations. On the basis of their work the researchers suggest ways of avoiding or correcting this bias.

GWASs are increasingly employed to reveal relationships between single nucleotide polymorphisms (SNPs) and particular disease conditions. The technique seeks out statistical differences among sets of polymorphic genetic markers between sufferers of a disease and a control group. It has already supplied useful information about the genetic basis of asthma, cancer, diabetes, heart disease and mental illness. GWASs, however, assume that the two groups being compared are drawn from the same homogeneous population—that is, there is no underlying pattern of genetic difference between them other than susceptibility to the disease condition being tested.

Although the population as a whole does not show great genetic diversity, previous work by other researchers suggested that the Japanese fall into two genetic categories, perhaps reflecting two migration events. But these studies were of limited regions of the genome. So the RIKEN researchers embarked on a much broader investigation with greater relevance to GWASs. They published their results in a recent issue of *The American Journal of Human Genetics*. Given the good quality of SNP data on the Japanese population, the researchers used statistical techniques mainly based on principal components analysis to check the homogeneity of genetic structure in a sample of 7,003 people drawn from all over Japan. They found strong evidence of the dual nature of the Japanese population. One genetic group, the Hondo cluster, includes most people from the main islands of Japan; the other, the much smaller Ryukyu cluster, includes most individuals from Okinawa and nearby islands (Fig. 1).

The research also demonstrated how such population stratification could impact the results of GWASs using Japanese subjects. As a consequence, the researchers propose measures to avoid bias, such as excluding members from the Ryukyu cluster if they occur in small proportion, equalizing Ryukyu numbers in both disease and control groups, or using statistical techniques to compensate for any potential bias.

“The aim of our analysis is to improve methods for conducting GWASs,” says first author Yumi Yamaguchi-Kabata. “We now wish to broaden the work to encompass different local regions in Asia.”

A RIKEN-led team of molecular biologists has determined the specific bonding leading to the formation of a protein complex involved in distributing pigment throughout the skin. Disruption of this membrane transport complex leads to the rare, lethal Griscelli syndrome for which there is no effective treatment. Patients show symptoms of albinism, suffer immunodeficiency, and typically die in early childhood. The work may stimulate the development of therapeutic drugs for the condition.

Members of the Rab protein family—of which there are more than 60 in humans—are thought to be essential to membrane trafficking, an important form of communication and distribution within cells. Rab proteins are typically bound to membranes either in an inactive guanine diphosphate form or an active triphosphate form which works through specific effector molecules to promote membrane trafficking.

The pigment melanin, which protects against radiation damage, is made and distributed within vesicles called melanosomes in skin color cells known as melanocytes. Rab27, which comes in two forms A and B, binds into a complex with the effector protein Slac2-a and myosin Va to transfer melanosomes onto actin filaments. The complex then transports the melanosomes along the filaments to where Rab27 uses another effector molecule to anchor them to the outer membrane of the cell.

Researchers from the RIKEN Systems and Structural Biology Center in Yokohama together with colleagues from Tohoku University were able to crystallize the Rab27B/Slac2-a complex and solve its structure using x-ray diffraction (Fig. 1). As active Rab27 proteins are notoriously difficult to crystallize, this was the first mammalian complex where the binding of such a protein with its effector molecule could be thoroughly investigated. The results were published recently in the journal Structure.

The researchers found three contact regions between Rab27B and Slac2-a, of which only one was involved in specific recognition. Mutations affecting any of the several specific intermolecular hydrogen bonds in this region were fundamentally disruptive, and some of them led to Griscelli’s syndrome. The group was able to verify the structure by engineering it to bind Slac2-a. The Rab3A amino acid sequence had to be altered by only four amino acid residues in the critical binding area to form the complex with Slac2-a.

“We are hoping that pharmaceutical companies will be able to use our structure as a basis for drugs which can be used to treat conditions like Griscelli’s syndrome,” says first author Mutsuko Kukimoto-Niino.

Decoding the rhythm of life
Genomic studies have now provided enough understanding to design clock genes

An international team of systems biologists led by researchers from the RIKEN Center for Developmental Biology in Kobe has used statistical methods to predict, seek out and finally build new DNA sequences that can regulate daily or circadian rhythms in cells. The group used data accumulated across a range of genomes to gain a greater understanding of the general biological principles of the control of circadian systems.

A network of genes ensures that the rhythms of organisms—sleep and wakefulness, changes in body temperature and blood pressure, the secretion of hormones and regulation of fertility—are attuned to daily and seasonal cycles. In humans common problems, such as jet lag and lack of alertness of shift workers, arise when the body’s circadian rhythms are not properly adjusted to the external environment (Fig. 1). Permanent disruption can lead to more serious disorders and has been implicated in bipolar disorder.

The RIKEN researchers conducted studies on the regulation of the estimated 5 to 10% of mammalian genes that show circadian rhythms. They worked in collaboration with scientists from the University of Pennsylvania, Kinki University in Osaka and INTEC Systems Institute in Tokyo and published their findings in the Proceedings of the National Academy of Sciences.

Members of the group began by constructing a database of promoters and enhancers—segments of DNA involved in regulating genes—from several mammalian genomes (http://promoter.cdb.riken.jp). They then used a statistical technique known as the Hidden Markov Model, with which they could search their database for core DNA sequences associated with three kinds of transcription factors involved in regulating activity with different daily peaks—E-box (morning), D-box (daytime) and RRE (night). This approach provided an estimate of the probability that any sequence it found was associated with such a regulator.

After picking the 10 most probable candidate genes in each category, the researchers were able to add them into seven different mouse tissues. Thirteen of the 30 regulatory candidates demonstrated strong circadian activity.

Taking the work a step further, they then used their model to design two artificial DNA sequences for each of the three kinds of regulatory genes—one predicted highly rhythmic activity, the other lower activity, but neither of which appears naturally in mouse or human genomes. When the researchers tested the activity of genes containing these synthetic sequences, their results showed that the sequences flanking the core of the regulators are significant in determining the amplitude of their impact on gene activity.

Focusing on glial cells to overcome an intractable disease, ALS

KOJI YAMANAKA

Unit Leader
Yamanaka Research Unit
Molecular Neuropathology Group
Brain Science Institute

Amyotrophic lateral sclerosis (ALS) is a devastating disease. Once ALS develops, the motor neurons that control the movement of muscles gradually start to die off, causing paralysis of the muscles in the hand and leg. The patient suffers from difficulty in using arms and legs, and in eating food and speaking. In about two to five years after the development of ALS, the muscles that control breathing are paralyzed, necessitating the support of a respirator. However, Koji Yamanaka and colleagues at the RIKEN Brain Science Institute have focused on glial cells that neighbor the motor neurons. They discovered that the glial cells cause damage to the nerve cells, thus accelerating progression of the disease. Unfortunately, no effective treatment has been found. So far, research into understanding ALS has focused mainly on motor neurons. However, Koji Yamanaka, Unit Leader, and colleagues at the Brain Science Institute have focused on cells neighboring the motor neurons, and have met with success in their discovery that the glial cells cause damage to the nerve cells, thus accelerating the progression of the disease. This discovery shows great promise in the development of new treatments to prevent the progression of ALS.

ALS, an incurable disease that exclusively destroys motor neurons

In the spring of 1939, Lou Gehrig, a Major League Baseball player for the New York Yankees in the US, was mired in a prolonged batting slump. His fans and team-mates were very surprised because he was a real slugger, who enjoyed many seasons with high batting averages; his batting record included 23 grand slam home runs, a Major League record, and a consecutive game-playing streak of 2,130. He was called 'Iron Horse,' but it was ALS that prevented him from continuing his playing streak. Lou Gehrig retired in June that year. Two years later he died young, at the age of 37 years.

In the US, ALS is known as ‘Lou Gehrig’s disease’ and is one of the neurodegenerative diseases caused by the gradual death of nerve cells. In Alzheimer’s disease, which is a well-known neurodegenerative disease, the patient develops dementia as a result of the gradual death of memory-related nerve cells. In ALS, in contrast, the patient becomes paralyzed because of the gradual death of the motor neurons in the brain and the spinal cord that control the muscles throughout the body.

There are about 6,000 patients with ALS and it is estimated that about 2,000 people may develop ALS every year in Japan. Patients with ALS develop the disease mostly at about 60 years of age, but young people can be affected, like Gehrig.

About 10% of patients with ALS develop the disease because they have inherited the causative genes, but no abnormal genes were found in the remaining 90%. “In other words, anybody can develop ALS,” says Yamanaka, who has worked as a neurologist and has treated patients with ALS.

Neurologists are the medical doctors who have been trained in the diagnosis of diseases of brain, spinal cord, and muscle, and their treatment with drugs. In fact, however, there are many other diseases that cannot be treated with drugs because the causes are unknown. “I faced a big dilemma in clinical practice,” says Yamanaka, who seriously considered what he could provide for patients with ALS. “So, I thought I would like to elucidate the cause of the neurodegenerative disease to develop new cures.”
Yamanaka trained and worked as a neurologist for four years. Then he devoted himself to basic research and started the study on ALS in 2001. Why did he select ALS as his subject of research? “I chose ALS because it is an incurable disease. ALS progresses quickly, and the symptoms of the patient worsen day by day. From the time the patient makes a clinical visit, he or she will be unable to walk within the first year, will be bed ridden within the following year, and won’t be alive within three years from the first visit. I was greatly motivated by shocking experiences when I was responsible as a neurologist for treating patients with ALS.”

Focusing on cells surrounding motor neurons

In 1993 there was a discovery that greatly contributed to the development of research into ALS. It was found that the relatives of patients with ALS had mutant-type SOD1 genes, where SOD1 is an enzyme that is capable of detoxifying active oxygen. About 2% of patients with ALS are estimated to develop the disease because of mutant-type SOD1 genes.

Model mice with mutant-type SOD1 genes that developed ALS were then generated. The model mice served to provide the first effective method for investigating the details of the process by which ALS develops and progresses. How do mutant-type SOD1 genes cause ALS to develop? Model mice from which normal SOD1 genes had been deleted were also created, but the mice did not develop ALS. This disproves the possibility that ALS develops as a result of the loss of function of SOD1 genes and hence a loss of the ability to detoxify active oxygen. On the basis of a subsequent study, researchers now think that the SOD1 proteins change shape because of the mutation of the gene, acquiring unknown toxic properties instead of functioning as enzymes, and the accumulation of such toxic proteins causes damage to motor neurons, leading to the development of ALS.

“In order to look for clues to alleviate damage to motor neurons and to slow the onset of ALS, mouse models of ALS were used in experiments. This achieved some results, but not amazing ones. Researchers began to think that they should focus on targets other than motor neurons. This was just when I started my study on ALS in 2001.”

Among cell types other than neurons, astrocytes and the abundant glial cells support neurons by providing nutritional factors. Microglial cells clear, in turn, clear damaged and dead cells. Focusing on glial cells to study ALS was challenging, according to Yamanaka. “It was well known that an increased number of glial cells are found in the spinal cord lesion of someone who has died from ALS. This used to be considered a secondary phenomenon arising from the death of motor neurons, because glial cells were not considered to be an important contribution to ALS.”

Why, then, did Yamanaka focus on the cells around motor neurons, such as glial cells? “Mutant-type SOD1 genes exist not only in motor neurons but also in all the cells in ALS model mice. Thus it is natural to think that mutant-type SOD1 genes in the cells around the motor neurons, for example in glial cells, are related in some way to ALS. The idea gave me an opportunity to start the study on ALS.”

![Figure 1: Effect of removing mutant-type SOD1 genes from astrocytes.](image-url)

In ALS model mice, when mutant-type SOD1 genes were removed exclusively from their astrocytes they developed the disease almost at the same time, but showed a slower progression and doubling in the duration of illness, in comparison with ALS mice with mutant-type SOD1 genes in all of their cells.
Unexpected research findings

Yamanaka and colleagues began to create ALS model mice from which their mutant-type SOD1 genes had been deleted certain kinds of cell groups in order to investigate how these genes function in the cells around motor neurons. In 2003 the team successfully created model mice in which mutant-type SOD1 genes have been removed exclusively from the motor neurons.

"It was technically impossible to delete mutant-type SOD1 genes from all the motor neurons, but we succeeded in deleting 30–50% of them. However, we thought that the mice would not develop ALS because the causative genes had been deleted in such large amounts. The experimental results were surprising: a delay was observed in the onset of ALS, but ALS progressed at the same speed."

Many researchers have studied on ALS over the years, in the hope of finding an effective way to slow its progression, by selecting motor neurons as their target for treatment. The experimental results, however, suggest that focusing only on motor neurons in this way achieves only a delay in the time of onset of ALS, but provides no effective method of stopping ALS from progressing. "Patients visit a clinic after they notice the onset of ALS. Thus it is no use providing medical treatment to such patients to delay the time of onset of ALS."

Glial cells are key components in the progression of ALS

Yamanaka and his colleagues also succeeded in creating model mice in which mutant-type SOD1 genes had been deleted from the microglial cells. There was no change in the onset of ALS, but its progression was definitely slowed.

Furthermore, the experiment with the model mice in which mutant-type SOD1 genes had been deleted from the astrocytes also showed a slower progression of the disease, with a doubling of the duration of the illness (Fig. 1).

This discovery by Yamanaka and his unit members suggests that motor neurons play the key role in the development of ALS, but two other players promote the progression of ALS. In other words, microglial cells and astrocytes are closely related to the progression of ALS. "It is now considered that astrocytes activate the microglial cells, and the activated microglial cells, in turn, release toxic substances such as proteins that produce nitric oxide or inflammation, thus causing further damage to motor neurons and accelerating the progression of ALS," explains Yamanaka (Fig. 2 and Fig. 3).

Yamanaka and his colleagues demonstrated, for the first time ever, the importance of microglial cells and astrocytes as a target of treatment for stopping ALS from progressing. However, they used ALS model mice into which mutant-type SOD1 genes had been introduced, but only 2% of patients with ALS develop the disease as a result of mutations in SOD1 genes. Their final goal is to develop a new cure that will be effective for almost all hereditary ALS types as well as non-hereditary types. "Pathological changes are observed in astrocyte or microglial cells in patients with ALS who have developed non-hereditary disease. In these patients with ALS, it is considered that glial cells have a toxic effect on the motor neurons and stimulate the progression of the disease. To confirm this, we are planning to create model mice that develop ALS resulting from causes other than mutant-type SOD1 genes."

Working to overcome ALS

In February 2008, Yamanaka released his findings to the press, saying that two types of glial cell are effective as targets of treatment for ALS, and received a great response. He received emotional requests from patients and their relatives asking for the earliest development of effective cures. For example, he received an autographed letter from a patient suffering from paralysis in his hands as a result of ALS. Some patients said that they would be willing to serve as a subject for experiments.

Now that we understand that the next targets are astrocyte or microglial cells, the next step will be to determine the molecular mechanisms occurring in the abnormal astrocyte or microglial cells that are producing the progression of ALS, and to identify the target molecules for treatment.

Yamanaka asserts, "Besides SOD1 genes, there should be a definite difference between ALS model mice and normal mice, for example, in terms of the types and amounts of genes or molecules that act in the astrocyte and microglial cells, or the places in which these cells are functioning. By investigating these factors, we should be able to elucidate the mechanism that promotes the progression of ALS and identify the target molecules for treatment."
Once these target molecules are known, their functions can be controlled so that glial cells will return to their normal state, which will enable the development of cures that can slow the progression of ALS.

The study by Yamanaka has raised expectations for regenerative treatment for ALS. It has been considered that if the treatment of ALS is targeted only to motor neurons, regenerative methods in which cells are transplanted to restore function cannot be effective because of the low rate at which transplanted cells form a correct network system and because of the low rate of extension of axons that transmit instructions from motor neurons to muscles.

“An axon extends its tip only by 1 mm a day. Some axons have a length of 1 m, which means that up to 1,000 days (about three years) are necessary for an axon to reach its final length. This is too long: by that time ALS will have progressed to its terminal stage.”

If glial cells are closely related to the progression of ALS, as Yamanaka has indicated, glial cells, if successfully transplanted, should have a therapeutic effect. “Glial cells can immediately start to function at the place where they are transplanted, and stop ALS from progressing.”

Contributing to a new perspective on Alzheimer’s disease

Alzheimer’s and Parkinson’s diseases are also classified as neurodegenerative diseases that gradually destroy nerve cells. “Most current studies on neurodegenerative diseases other than ALS also focused exclusively on nerve cells. Our research findings on ALS have shown that toxic substances released from abnormal glial cells can destroy nerve cells, and this fact is significantly affecting research trends in other neurodegenerative diseases. A therapeutic agent that makes glial cells return to normal will possibly be very effective in treating not only ALS but also other neurodegenerative diseases such as Alzheimer’s or Parkinson’s.”

When can a therapeutic agent against ALS be developed? “First of all, our mission is to elucidate the detailed mechanism of ALS. Then we will proceed with a study of ALS so that a therapeutic agent against ALS can be verified in a clinical test in ten years.”

Yamanaka and members of his unit are making a steady progress on their basic studies towards overcoming ALS, based on compelling requests from patients with the disease.

About the researcher

Koji Yamanaka was born in Tsu, Japan, in 1967. He graduated from the Department of Medicine, Kyoto University, obtained his MD in 1992, and completed his PhD in 2000 from the same university. After five years of postdoctoral training at the Department of Medicine and Neuroscience, University of California at San Diego, he was appointed as a unit leader at RIKEN Brain Science Institute, where he started his new career as a principal investigator in 2006. His research focuses on the molecular pathogenesis of neurodegenerative diseases, and especially amyotrophic lateral sclerosis, using in vitro, cell and animal models.
Prime Minister Aso visits RIKEN facility in South Korea

Prime Minister Taro Aso visited South Korea on January 12, visited RIKEN’s Flucto-Order Functions Asian Collaborative Research Team of the Advanced Science Institute (ASI), together with Byong Man Ahn, South Korea’s Minister of Education, Science and Technology and other dignitaries. This Collaborative Research Team is the third overseas base for RIKEN, and was set up inside the new Fusion Technology Center at Hanyang University in Seoul.

The team is conducting research on new information processing devices, such as those exploiting the phenomenon of self-organizing behavior. The research will be useful in developing electronic devices that utilize molecular fluctuation and instability. It is also expected to produce new functional materials and information-processing technologies.

The focus of the prime minister’s visit was on cooperative activities in Asia. Prime Minister Aso and RIKEN President Ryoji Noyori heard about the activities of the Fusion Technology Center from Chong Yang Kim, President of Hanyang University. In addition, Haiwong Lee, a professor at Hanyang University, and Masahiko Hara, the director of the Advanced Science Institute’s Global Collaborative Research Group, took the visitors around the laboratory and explained the research activities taking place there.

International approach to balancing gender participation in research

The US-Japan Roundtable Discussion on Equal Participation in Science and Engineering was held from February 16 to 18 at Hokkaido University and was joined by RIKEN. The conference was jointly sponsored by the US National Science Foundation and the Support Office for Female Researchers in Hokkaido University.

At the Discussion, six American and seven Japanese panelists with experience in promoting gender equality discussed the issues and problem areas related to gender-equal participation, and gave ideas on how the two countries can cooperate to address these issues.

After presentations were made on equal participation promotion programs in Japan and the US, workshops were held on career development, work-life balance, and leadership training.

RIKEN has a gender equality program supporting the various needs of all employees. Not only does it actively support its female researchers, RIKEN is engaged in creating work environments that allow both men and women to realize their full potential. For example, RIKEN subsidizes a research support person for pregnant research staff or staff responsible for the care of small children, and work attendance exemptions are available for childcare.

RIKEN BRC International Symposium examines microbial resources

An international symposium organized by the RIKEN BioResource Center (BRC) drew over 230 microbiologists and other interested parties to Tokyo in early February to discuss the issue of microbial resources in Asia and the wider world.

The symposium included three main sessions: ‘Microbial Resources and Their Possibilities’, ‘Microbial Resources in Asia and the role of BRC’ and a lecture by Yoshihito Benno, former head of the Microbe Division / Japan Collection of Microorganisms (JCM), RIKEN BRC. In the first of these, five speakers introduced the latest results of cutting-edge research in basic and ecological microbiology capable of delivering benefits to the environment, such as solving the problem of marine oil pollution, and probiotic lactic acid bacteria related to human health.

Following this in the second section, researchers from the China General Microbial Culture Collection Center, the Microbial Genomics and Applications Center in South Korea and the Thailand Institute of Scientific and Technological Research reported on microbial resources in their respective countries. The Chinese delegation introduced work being carried out on an information network among the culture collection of Asian countries, while the topic of isolation of microbes from the environment and their meta genomic analysis formed the basis of the contribution from South Korea. Finally, the Thai contingent introduced some aspects of their recent activities in microbial research and corresponding applications.

A common thread linking all the presentations was the vital role played by the JCM in Asia, and the importance of the Asian Network on the Microbial Resource (ANMR) project, in which RIKEN was the core institution in fiscal 1995–99, in forming stronger relationships among Asian countries.

In the last section, Yoshihito Benno, head of the Microbe Division / JCM until his retirement at the end of March 2009, gave a lecture on his long career as a scientist and administrator, including his 36 years of research on the function of intestinal bacteria, during which time he made huge contributions to microbial taxonomy using both culture-based methods and molecular techniques. Benno also looked back on developments in the organization and capabilities of the RIKEN BRC, including the successful and smooth integration of the JCM into the BRC 2004, the selection of the JCM as a core ‘General Microbes’ facility of the National BioResource Project by the Ministry of Education, Culture, Sports, Science and Technology, and the development of an ISO 9001-quality management system. In addition to reflecting on the past, Benno also spoke of the future, talking about the possibility of developing a new health check system using culture-independent analysis of human gut microbiota.
Dear Dr. Okanoya,

It’s been a year since I said good-bye after working in your lab as a summer intern. Your kindness and the incredible people you have gathered in your laboratory made my stay unforgettable. I could go on about how much I liked the diversity of fields and ideas, and the open-mindedness with which you approach the question of language evolution, a fascinating subject. This is all true, but if I can say this, I loved the atmosphere even more. It was casual and friendly, and I loved the laughter so often exploding from the middle room.

I had no idea what to expect from you, your lab and Japan when I applied for the internship. I had heard many conflicting things about your country and the Japanese people. When I got there, though, I met some of the most gentle, caring and intelligent people I’d ever encountered. This was my initial impression and it only became stronger as I got more familiar and comfortable there. Everybody was welcoming and kind.

You made sure I was well acquainted with the scientific work at your lab, and I had many interesting discussions with people from different disciplines, including animal behavior, electrophysiology, microbiology and neural network modeling. I learned a lot during my short stay in your lab, and a long-lasting collaboration was born between our labs. While there, I worked with a couple of people closely and often consulted with others. I loved the supportive environment and the frequent communication between the lab members. Many of us ate lunch together and we arranged weekly English lessons (I have an English teaching background), which was fun and brought us even closer. I can’t express how much this meant and still means to me.

I went to Japan with my husband and small daughter, and my whole family was so kindly accepted into the little community of your lab. Thank you! I think coming to RIKEN with a family is even better than coming alone. There is an incredible day care center on campus staffed with the warmest and most devoted teachers who arrange fun activities for the children and love them as if they were their own. When I think of that place, my heart fills with happiness.

Ever since I returned to the United States, I’ve been thinking about Japan and wishing to go back. I began taking Japanese lessons at the local Japanese Cultural Institute, Tenri, and I am preparing to apply to RIKEN’s foreign post-doc program. Hopefully, one day I can return and work with you again.

Until then, please accept my warmest regards,

Olga Feher
Laboratory of Animal Behavior
City College of New York
New York, USA
RIKEN, Japan’s flagship research institute, conducts basic and applied experimental research in a wide range of science and technology fields including physics, chemistry, medical science, biology and engineering. Initially established as a private research foundation in Tokyo in 1917, RIKEN became an independent administrative institution in 2003.

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For further information on the research presented in this publication or to arrange an interview with a researcher, please contact
RIKEN Global Relations Office
2-1, Hirosawa, Wako, Saitama, 351-0198, Japan
TEL: +81 48 467 9443
FAX: +81 48 462 4715
E-Mail: rikenresearch@riken.jp

www.rikenresearch.riken.jp