RIKEN RESEARCH

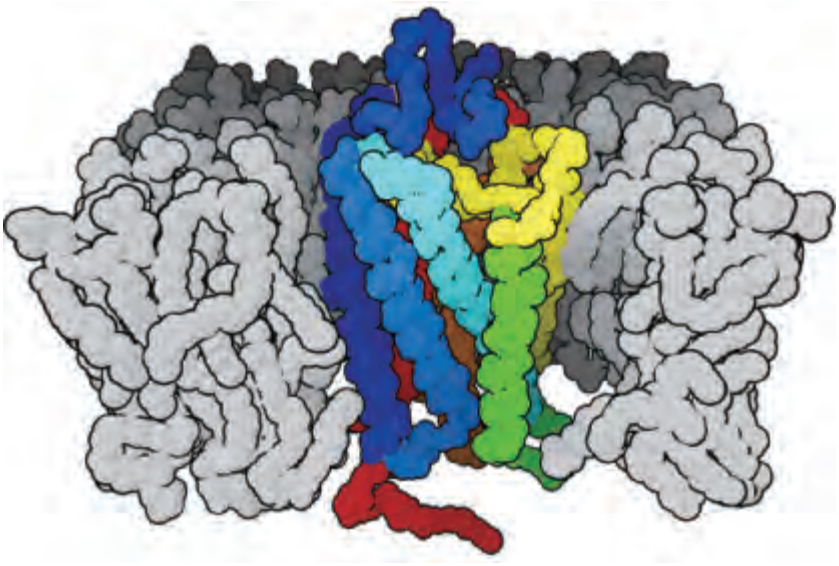
NOVEMBER
2009 Volume 4 Number 11

Interfacial relations

www.rikenresearch.riken.jp

Getting membrane proteins into the fold

A new preparation method promises to bring a challenging but clinically important subset of proteins within easier reach of scientists



en.wikipedia.org

Figure 1: Schematic of a membrane-bound protein, which relies on the hydrophobic environment within the cell membrane. These proteins are notoriously difficult to overexpress and purify in large quantities.

Membrane-embedded proteins execute many of the most important cellular functions, detecting external environmental cues, mediating communication and signaling, and facilitating molecular import and export. Accordingly, they also represent important targets for drug development, and an estimated 60% of currently available drugs are believed to target membrane proteins.

On the other hand, experimental investigation of the structure and function of individual membrane proteins is routinely thwarted by the general difficulty of preparing large quantities of properly folded protein. This is largely due to the chemical composition of these proteins, which have large hydrophobic surfaces that are chemically averse to being exposed to water; these protein segments are stable when embedded within the equally hydrophobic lipid-based cell membrane (Fig. 1), but tend to assemble into irregular, improperly folded clumps when prepared in solution.

Most water-soluble proteins can readily be produced either within modified cell lines or through the use of cellular extracts that contain the full complement of machinery required for protein synthesis. However, the yield of membrane proteins obtained from cultured cells is generally inadequate, and scientists have had to tinker extensively with extract-based production methods to obtain usable quantities of functional protein.

Now, a research team led by Shigeyuki Yokoyama at the RIKEN Systems and Structural Biology Center in Yokoyama has developed an efficient preparation method that produces high yields of functional membrane proteins[1]. "Up until now, aggregation and misfolding of membrane proteins during cell-free protein synthesis have been avoided by adding either lipid-based liposomes or detergents," explains Yokoyama. However, he adds that such workarounds were not a complete solution, and challenges remained in selecting the appropriate detergent for a given protein, or ensuring that newly synthesized proteins were properly folded and inserted into the membranes of liposomes.

Best of both worlds

Yokoyama and colleagues solved this problem by developing a hybrid technique that incorporates elements of both preparation methods, synthesizing their proteins in a bacteria-derived extract containing both detergent and lipid molecules. During production, hydrophobic stretches of the newly formed protein are protected by this lipid-detergent mixture; once synthesis is complete, the detergent is removed from the sample via dialysis and the remaining lipids subsequently assemble into bubble-shaped liposomes with the mature proteins securely embedded within these artificial membranes (Fig. 2).

The researchers then demonstrated the efficacy of their technique with bacteriorhodopsin (BR), an archaea-derived photosynthetic pigment protein. The light-responsive properties of BR made it a particularly useful test subject for this method—improperly folded or aggregated BR appears yellow, while preparations of mature BR appear purple. In initial experiments, they were able to demonstrate successful production of properly folded BR with a variety of detergents, and although the overall folding efficiency was somewhat lower than previously described preparations, the overall quantity of protein produced was significantly greater—as much as 80-fold greater, depending on the detergent. Subsequent analysis confirmed that much of this properly folded BR was successfully integrated into the

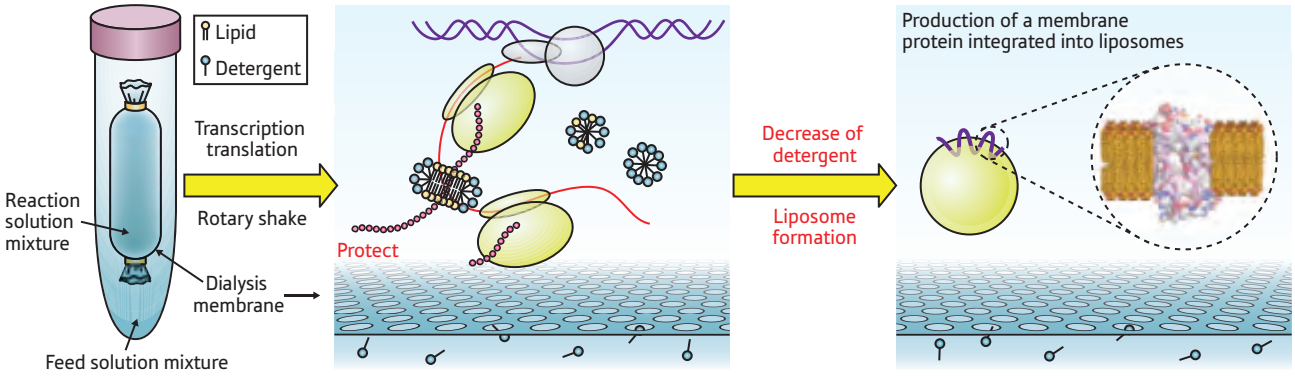


Figure 2: An overview of the new protein production process. Protein is produced in a tube via a cell-free transcription and translation reaction (left). This reaction is performed in the presence of lipids and detergent, which protect hydrophobic protein segments and help initial folding along (middle). When detergent is removed by dialysis, the properly folded proteins end up embedded within liposomes, replicating their normal integration into cell membranes (right).

membranes of liposomes following detergent removal.

Yokoyama and his colleagues' protein preparations also retained proper functional characteristics. Naturally occurring BR undergoes a series of chemical transitions known as a photocycle, in which excitation by light at particular wavelengths induces a series of subtle structural changes; these shifts can in turn be quantified by changes in the light-absorption properties of the protein. They found that their synthetic BR mirrored the photocyclic characteristics of native protein, and even displayed the capacity to act as a light-activated proton pump—a key component of its photosynthetic activity.

Making a big production of it

Whether researchers are looking to derive protein in crystalline form for structural analysis or in solution for functional characterization, the ability to obtain high-purity preparations is essential. In this regard, the Yokoyama team's technique passed with flying colors, with a 75-fold greater yield of functional BR protein relative to a previously described high-yield liposome-based method.

Yokoyama indicates that since the initial publication of this work, his group has had the opportunity to test their method on a variety of membrane-bound receptors and channels, and they are currently in the process of characterizing the efficiency of these preparations. Fortunately, the system is sufficiently flexible that a number of adaptations can be introduced to optimize production for any given protein. "We will test other lipids or steroid detergents for the functional overproduction of other types of membrane proteins," he says. "Moreover, by controlling lipid bilayer formation and protein synthesis speed through changes in reaction conditions such as temperature, we can improve this system to be suitable for many types of membrane proteins."

Even as Yokoyama and his colleagues work out the details of refining their overexpression system, they have already begun to contemplate future targets for which this method might prove valuable—and with so many poorly characterized membrane proteins, the field is wide open. "We hope to apply this system to biochemical, biophysical and structural studies of human G-protein coupled receptors, which are important targets for drug design, and proteins that transport small molecules such as nutrients, across the membrane," he says, "as well as complexes incorporating membrane proteins, because the cell-free system makes it easy to co-express more than one protein." ■

1. Shimono, K., Goto, M., Kikukawa, T., Miyauchi, S., Shirouzu, M., Kamo, N. & Yokoyama, S. Production of functional bacteriorhodopsin by an *Escherichia coli* cell-free protein synthesis system supplemented with steroid detergent and lipid. *Protein Science* **18**, 2160–2171 (2009).

About the researcher

Shigeyuki Yokoyama was born in Tokyo, Japan, in 1953. He received his BS and PhD degrees from the University of Tokyo in 1975 and 1981, and following completion of five years of postdoctoral work, became an associate professor in 1986 and a professor in 1991 in the Department of Biophysics and Biochemistry, University of Tokyo. In 1993, he was appointed chief scientist of the RIKEN Cellular Signaling Laboratory, and later project director of the Protein Research Group in the Genomic Sciences Center. He played a pivotal role as science director of the RIKEN Structural Genomics / Proteomics Initiative (RSGI) and director of the Highthroughput Factory supporting the Protein 3000 project. Since 2008, he has acted as director of the Systems and Structural Biology Center (SSBC) at the RIKEN Yokohama Institute.

Pores for thought

Porous coordination polymers that strongly adsorb polar guest molecules can be made using a ligand with separated positive and negative charges

A porous coordination polymer (PCP) that strongly adsorbs methanol, a model guest molecule, has been prepared by Masakazu Higuchi from the RIKEN SPring-8 Center in Harima and co-workers from the University of Kyoto, the Japan Synchrotron Radiation Research Institute and Osaka Prefecture University[1].

The new material is important because porous materials that can adsorb guest molecules offer opportunities in finding ways to store hydrogen fuel, and to sequester waste gas such as carbon dioxide, which can reduce the impact of burning fossil fuels. Porous coordination polymers (PCPs) provide a particularly attractive option in both endeavors because they contain micropores and their surfaces can be designed to have specific properties.

Also known as metal–organic frameworks (MOFs), PCPs are formed between metal ions—often from transition metals such as zinc—and well-defined organic ligands that can bond to more than one metal atom. With sufficiently rigid ligands, such that a single ligand cannot just coordinate to a single metal ion, it is possible to produce a continuous network of metal ions held together by the ligands. It is within the pores of these PCPs that guest molecules such as gases can be accommodated.

For guest molecules to be adsorbed efficiently they must interact with the pore walls. "We thought that electrostatically charged walls would be beneficial, but this introduced a new problem," explains Higuchi, "the overall structure must be electrically neutral and the counter-ions required to achieve this occupy the pores of the PCP meaning that they are blocked to guest molecules."

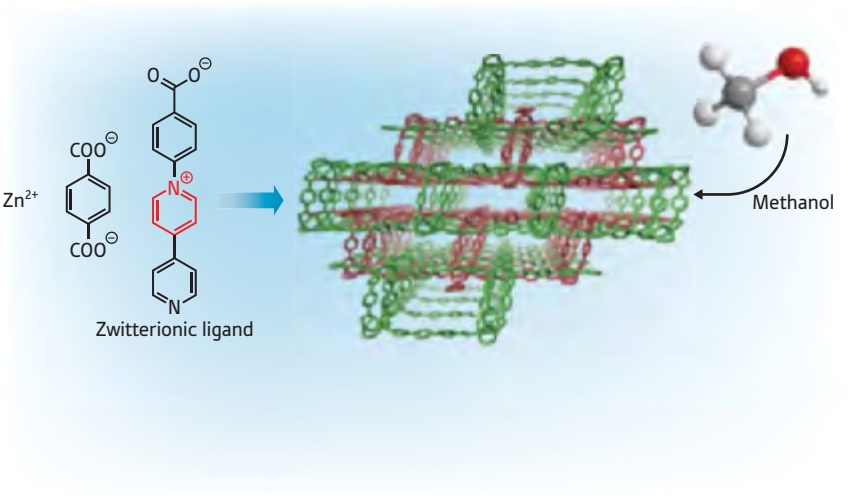


Figure 1: Schematic showing the continuous porous structure (center), which can adsorb methanol, formed by reaction of zinc ions (Zn^{2+}) with terephthalic acid and 1-(4-carboxyphenyl) – 4,4'-bipyridinium ligands (left).

Higuchi and colleagues' PCP is based on the coordination of zinc ions with a zwitterionic ligand, which is electrically neutral, but carries separated positive and negative charges (Fig. 1). They showed that guest molecules of methanol adsorb more strongly than a similar PCP made with uncharged ligands. It can also adsorb more guest molecules because the pores are not blocked by counter-ions.

The zwitterionic ligand used in the new material described by Higuchi and his colleagues means that the pore walls are highly charged but additional counter-ions are not required. They have also shown that the material adsorbs methanol more strongly than a similar PCP with uncharged pore walls. "In the future, we plan to investigate how other guest molecules interact with the charged pore surface" says Higuchi. "Ultimately, we hope to see this develop into a material that can be made on an industrial scale."

1. Higuchi, M., Tanaka, D., Horike, S., Sakamoto, H., Nakamura, K.,Takashima, Y., Hijikata, Y., Yanai, N., Kim, J., Kato, K. *et al.* Porous coordination polymer with pyridinium cationic surface, [Zn_2(tpa)$_2$(cpb)]. *Journal of the American Chemical Society* **131,** 10336–10337 (2009).

Interfaces: Different for every molecule

A novel spectroscopic technique reveals a new fundamental property of air/water interfaces

Figure 1: Owing to copious interfaces between water droplets and air, the dependence of polarity at interfaces on the solute molecule could have important consequences for atmospheric science.

Contrary to expectations, structurally different molecules can display different solvent properties at an interface between air and water, researchers in Japan have discovered[1]. Tahei Tahara and colleagues from the RIKEN Advanced Science Institute in Wako showed that polarity at this interface cannot be defined simply, because it depends on the nature of the solute molecule at the interface. The finding could have significant consequences for chemistry at interfaces, since the polarity of a molecule's environment affects how it reacts with other molecules. Fields such as atmospheric science, where air/water interfaces abound, will be particularly affected (Fig. 1).

The researchers made their discovery using an interface-selective spectroscopic technique that they developed earlier[2]. The spectra that the technique produces are of comparable quality to those of bulk solutions, enabling previously impossible comparisons between systems.

The researchers looked at the electronic spectra of five coumarin dyes at the interface between air and water; electronic spectra are essentially a graphical representation of a molecule's color. Coumarin dyes all share the same basic chemical structure and are used to probe the polarity of solvents because their spectra differ depending on the molecules' environment.

Tahara and colleagues found that the spectra of all five coumarin dyes at the air/water interface resembled a cross between the bulk spectra of coumarin in polar water and non-polar hexane. This is because the dye molecules were positioned partly in the polar water and partly in the non-polar air at the interface. However, the closeness of the spectra to either the spectrum in water or in hexane changed depending on the precise structure of each coumarin dye.

Previously it was thought that, in ordinary cases, molecules experience the same polarity—the average of that of polar water and non-polar air. The spectra Tahara and colleagues measured, however, showed that even molecules having similar structures experience substantially different polarity at the air/water interface.

The researchers found that the different molecules were positioned at slightly different angles at the interface of air and water so have different sections of their structures submerged and are, consequently, in quantitatively different surroundings.

"This work showed that, even at the same air/water interface, the interaction between the solute and solvent is significantly varied," says Tahara. This means the molecules experience different environments at the interface, similar to being in different solvents from a view point of the stabilization energy. "This fundamental understanding of molecular behavior will be very important when people consider chemical reactivity at liquid interfaces." ■

1. Sen, S., Yamaguchi, S. & Tahara, T. Different molecules experience different polarities at the air/water interface. *Angewandte Chemie International Edition* **48,** 6439–6442 (2009).
2. Yamaguchi, S. & Tahara, T. Precise electronic $\chi^{(2)}$ spectra of molecules adsorbed at an interface measured by multiplex sum frequency generation. *Journal of Physical Chemistry B* **108,** 19079–19082 (2004).

Cellular secrets exposed in living color

'On–off' fluorescent probes allow multicolor detection of nucleic acid strands within living cells

One of the best ways to watch the complex workings of living cells is to label components, such as DNA segments, with fluorescent probes—small molecules with active optical properties. Then, using a fluorescent microscope, movement of the labeled DNA can be tracked through the entire lifespan of a cell without disruption.

Unfortunately, conventional probes are always 'on'—emitting fluorescent light—regardless of whether the target nucleic acid is present or not. Now, Akimitsu Okamoto and colleagues from the RIKEN Advanced Science Institute in Wako have designed new fluorescent probes that turn on only when a specific nucleic acid strand is recognized[1]. Because these probes can be labeled with different fluorescent colors, it is now possible to image multiple processes in a cell simultaneously.

Okamoto's probes comprise a pair of identical fluorescent dye molecules, linked together by a Y-shaped organic chain to a DNA strand. When the probe is not attached to an RNA target, it is in the 'off' state and emits no fluorescent light. This is because the two dye molecules stack parallel to one another such that they can access each other's electronic states, suppressing the fluorescence through what is known as an excitonic interaction.

When the probe encounters a complementary RNA strand, however, it undergoes hybridization and forms a double helix. In this configuration, the two dye molecules on the probe become separated and stack between groups in the double helix. Immediately, fluorescence is restored and the probe turns 'on'.

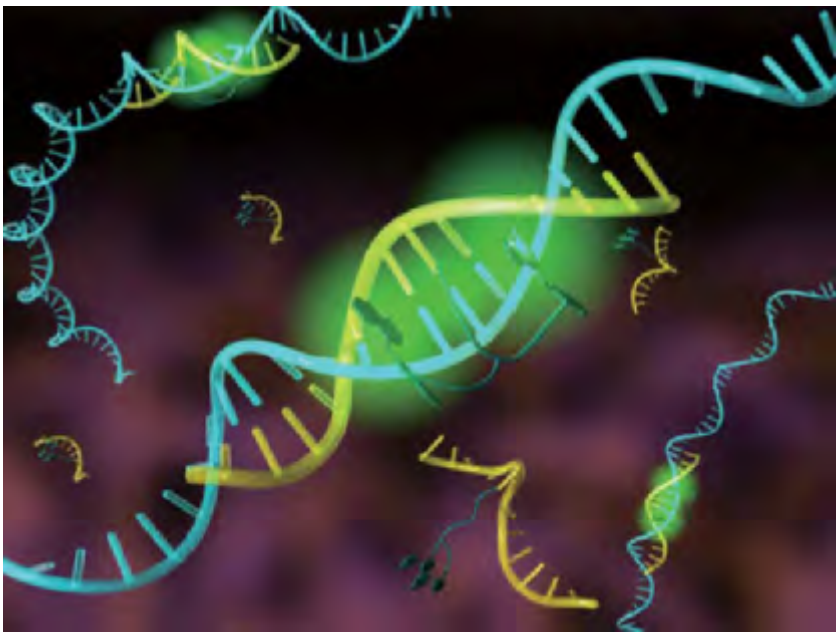


Figure 1: Schematic showing that new fluorescent molecular probes turn 'on' only in the presence of a specific RNA target.

Okamoto and his team found that by adding different types of dyes to the 'on–off' probes they emitted distinct fluorescent colors after hybridization. Using this technology, the researchers set out to visualize, in real-time, microRNAs, which are small nucleic acids that regulate gene expression.

In their experiment, three types of microRNA strands were injected into a living cell. Then, three probes were added; each one complementary to one of the microRNA strands. Fluorescence in three different colors was instantly seen in the middle of the cells (Fig. 1), demonstrating successful recognition of multiple targets.

According to Okamoto, while scientists know how RNA is synthesized, spliced and transported within a cell, much of this information is fragmented. Correlating this knowledge with time-dependent, multicolor imaging will help clarify gene expression mechanisms in living organisms.

"I want to see the life of RNA in cells," says Okamoto. "We need long-term observations to know when, where, which and how RNA works—from birth to death." ■

1. Ikeda, S., Kubota, T., Yuki, M. & Okamoto, A. Exciton-controlled hybridization-sensitive fluorescent probes: Multicolor detection of nucleic acids. *Angewandte Chemie International Edition* **48,** 6480–6484 (2009).

The brains behind rule-guided behavior

Several regions of the primate brain's prefrontal cortex have distinct functions in high-level cognitive tasks

Complex reasoning and abstract thought—hallmarks of human behavior—are thought to originate in the region of the brain known as the prefrontal cortex. Now, a research team from RIKEN in Japan and the University of Oxford in the UK has differentiated the roles of several sub-regions of the prefrontal cortex in the macaque monkey[1].

Led by Keiji Tanaka of the RIKEN Brain Science Institute in Wako, the team trained fourteen macaques in an analog of a diagnostic test of frontal lobe function known as the Wisconsin Card Sorting Test. In this test, after viewing an image, a monkey must select a matching image from a choice of three, according to an unspecified rule, either 'match shape' or 'match color' (Fig. 1). A reward is given only for applying the currently relevant rule, which must be a guess on the first trial.

After maintaining a success rate of at least 85% over several trials, the rule changes without notice. If the monkey makes its next choice using the previously correct rule, it is not rewarded. To receive further rewards, the monkey must switch the rule, remember to apply the new rule under varying conditions in subsequent trials, and be able to update the rule again.

The researchers compared the performance of normal monkeys to those with brain damage to specific areas of the prefrontal cortex. They discovered that damage to the cortex surrounding the principal sulcus impaired the ability to retain the current rule in working memory, whereas damage to other regions did not affect this ability.

Tanaka and colleagues also found a link between lesions to the orbitofrontal cortex and difficulties in rapidly updating the changing rules. These monkeys needed to successfully complete multiple consecutive successful trials before they modified their behavior. Monkeys with damage to the anterior cingulate cortex exhibited neither of these deficits; they made rapid, incorrect choices at a higher rate. According to Tanaka, these monkeys often ignore the rule and act impulsively, based solely on long-term habits.

Similar deficits have been observed previously about the effects of frontal lobe damage in humans, but with no clear localization. Now, the specific links between these specialized brain regions and separable components of cognitive flexibility in using behavioral rules can be used to more thoroughly diagnose patients and to help scientists understand how the brain works to solve complex tasks. ■

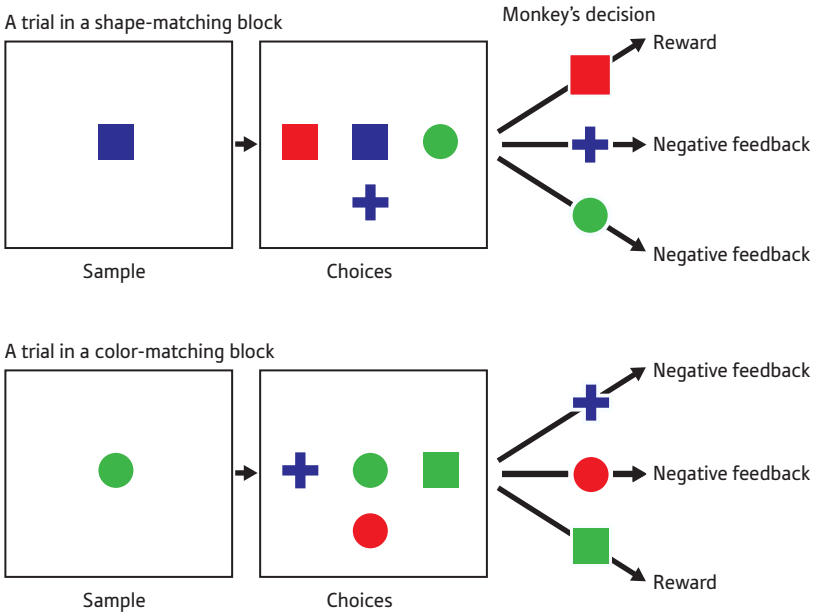


Figure 1: An example of the analog of the Wisconsin Card Sorting Test. Monkeys are rewarded after correctly applying the 'shape-matching' (top) or 'color-matching' (bottom) rule that will change without notice after several consecutive correct trials.

1. Buckley, M.J., Mansouri, F.A., Hoda, H., Mahboubi, M., Browning, P.G.F., Kwok, S.C., Phillips, A. & Tanaka, K. Dissociable components of rule-guided behavior depend on distinct medial and prefrontal regions. *Science* **325**, 52–58 (2009).

Helping neurons find their way

Variations in the spatial distribution of cellular signaling molecules provide the information needed to steer neuron growth within the brain

As the brain develops, neuronal axons extend outward in search of other neurons, all the while receiving 'directions' from the extracellular environment in the form of chemical signals that indicate when and where these growing axons should turn. For example, axons exposed to a gradient distribution of nerve growth factor (NGF) protein will automatically steer in the direction of highest NGF concentration.

"NGF is one of the most extensively studied molecules that direct axon elongation," explains Hiroyuki Kamiguchi of the RIKEN Brain Science Institute in Wako. "However, it has remained unclear for a long time how axons change the direction of elongation in response to NGF."

NGF-mediated turning is facilitated in part by the cellular signaling molecule inositol trisphosphate (IP_3), which in turn governs the intracellular release of calcium ions—an essential component of NGF's chemo-attractive action. By applying advanced methods for molecular-resolution live cell imaging, Kamiguchi and his colleagues have now gained valuable insights into how this process directs axonal guidance[1].

The researchers cultured chick-derived neurons expressing a genetically encoded sensor that fluoresces at specific wavelengths in the presence of IP_3, and then observed how individual neurons responded to an NGF gradient in the vicinity of the growth cone—the leading edge of a growing axon. They immediately noted the establishment of an asymmetric distribution of IP_3 within the growth cone and an elevated signal on the growth cone side exposed to higher NGF levels; this is mirrored by a similarly uneven distribution of IP_3-induced calcium release. This asymmetry correlates directly with axonal turning such that the growth cone steers in the direction established by the highest levels of NGF, IP_3 and calcium ion (Ca^{2+}) release (Fig. 1).

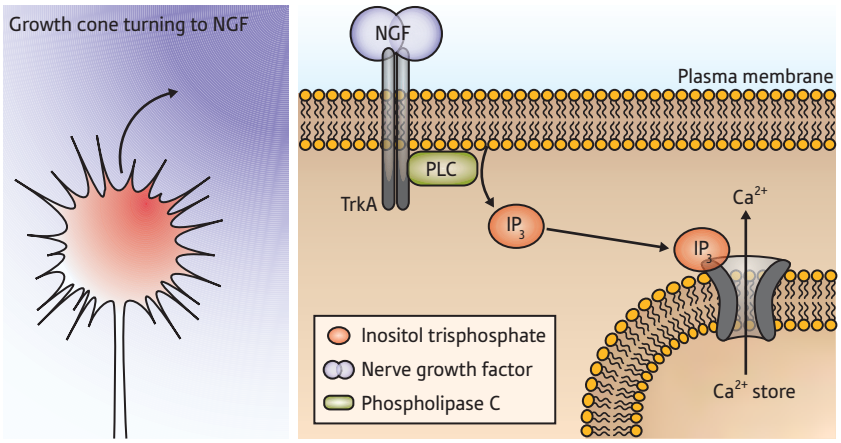


Figure 1: Schematic showing an axonal growth cone being guided in the direction of highest concentration of NGF (purple) via the establishment of an asymmetric internal gradient of IP_3 (red) (left). This action is mediated by elevated NGF receptor (TrkA) binding at the axonal surface, resulting in increased IP_3 production via the enzyme phospholipase C (PLC); this subsequently induces release of Ca^{2+}, which stimulates axonal turning (right).

The development of techniques for accurately detecting potentially subtle variations in IP_3 distribution was a key component of their success in this work. "We needed to detect 1% differences in fluorescence emission from the IP_3 sensor between both sides of the growth cone," says Kamiguchi.

However, he considers even the mere existence of such a gradient across the 10–20 micron width of the growth cone to be fairly surprising. "Because IP_3 diffuses so rapidly in cytoplasm, it has not been viewed as a highly localized messenger," he says. "This suggests the existence of robust degradation machinery to localize IP_3 signals to one side of the growth cone."

These insights into how neurons establish direction-specific signaling profiles should provide helpful starting points for understanding other models of cell polarization and migration. ■

1. Akiyama, H., Matsu-ura, T., Mikoshiba, K. & Kamiguchi, H. Control of neuronal growth cone navigation by asymmetric inositol 1,4,5-trisphosphate signals. *Science Signaling* **2**, ra34 (2009).

Innovation via genetic 'googling'

Intelligent search engines called PosMed and PosMed-plus make it easier for researchers to identify candidate genes for cloning

Figure 1: The cloning of species such as rice to acquire stronger, or more environmentally friendly, crops could be made easier thanks to the intelligent search engine PosMed-plus.

Many diseases are caused by genetic mutations. Researchers can use a technique called linkage analysis to identify rough intervals on the chromosome that might have mutated to cause each condition; however, these intervals often contain tens or hundreds of genes. Rather than laboriously testing each gene, it is useful for researchers to acquire as much knowledge as possible about the condition in question, so that they can narrow down their choice to the most likely candidate genes.

Now, Tetsuro Toyoda and co-workers at RIKEN's Bioinformatics And Systems Engineering (BASE) division in Yokohama have developed intelligent search engines that can identify candidate genes from huge genetic databases and over 17 million medical and biological documents[1,2]. The programs could not only help researchers to identify the genes responsible for diseases, but also highlight useful mutations that make crops more robust.

Toyoda explains some of the motivation behind his team's work: "The RIKEN Genomic Sciences Center promoted a project to generate genetically mutated mice on a large scale. The disadvantage is that the locations of the mutation that confer the phenotypes, such as diseases, were difficult to identify [using] a conventional genetics approach."

The BASE team worked alongside researchers at RIKEN's BioResource Center in Ibaraki to develop their search engine, called PosMed (Positional Medline), which accesses vast amounts of information on the genetics of humans, mice and rats. They also collaborated with RIKEN's Plant Science Center to develop a similar system for plants, called PosMed-plus (Positional Medline for plant upgrading science), which so far includes thale cress (*Arabidopsis thaliana*) and rice (*Oryza sativa*) (Fig. 1).

A researcher using PosMed or PosMed-plus can choose their species of interest, then type in a simple phrase representing a phenotype or function, for example 'diabetes' or 'drought tolerance'. The program then searches through text in existing literature databases and assesses the strength of each gene's connection to the phenotype in question. It can also highlight other genes that are expressed at the same times or places, or cited in the same papers, and even find similar genes in other species.

"Since the invention of the PosMed system, many mutations have been easily identified, because a researcher can prioritize the genes that need to be investigated with direct sequencing," says Toyoda.

The researchers hope to add more species to their databases soon. For example, Toyoda says: "Recently we are focusing on plants that are useful for green technologies—to combat climate change."

1. Yoshida, Y., Makita, Y., Heida, N., Asano, S., Matsushima, A., Ishii, M., Mochizuki, Y., Masuya, H., Wakana, S., Kobayashi, N. & Toyoda, T. PosMed (Positional Medline): prioritizing genes with an artificial neural network comprising medical documents to accelerate positional cloning. *Nucleic Acids Research* **37,** W147–W152 (2009).
2. Makita, Y., Kobayashi, N., Mochizuki, Y., Yoshida, Y., Asano, S., Heida, N., Deshpande, M., Bhatia, R., Matsushima, A., Ishii, M. *et al.* PosMed-plus: an intelligent search engine that inferentially integrates cross-species information resources for molecular breeding of plants. *Plant Cell Physiology* **50,** 1249–1259 (2009).

Know who your friends are

Neurons may be casual initially about their interactions, but get very picky when it comes to making a lasting synaptic connection

The brain is a self-assembling computer, in which different types of neurons extend their processes outward to interact with each other over the course of nervous system development, establishing tentative axon–dendrite connections that are subsequently formalized as mature synapses.

There are rules governing which types of connections should be established, although it remains unclear how neurons 'know' these rules. "Recognition seems to occur because neurons are always connected with [the] right partners, but the real mechanisms for this recognition remain unknown—and it is even unclear whether such 'recognition' really takes place," explains Masatoshi Takeichi of the RIKEN Center for Developmental Biology in Kobe.

The cerebellum primarily receives inputs from two kinds of axonal fibers: mossy fibers, originating from pontine nuclei in the cerebral cortex, and climbing fibers, which emerge from inferior olivary nuclei in the medulla. Each of these fiber types in turn associates with a specific subset of cerebellar cells; mossy fibers form synapses with granule cells (GCs), while climbing fibers connect to Purkinje cells.

Prior data indicate that these various cells interact indiscriminately early in development but then abort inappropriate connections as the brain matures, and Takeichi and graduate student Shoko Ito recently explored this phenomenon in the context of studying how cerebellar GCs find the right partner[1].

Co-cultures of GCs with pontine tissue showed little evidence of specific interaction between cells at first, but within several days began to exhibit signs of synapse formation. Interestingly, time-lapse movies revealed that dendrites from GCs appear capable of specifically recognizing mossy fibers, forming claw-like structures that physically latch onto these axons (Fig. 1).

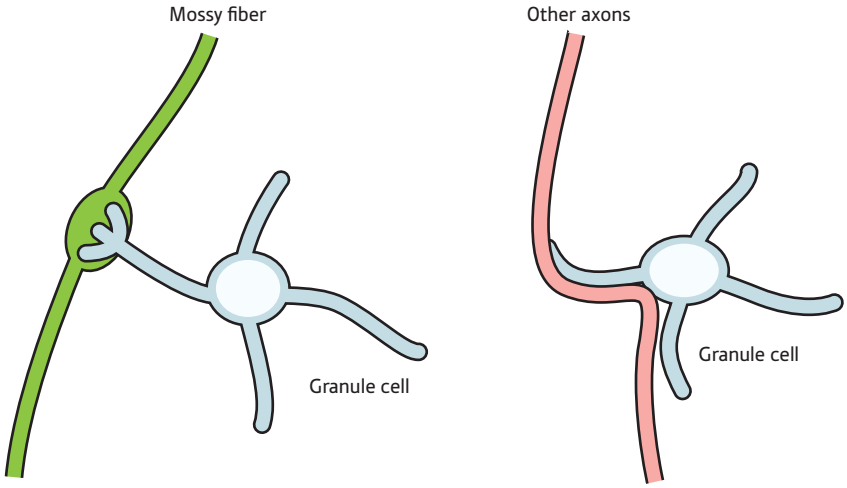


Figure 1: Schematic of cultured cerebellar granule cells showing that dendrites form synapses with mossy fibers and display the morphological characteristics seen *in vivo* (left), but fail to do so when encountering other axons, resulting in irregular synaptic contacts (right).

GCs showed markedly different behavior when cultured with climbing fibers or hippocampal cells, forming connections that displayed some characteristics of working synapses, but without the full range of morphological changes observed in dendrites from the pontine co-cultures. "Granule cells could form synapses with the correct positioning and morphology only when they met the mossy fibers," says Takeichi. "This finding was unexpected."

Overall, these findings suggest that although cerebellar cells can forge tentative links with a diverse array of axons, specific recognition mechanisms are in place to ensure proper synaptic wiring. "We have convincingly demonstrated that neurons do recognize their specific partners even *in vitro*, where environmental cues which could assist neuronal recognition are absent," says Takeichi. Exactly which factors facilitate this recognition remains a mystery, however, and he indicates that this will be a focus of future research from his laboratory. ■

1. Ito, S. & Takeichi, M. Dendrites of cerebellar granule cells correctly recognize their target axons for synaptogenesis *in vitro*. *Proceedings of the National Academy of Sciences USA* **106,** 12782–12787 (2009).

Solving the riddle of the turtle shell

An investigation of developing embryos reveals that tissue folding and movement is the key to the turtle's unusual body plan

The long-standing mystery of the evolution of the turtle shell has been resolved by researchers from the RIKEN Center for Developmental Biology in Kobe. The answer involves a folding process during embryonic development accompanied by a progression of relatively straightforward changes, not the major evolutionary leap that had previously been proposed.

Turtles, birds, mammals, lizards and crocodiles, known collectively as the amniotes, share a common ancestor. Compared with the other members of this group, however, the turtle skeleton seems inside out. The dorsal shell is generated by a fusing of the ribs, and the shoulder blade, or scapula, is contained inside the rib cage. In the other animals, the scapula is outside the rib cage.

Shigeru Kuratani and his colleagues thought the key to the evolution of this radical change may lie in the embryological development of the turtle. As detailed in a recent paper in *Science*[1], they used tissue-specific stains and the activity of pivotal genes to compare the development of bones and muscles in Chinese soft-shelled turtles to equivalent embryonic stages in chickens and mice.

What they found was a delicate interplay of tissue folding and movement. In the chicken and mouse, the ribs grow out from the spinal column and follow the body wall around the sides to the chest of the developing animal forming a cage that leaves the shoulder blade outside. But the turtle ribs stop short, sticking straight out from backbone without bending. Then, a folding process occurs along the sides of the turtle pinching in the body wall between the ribs and the shoulder blade, leaving the ribs over and above the scapula (Fig. 1). After the short turtle ribs and interspersed skin tissue fuse to form the bony dorsal shell, the fold forms its outer lateral extent.

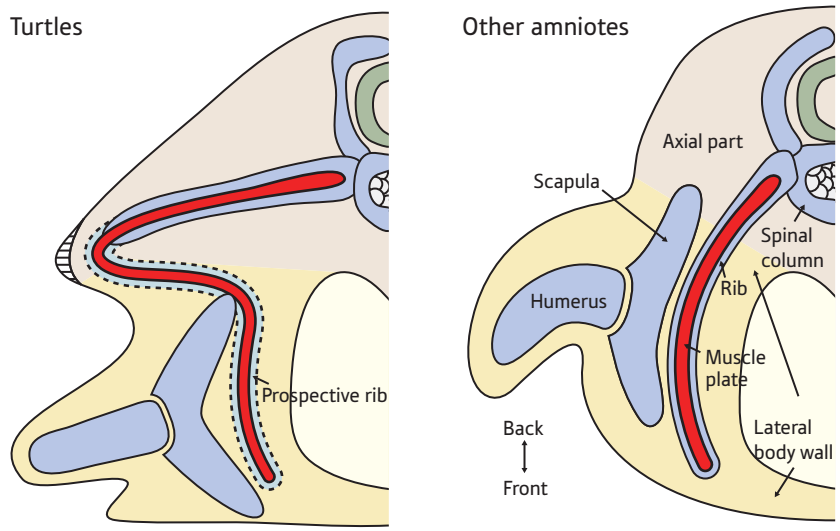


Figure 1: Cross section of the body plan of turtles (left) and other amniotes (right) showing the relative positions of ribs, shoulder blade (scapula) and muscle plate during embryological development.

Some of the muscular connections between the ribs and other parts of the skeleton remain intact during the folding process. But the resultant positioning of bones in the turtle has allowed other functional muscular connections to evolve. A 220-million-year-old turtle fossil discovered last year in China, which has a shell only on the underside, could easily represent an intermediate stage in development. "A developmental stage of the modern turtle, when the ribs have not encapsulated the shoulder blade yet, resembles this fossil species," Kuratani says.

What causes the folding process in turtle development is unknown. "That will be the subject of a future study," Kuratani says.

1. Nagashima, H., Sugahara, F., Takechi, M., Ericsson, R., Kawashima-Ohya, Y., Narita, Y. & Kuratani, S. Evolution of the turtle body plan by the folding and creation of new muscle connections. *Science* **325**, 193–196 (2009).

Space observatory's detector technology goes into single-molecule imaging

Astrophysicists at RIKEN lend their expertise to biologists to develop one of the world's fastest and most sensitive cameras to observe cell behavior at the nanometer scale.

Since 1999, Yoshiyuki Takizawa has been working on the Extreme Universe Space Observatory (EUSO), an international project to develop a super wide-field telescope capable of observing large volumes of Earth's atmosphere in order to detect the arrival of high-energy cosmic particles. Along with other international astrophysicists, Takizawa has been developing a photon detector that will be a critical part of the new 2.5-meter EUSO telescope onboard the Kibo Japanese experiment module of the International Space Station. The module is to be launched by 2015. The detector will consist of six thousand 1-inch-square photomultiplier tubes, and will allow an area of about 400 kilometers in diameter of Earth's atmosphere to be imaged in each shot.

The technologies for photon detection and the associated readout algorithm are so innovative that Takizawa, a research scientist at the Computational Astrophysics Laboratory of the RIKEN Advanced Science Institute (ASI) in Wako, decided to apply his expertise to observing a completely opposite object: molecules of nanometer size.

From space to single molecules

The original idea came from Takizawa's supervisor, Toshikazu Ebisuzaki. Having conversations with various researchers, Ebisuzaki learned that biologists are frustrated with their inability to observe many important biological phenomena.

In collaboration with three biologists at RIKEN's Wako campus—Yasushi Sako, Kiminori Ushida and Etsuko Muto—Takizawa obtained a research grant from the RIKEN Strategic Research Programs for Research and Development, or the 'President's Fund' as it is known. The two-year interdisciplinary project, commenced in October 2008, aims to develop an ultra-sensitive camera based on Takizawa's detector. "Unlike space science, a research target is on hand in biology. It is appealing to share the joy with biologists to uncover new mechanisms of living things," Takizawa says.

The project comprises a dozen researchers from four laboratories (Fig.1). The new camera will be able to image molecules of 1–100 nanometers in size in just one microsecond, making it 10–100 times faster than equivalent detectors. Although there are existing technologies for looking at single molecules, none has so far delivered these capabilities.

Figure 1. Collaborators on the molecular biology imaging project. From right: Yasushi Sako, Kenji Okamoto, Yoshiyuki Takizawa, Itsushi Minoura, Kiminori Ushida, Yasushi Watanabe and Kayo Hibino.

Biological purposes

The three biologists collaborating with Takizawa study different subjects but share a fundamental objective to observe molecular behaviors with microsecond resolution. "We have individually chosen research topics best suited to test the detector's performance," says Ushida, a senior scientist at the Supermolecular Science Laboratory of the RIKEN ASI.

Three years ago, Ushida developed a novel method of fluorescence correlation spectroscopy to directly observe anomalous diffusion in hyaluronan solution, which plays an important role in controlling the transport of molecules in many biological media such as extracellular matrices. In the past, such phenomena were difficult to detect, forcing researchers to depend on hypotheses based on the simple rule that diffusion coefficients are constant. "I'd like to handle the diffusion of a single molecule in a precise manner using the best optical spectroscope, which will be possible with the new detector," says Ushida.

Sako, a chief scientist at the Cellular Informatics Laboratory of the RIKEN ASI, is using single-molecule fluorescence microscopy to observe the orientation of membrane proteins, such as hormone receptors, through the movements of molecules and the changes of their structures and shapes. Researchers have many assumptions for the binding process between a hormone receptor and a specific hormone in membranes, but no one has directly seen such a reaction, Sako says.

Muto, team leader of the Laboratory of Molecular Biophysics at the RIKEN Brain Science Institute, and her colleagues are looking at the relationship between motor proteins and microtubules using dark-field microscopy. Microtubules exist within neurons and play an important role in the transport of intracellular substances. Malfunction of the transportation system is known to trigger certain diseases. "Higher temporal

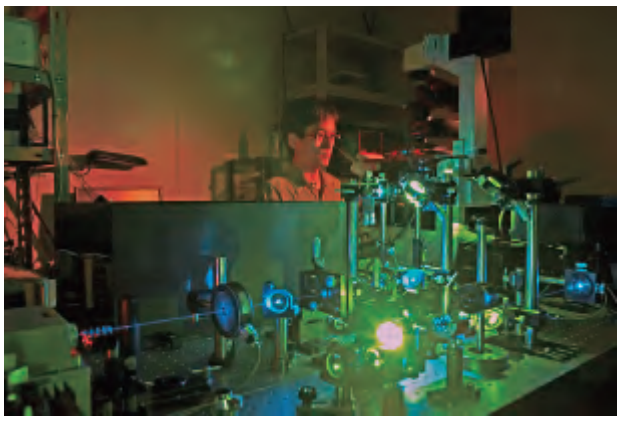


Figure 2: A prototype microscope system. Kenji Okamoto of the Cellular Informatics Laboratory demonstrates the experiment to test the performance of a prototype detector attached to a high-end optical microscope.

resolution could enable us to discover new types of fluctuations at smaller timescales," says Itsushi Minoura, a postdoctoral fellow in Muto's laboratory.

A prototype is now being used with a microscope in Sako's laboratory (Fig.2). When they want to test the device, Kenji Okamoto, a researcher in Sako's lab, sets up the device and gives meticulous instructions on its use. Another researcher in Sako's lab, Kayo Hibino, is busy making novel fluorescent probes and particle probes that she will use to label sample cells so researchers can maximize the quality of images.

Critical technologies

The participants say the project wouldn't have started without the 'ubiquitous circuit board' developed by Yasushi Watanabe, a research scientist in the Radiation Laboratory of the RIKEN Nishina Center for Accelerator-Based Science. The board is mounted with a DAP/DNA chip that has the special ability to reconfigure its signal processing logic circuit in one clock cycle—just six nanoseconds. Equivalent system chips, such as field-programmable gate arrays, require up to a few seconds to rewrite configuration programs. Watanabe's circuit board is innovative in that it is capable of storing numerous programs and reading out optimal programs according to the type of signal detected. The device, also developed with the support of the President's Fund, was completed in 2007.

Takizawa and his colleague Yoshiya Kawasaki are developing a key technology: a G-APD photon detection system (Fig. 3). The system consists of a Geiger-mode avalanche photodiode (G-APD), an application-specific integrated circuit (ASIC) and Watanabe's ubiquitous circuit board. The ASIC is another type of system chip that was originally developed jointly by the Ebisuzaki laboratory and the Institute of Space and Astronautical Science (ISAS) of the Japan Aerospace Exploration Agency. Takizawa and Kawasaki have upgraded the chip design with ISAS collaborators and adjusted it for use in the current project. They are now pursuing "the most delicate part of the detector system development," as Takizawa says, to connect their new circuit to the ubiquitous circuit board in order to relay ultra-fast signals without electrical noise.

The first prototype will likely be completed by the end of 2009, but the participants won't be satisfied to leave the development there. "The most important thing in this project is not simply to make the prototype camera work, but what we will do next," says Sako. Takizawa adds, "We'd like to keep improving our technologies so as many people as possible can benefit from our work."

About the President's Initiatives

In fiscal 2003, RIKEN launched the Strategic Research Programs for Research and Development, dubbed the 'President's Fund', to encourage researchers to obtain internal competitive grants and raise their motivation toward research. Proposals for funding are invited in four categories: interdisciplinary research (2 years, maximum 50 million yen), challenging research (2 years, maximum 30 million yen), pre-project research (1 year, less than 5 million yen) and top-down research under the president's direction. As of fiscal 2008, a total of 147 projects have been awarded.

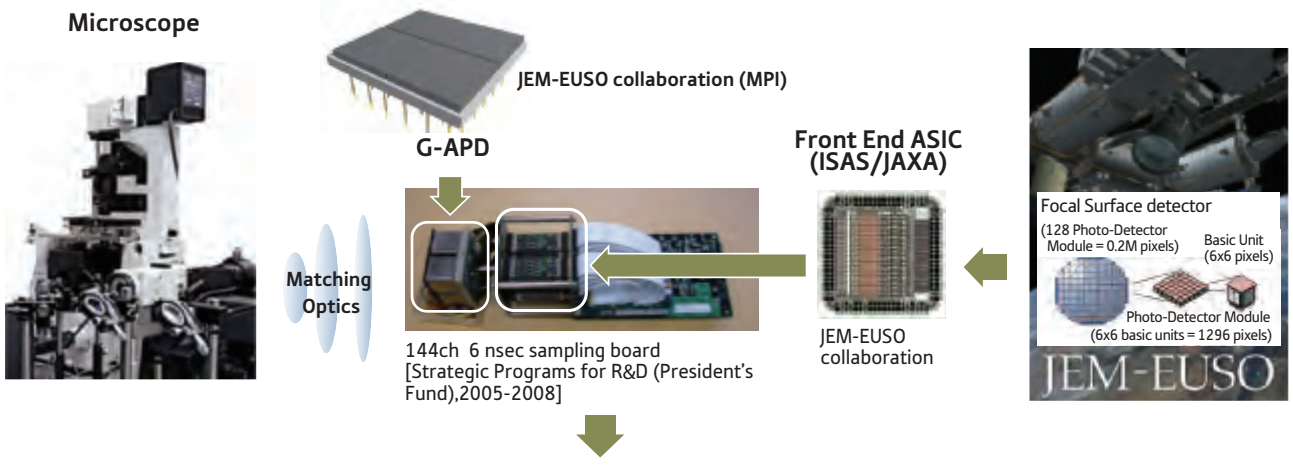


Figure 3: The G-APD photon detection system. Yoshiyuki Takizawa and his colleague Yoshiya Kawasaki of the Computational Astrophysics Laboratory are now developing this key technology. The first prototype is expected to be completed in a few months.

Deducing the evolution of the cerebral cortex from the thalamus

Tomomi Shimogori

Unit Leader
Shimogori Research Unit
RIKEN Brain Science Institute

What is the difference between humans and animals? For example, it is impossible for mice or dogs to speak with human words, not only because the shape of their mouths or vocal cords is different, but also because their cerebral cortex lacks the speech area that processes complex language. "We want to know how new areas such as the speech area formed in the cerebral cortex of humans in the process of evolution," says Tomomi Shimogori, unit leader of the Shimogori Research Unit. She and her colleagues are trying to understand the mechanism that creates new areas in the brain and to understand the functions that exist only in the human brain. In the future, this research is expected to contribute to our understanding of the causes of diseases such as autism, which are thought to be rooted in the initial development process of the brain, and to develop better treatments.

The research papers on developmental biology were boring

The cerebral cortex covers the surface of the human brain and is patterned with many grooves. "If the cerebral cortex were stretched and spread out, it would be about the size of a newspaper sheet," says Shimogori. "If we continue with the newspaper example, the cerebral cortex has a different area for each kind of information processed, like the economics page, the local news page and the weather page. The cerebral cortex has not only become larger in humans than in mice or dogs, but has developed many new areas that process various kinds of information. Humans have thus acquired a high degree of knowledge and language ability."

However, whether in mice, dogs or humans, the first stage of brain development is the formation of neural tubes in the cells of the first layer of the cerebral cortex. These neural tubes thicken and transform into complex shapes to form the brain. The areas of the cerebral cortex are formed completely when an organism is born. The cerebral cortex is large and has many areas in humans but is small and has only a few areas in dogs and mice because of differences in the development processes of the brain.

Shimogori, who previously researched cancer and other diseases in a pharmacology department, began researching brain development after she was appointed to a postdoctoral position at the University of Chicago in the US in 1998. "The development of the brains of mouse fetuses is very beautiful to watch. The neural tubes thicken and change shape before your very eyes, the cells migrate to each location, and the dendrites elongate and connect accurately to other cells. Then they begin to function as a brain. I thought it was such a beautiful and mysterious phenomenon. But the research papers on developmental biology that were being published at that time were all about research using knockout mice, and none of those papers were interesting."

In the 1990s, development biology was thriving with research on the functions of genes during the developmental process. In these

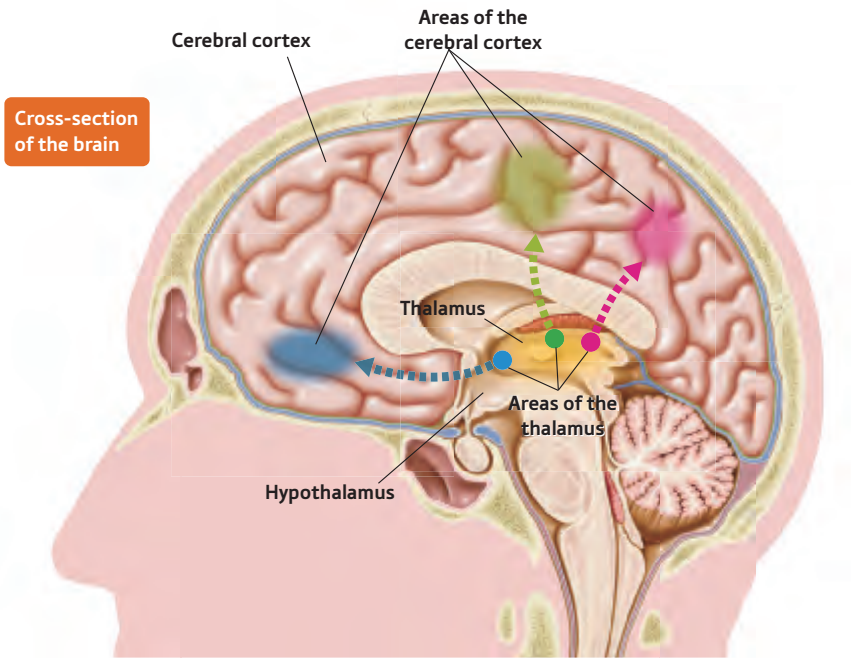


Figure 1: Connection between areas of the thalamus and respective areas of the cerebral cortex. The thalamus has a different area for each kind of information to be processed. Dendrites of neurons in the thalamus elongate, connect to each area of the cerebral cortex, and transmit information.

studies, 'knockout mice' were created by destroying specific genes, and the treated mice were compared with normal mice. However, the method used in those days for making knockout mice was problematic in that the functions of the specific genes were lost in all of the cells from the beginning stage of development.

For example, it has been pointed out that the fibroblast growth factor *FGF8* may perform important functions during the formation of the cerebral cortex. However, if the gene that makes *FGF8* is destroyed in the beginning stage of development, the fetus will die before the cerebral cortex is formed. "*FGF8* performs important functions that are essential to the development of not only the brain but also various other regions of the body. Consequently, it was not possible to study how *FGF8* functioned in the cerebral cortex by the methods used in those days to create knockout mice."

Controlling areas of the cerebral cortex

"If methods can be developed for manipulating the activity of genes during specific periods and in specific regions, I think more interesting research will be possible." Shimogori took notice of a gene transfer technique called 'electroporation', which was being applied to chicken fetuses. Electroporation is a method of injecting genes directly into specific regions of embryos and introducing the genes into cells by applying electrical stimulation. Using electroporation, it is possible to exclusively overexpress specific genes during specific periods, or to inject other genes to inhibit the activity of specific genes. "My goal was to apply this method to mice."

In chickens, it is relatively easy to perform embryo manipulation, such as the introduction of genes, because development occurs within the eggshell. However, this operation is difficult in mice because development occurs in the uterus. It is necessary to remove the uterus with the fetus from the belly of an anesthetized mother mouse, introduce a gene into the fetus, and put the fetus back into the mother's body. "I made my own electrodes for applying electrical stimulation, and through trial and error I eventually succeeded in establishing a method for introducing genes into mice."

Using this method for introducing genes, Shimogori manipulated expression of *FGF8* in the cerebral cortex of mice and studied how changes occurred in the way that areas were formed within it.

As a result, when she overexpressed *FGF8*, an area called the 'barrel field', which processes sensory information from the whiskers, moved behind its original position, and the motor area and frontal lobe that had formed in front of it expanded. When she introduced a gene that inhibited the activity of *FGF8*, on the other hand, the barrel field moved forward and the visual field that had formed behind it expanded. "*FGF8* is secreted from the front of the region that becomes the cerebral cortex. Once the *FGF8* is secreted, its concentration in the cerebral cortex becomes higher on the front side and lower on the back side. This difference in concentrations may cause differences in which genes are expressed and thus may cause different areas to form in the front and back areas of the cerebral cortex."

In addition, when Shimogori expressed *FGF8* in the back side of the cerebral cortex, where it was not originally expressed, a new barrel field formed. "By changing the way that *FGF8* is expressed, I was able to change the position and size of the areas of the cerebral cortex. If we put this in terms of a newspaper, I enlarged the weather page and created an additional economics page. But I was unable to create a new area."

Focusing on the thalamus, which inputs information into the cerebral cortex

How did new areas form in the cerebral cortex during the process of human evolution?

"Although new areas may be formed in the cerebral cortex, they do not function unless they are connected to other areas. For example, the visual area requires input of visual information that is received from the outside world through the eyes. Except for smell,

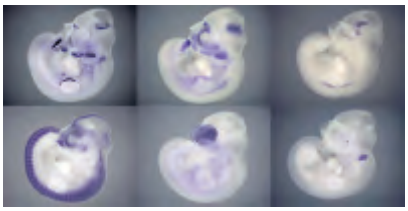


Figure 2: Genes expressed in mouse fetuses ten days after fertilization.
The period and region of expression is studied by individually staining various genes involved in thalamus formation.

sensory information from the outside world such as sight, hearing, touch and taste is collected in the thalamus and sent to the cerebral cortex (Fig. 1). "I thought that the reason new areas form in the cerebral cortex during evolution might be that changes in the habitat environment cause new information to enter from the thalamus."

In 2004, Shimogori launched a research unit at the RIKEN Brain Science Institute and started full-blown research on the thalamus. "The thalamus is difficult to study because it is in the depths of the brain. Consequently, thalamus research is not progressing as fast as research on other regions of the brain. Also, the number of thalamus researchers is very small."

The thalamus is divided into areas according to the information being processed. The dendrites of the neurons in each of these areas elongate, connect to their respective areas in the cerebral cortex, and transmit information to those areas. The size and number of areas in the thalamus are known to differ among animals. So how are new areas formed in the thalamus? "To answer that question, it is first necessary to investigate how the thalamus is formed and how each area is formed within it. During the development process, cells change in shape and properties, and move great distances, so we follow this phenomenon while extrapolating what kinds of cells these cells will become. For this purpose, landmarks are necessary."

Those landmarks are the genes that are expressed in the thalamus. Shimogori and her colleagues discovered at least 1,000 kinds of genes that are expressed in the mouse thalamus and studied when and where their expression began and how their patterns of expression changed (Fig. 2). "If we can understand which genes are expressed exclusively in each area, we can enhance and suppress the expression of those genes and study what kind of effect this enhancement and suppression has on the formation of areas in the cerebral cortex, which is an access point."

For example, in the thalamus of higher animals, there is a particular area called the 'pulvinar', which does not exist in rodents such as mice. In humans, part of this pulvinar is highly developed and is connected to the lateral prefrontal cortex area of the frontal lobe, which exists only in the cerebral cortex of primates. Accordingly, if it is known which genes are necessary for pulvinar formation, the way that they influence formation of the frontal lobe can be investigated.

Did a new way of using genes cause the brain to evolve?

To deduce the evolution of the cerebral cortex from the thalamus, it is necessary to study and compare the formation process of the thalamus in various species of animals. Shimogori and her colleagues are now investigating how genes corresponding to genes discovered in the mouse thalamus are expressed in the chicken thalamus.

If the differences in gene expression between mice, which are mammals, and chickens, which are birds, can be compared, the characteristics of thalamus formation in mammals should become apparent.

Through collaborative research with Norihiro Okada and his colleagues from the Tokyo Institute of Technology, Shimogori and her colleagues have determined that whereas *FGF8* is expressed strongly in the hypothalamus of mice, it is hardly expressed at all in the hypothalamus of chickens.

DNA fragments called 'retrotransposons' are what enhance expression of *FGF8* in the hypothalamus of mice. Retrotransposons make copies of themselves and enter other locations in the genome (total genetic information). When a retrotransposon enters a switch region that controls the expression of genes, it can sometimes enhance and suppress that expression. In mice, retrotransposons enter the genetic switch of *FGF8* and enhance its expression in the thalamus during development (Fig. 3).

The mapping of genomes of various animals has progressed remarkably in recent years and has led to the discovery that all animals are very similar in terms of the number of genes that they have and in the chemical substances and base sequences that constitute their DNA, even when the animals differ greatly in abilities and form. So, what causes differences in abilities and form among animals? Researchers are beginning to think that these differences are caused by differences in the time, location and strength of their gene expression, namely, by differences in the way that genes are expressed. One of the main causes of these differences in the use of genes is retrotransposons. The

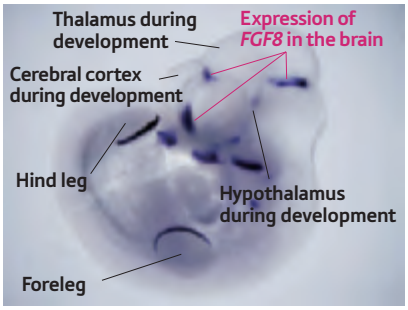


Figure 3: *FGF8* expressed in the thalamus of a mouse fetus.
Retrotransposon, which enhances expression of *FGF8* in the thalamus of mouse fetuses, has also been discovered to enhance expression of *FGF8* in the hypothalamus, which governs instinctive behaviors such as feeding, sexual behavior and sleep. There are differences among animals in the way that *FGF8* is expressed in the hypothalamus, and this may cause differences in their behavior.

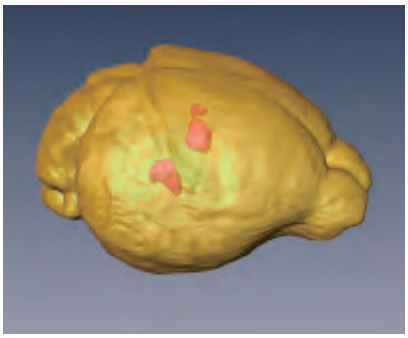


Figure 4: Presentation from the gene expression database showing the development of the thalamus.
The time and location of specific genes (pink) in the brain (yellow) of a mouse fetus can be displayed as a stereoscopic image (currently experimental). The database forms a research foundation that will play an important role in research at public research institutes such as RIKEN.

genomes of mammals contain many retrotransposons, which in humans make up 40% of the genome. They are thought to be deeply involved in the evolution of mammal brains.

So what happens when *FGF8* expression in the mouse thalamus is enhanced by retrotransposons? Shimogori and her colleagues discovered that when *FGF8* expression is further enhanced by a gene transfer method, areas inside the thalamus move behind their original position. In contrast, if the activity of *FGF8* is suppressed, the areas move to the front of their original position. *FGF8* plays an important role in forming areas in the thalamus in the same way that it does in the cerebral cortex.

In contrast, *FGF8* is hardly expressed at all during thalamus formation in chickens. What differences does *FGF8* cause in the formation of mammal and bird brains? "We can explore that question by injecting *FGF8* into the thalamus of a chicken during its development. I want to conduct that experiment in the future."

At the Responsive Matter Chemistry and Engineering Research Group, researchers deal in difficult, painstaking work in the creation of new molecular devices with specific functions. "We have produced very exciting results, including a new molecule that exhibits an interesting phenomenon that was never expected. Unfortunately, however, we cannot speak about the phenomenon yet because the results have not been published." Look for the results to be published by the Responsive Matter Chemistry and Engineering Research Group in the near future.

Did the function of sorting information in the thalamus cause the evolution of the cerebral cortex?

Shimogori points out the importance of figuring out the unknown functions of the thalamus and simultaneously exploring the process of thalamus formation. "The thalamus may have acquired the ability to sort information during evolution. This may have caused the evolution of the cerebral cortex."

For example, the thalamus developed the ability to sort information about the outline, color and depth of objects from visual information and send it to different locations in the cerebral cortex. New areas may have developed in the cerebral cortex as a result. "Among primates who lived in treetops, some may have emerged that acquired a new area in the brain that enabled stereoscopic vision by extracting depth information in the thalamus and sending it to the cerebral cortex. Thus, probably only the monkeys who had acquired advanced stereoscopic vision were able to move around efficiently from tree to tree and quickly find fruit, thus winning the struggle for survival."

Expanding a research network based on a research foundation

Through collaborative research with Johns Hopkins University in the US, Shimogori and her colleagues are preparing a database that will show the time and location of the beginning of expression of genes that function in the development of the thalamus. It will also show changes in the expression patterns of these genes. The information will be displayed in the form of stereoscopic images, and Shimogori and her colleagues are already preparing to release this database on the Internet (Fig. 4). This will be the first thalamus database of its kind. "Five years have passed since this research unit was launched at RIKEN, but during that time, we have mainly been collecting tools for researching the thalamus. Some might think, 'You are still collecting tools after five years?' But in order to understand the complex network that is the brain, a solid research foundation is essential. It is also necessary to promote collaborative research with researchers from various fields and expand this research network based on this foundation."

Full-blown research can then be pursued on deducing the evolution of the cerebral cortex from the thalamus. In the future, this research is also expected to contribute to medicine. "For example, conditions such as autism in humans may be caused by errors in the formation of specific areas or nerve connections in the brain. Knowing what areas exist in the human brain and how neurons are connected in order to function properly might be useful for understanding the causes of diseases such as autism, and for developing treatments for them."

About the researcher

Tomomi Shimogori was born in Chiba, Japan, in 1970. She graduated from the Hoshi Collage of Pharmacy, Tokyo, in 1993 and obtained her PhD in pharmaceutical science from Chiba University in 1998. After six years of postdoctoral training at the Department of Neurobiology, University of Chicago, USA, she returned to Japan to join the RIKEN Brain Science Institute as unit leader exploring the mechanism of thalamic development and its contribution to cortical evolution. Her research focuses on the developmental mechanisms patterning the mouse thalamus, comparative analysis of developing mouse and chick thalamus, and the role of thalamocortical axons in cortical plate development.

The Nishina School offers students a unique introduction to nuclear physics

The RIKEN Nishina Center for Accelerator Based Science held its second annual Nishina School between September 29 and October 8, 2009. Initiated as part of an agreement between RIKEN and China's Peking University signed in February 2008, the Nishina School offers students a unique opportunity to acquire hands-on experience in theoretical and experimental nuclear physics. Seven final-year Peking University students travelled to Tokyo this year to attend the school.

A total of eight lectures were delivered, covering topics including nuclear theory, data acquisition, particle accelerators and scintillator detectors. The students were also given the opportunity to conduct two experiments, participate in various training sessions, and join a tour of RIKEN's Rare Isotope Beam Factory.

In a final presentation summarizing the students' experiences at RIKEN, Peking University student Chen Zhongjing expressed how impressed he was by the research environment. "We may be back here again in the future to do research in nuclear physics," he predicted.

While the research itself was challenging enough, developing the skills needed to communicate the results of experiments was also a learning experience. "For most of us, this is the first time to explain physics in English," said Zhongjing.

Haiwang Yu, another Peking University student, praised RIKEN's research equipment. "Most of the instruments at our university are designed for teaching, not for research," he said. "At the Nishina School, we start with instruments used in real research, so we learn what research is really like."

One of the most popular components of this year's school was a game surrounding an experiment on positron emission tomography (PET). In analogy to the use of PET in cancer imaging, teams took turns using coincidence measurements from a circle of scintillator detectors to find a gamma-ray source (the 'tumor') hidden under one of many paper cups placed by the opposing team.

Melissa Furukawa, an undergraduate student at McMaster University in Canada who attended the Nishina School as a guest, was on the team who won the game. "Doing these kinds of experiments really helps you to understand how the detectors work," she said.

Aside from lectures and experiments, students also spent their weekends visiting landmarks around Tokyo and learning about various Japanese traditions. A ceremony in the evening of October 8 brought this year's Nishina School to a close.

Symposium on emerging and reemerging infectious diseases held in Tokyo

On October 9, 2009, the Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT) and the RIKEN Center of Research Network for Infectious Diseases (CRNID) held a one-day symposium entitled "Building an Africa-Asia Knowledge Network on Infectious Diseases" at the Marunouchi Building in Tokyo. The symposium capped off five years of research under the Program of Founding Research Centers for Emerging and Reemerging Infectious Diseases initiated by the MEXT in 2005, and featured talks by leading Japanese and international scientists.

The pressing need for research in the area of emerging and reemerging infectious diseases has been reiterated in recent years with the worldwide spread of the 2009 pandemic influenza and the severe acute respiratory syndrome (SARS). "While infectious diseases heed no national borders," RIKEN CRNID Director Yoshiyuki Nagai pointed out in his introduction, "there are borders in research on infectious diseases." The CRNID, which coordinates a network of 12 research centers across eight countries in Africa and Asia, was created to bridge these borders. Through partnerships fostered by the network, Japanese universities and research institutes work with universities in target countries toward advances in on-site diagnosis and treatment.

The issue of Asian countries becoming 'hot spots' for disease emergence was emphasized in a presentation by Paul Brey, director of the Institut Pasteur in Laos, who noted that factors such as animal reservoirs and the consumption of wild animals accelerate the spread of infection. Brey drew attention to the need for the "networking of networks" in research, a sentiment echoed in presentations on the situations in Vietnam, India, Indonesia and the Philippines. Naoto Keicho, director of the Department of Respiratory Diseases at the International Medical Center of the Japan Research Institute, drew attention to the importance of international cooperation in his studies on tuberculosis in Vietnam. "We cannot focus only on Vietnam," he said, "we have to think about all of Asia."

In the second half of the symposium, Yoshihiro Kawaoka, director of the International Research Center for Infectious Diseases, emphasized the difference between the 2009 pandemic influenza and the seasonal flu, stressing the danger that this year's pandemic could mutate into a more virulent strain. Koichiro Kudo, director of the Disease Control and Protection Center, reported on his experience visiting Mexico in August to investigate actual cases and on-site treatment methods. These presentations set the stage for a panel discussion focusing on global and domestic responses to the pandemic, including a debate on the effectiveness of vaccines and other preventive measures.

In closing remarks, Nagai highlighted the need for emerging and reemerging infectious diseases to be granted a higher priority in government policy, and looked ahead to the next phase of research in the project of founding research centers.

Invitation to conduct drug-discovery research using RIKEN technology

As part of plans to launch its new Drug-discovery Technology Program in 2010, RIKEN is making its life sciences technology infrastructure available to outside users on a trial basis to facilitate drug-discovery research and development. The new program will be headed by Toshio Goto, currently a senior advisor of RIKEN.

The key elements of the technology infrastructure include an extensive chemical bank that researchers can use to search for drug-discovery targets, in silico dry/wet screening using computer and compound arrays, compound and protein interaction analysis using nuclear magnetic resonance, and disease analysis and positron emission tomography using model experimental animals.

Users of the infrastructure will also have access to drug-discovery coordinators, who will provide consultation and planning support as well as assistance in drafting proposals.

At the end of the trial period, opinions and suggestions submitted by the trial users will be incorporated into the redevelopment of RIKEN's drug-discovery platform, which will ultimately be made available to outside users in or soon after the 2010 financial year.

Dr Toshikazu Ebisuzaki
Chief Scientist
Computational Astrophysics Laboratory
Advanced Science Institute, RIKEN
Wako, Saitama, Japan

Dear Dr Ebisuzaki,

From my post at the Joint Institute for Nuclear Research (JINR) in Dubna, near Moscow in Russia, I was invited to Japan in 1996 as a visiting professor. At that time, I already had a good impression of Japan, and liked it most for its absolutely peaceful atmosphere. That period was one of economic instability and local conflict in my own country, which caused many scientific activities to stop or be severely impeded. Japan was a kind of sanctuary for me and allowed me to continue in scientific research.

From 1998 to 2004, I worked with you at the Computational Astrophysics Laboratory at RIKEN. From the beginning of my research at RIKEN I was involved in molecular simulation of physical and biological system s. RIKEN's research environment was excellent, with research laboratories equipped with modern facilities and a friendly atmosphere. I was especially impressed by the high level of computer simulation study at your Laboratory.

In the development of computing facilities and simulation techniques, Japan is unique in the world. I think that no other country is able to achieve such progress in computing technologies as I have seen in Japan today. At RIKEN, I worked in molecular simulation with Japanese researchers and colleagues from many other countries. I was involved in studies using high-performance parallel/vector and special-purposes machines. The simulation techniques and methodologies we used are applicable in all branches of scientific research.

In 2004, I returned to the JINR Dubna, where I now act as head of Computer Molecular Modeling, a new research unit created within the Laboratory of Radiation Biology. I still work in collaboration with research colleagues from Japan, arranging mutual visits and cultural exchanges, and conducting scientific research based on molecular simulations. Building on my experiences at RIKEN, I like to think that we established a new interdisciplinary branch of science at the JINR — molecular dynamics simulation. We continue to perform molecular dynamics studies in close international collaboration with colleagues from Japan, culminating with our regular Japan–Russia International Workshops at the JINR Dubna.

RIKEN Wako campus I think is one of the special places in Japan, perhaps in the world, where the accumulation of international scientists is very high. This is a sign of high-level standards that attract scientists from all countries. In this sense RIKEN serves as a strong basis for supporting international cooperation among different nationalities and research centers. My time with RIKEN in Wako was one of the best times of my life; the beautiful greenery, the very good living conditions and the excellent research environment. What else do you need to be happy?

Your sincerely,

Dr Kholmirzo Kholmurodov
Computer Molecular Modeling Sector
Laboratory of Radiation Biology
Joint Institute for Nuclear Research
Dubna, Moscow, Russia

RIKEN

www.riken.jp

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.

RIKEN RESEARCH is a website (www.rikenresearch.riken.jp) and print publication intended to highlight the best research being published by RIKEN (www.riken.jp). It is written for a broad scientific audience and policy makers interested in science and aims to raise global awareness of RIKEN and its research.

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 48 462 1225
FAX: +81 48 462 4713
E-Mail: rikenresearch@riken.jp

www.rikenresearch.riken.jp