RIKEN RESEARCH

JULY 2011
VOLUME 6 NUMBER 7

HIGHLIGHT OF THE MONTH

Biology: Insights into the story of the eye

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Biology

Insights into the story of the eye

Cultures of embryonic stem cells can be coaxed to spontaneously develop into a mature, properly organized retina

Although it is well established that embryonic stem cells (ESCs) have the capacity to develop into every adult cell type in the body, mysteries abound regarding the process by which the differentiation of these cells is coordinated during the formation of complex tissues.

The eye, for example, is a very sophisticated organ, consisting of several highly organized layers of specialized cell subtypes, which initially develops from a largely homogeneous embryonic tissue known as the ectoderm. This process initiates with the formation of a bubble-like optic vesicle, which subsequently folds back on itself to form a two-layer 'optic cup', with the inner surface forming the sensory tissue of the neural retina and the outer surface forming the supporting tissue of the retina pigment epithelium (RPE).

How this actually happens remains unclear and controversial. "One strong hypothesis was that the invagination of the retina is caused by pushing from the lens, which itself invaginates from the surface ectoderm," explains Yoshiki Sasai of the RIKEN Center for Developmental Biology, Kobe. He adds that although some studies support this idea, others have proven ambiguous or contradictory. However, recent stem cell research from Sasai and colleagues not only offers powerful new insights into retinal development but also provides surprising evidence in favor of an alternative hypothesis[1], originally proposed over 100 years ago.

Getting organized

At the turn of the 20th century, German developmental biologist Hans Spemann, a legend in the field of embryology, uncovered evidence indicating that invagination of the optic cup and subsequent lens formation occur spontaneously, without any external force. "This mechanism has been under debate for many decades since that time," says Sasai. Using a specially designed strategy for the cultivation of mouse ESCs, his group has now provided vital support for this model by demonstrating successful differentiation of an appropriately layered and structured mouse retina *in vitro*, in the absence of external physical drivers.

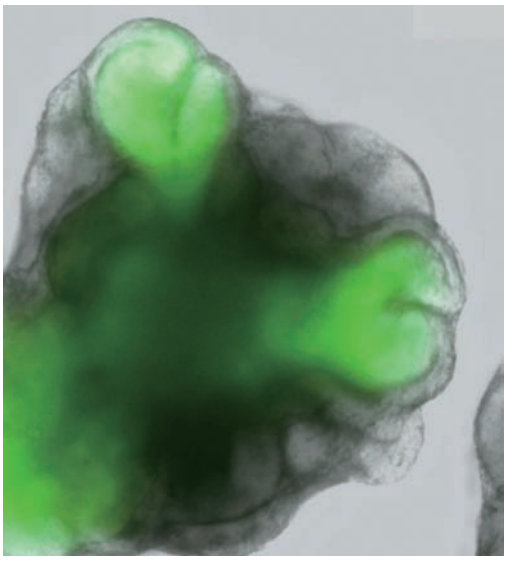

Figure 1: Optic vesicles, formed by ESCs cultivated over nine days under modified SFEB conditions, have undergone invagination to yield optic cup-like structures. Green fluorescence indicates expression of the retina-specific gene Rax in the optic cups.

Sasai and colleagues' technique was an adaptation of serum-free culture of embryoid body-like aggregates (SFEBq), a cell culture system that they had developed previously to coax the development of ESCs into organized layers of cerebral cortical neurons[2]. "The retina is another clearly stratified structure, and we tried to see whether we could achieve more complete development of layered structures that resemble postnatal tissue from stem cells," explains Sasai.

After a week under these improved culture conditions, their ESCs developed into hollow spheres of neuroectoderm epithelial cells, precursors of nervous system tissue, which subsequently sprouted dome-shaped vesicles expressing the retina-specific gene *Rax*. Over the next few days, these vesicles spontaneously folded inward to form structures that closely resemble the optic cup observed in natural embryonic development (Fig. 1). Both of these newly formed layers exhibited gene expression profiles matching their natural counterparts, with no evidence for the onset of lens formation or other ectodermal influences that might represent physical triggers for this developmental process.

By closely observing this *in vitro* optic cup formation with a powerful

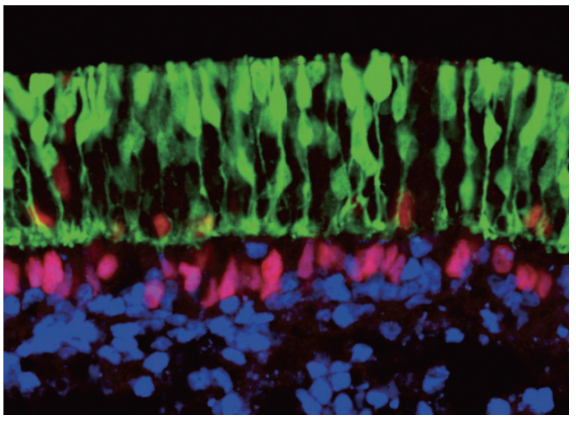

Figure 2: Cultured neural retina layers develop into highly organized structures composed of distinct layers. Fluorescent labeling indicates the photoreceptors in the outer nuclear layer (green), the bipolar cells of the inner nuclear layer (red) and the ganglion cell layer (blue).

fluorescence microscope, the researchers were able to characterize the details of invagination at various stages. Initially, the optical vesicles consisted exclusively of a single type of epithelial cell, but as the outermost edge of the vesicle flattened, indented and finally underwent full invagination, the cells formed distinct subpopulations that also closely resemble their counterparts in the naturally developing neural retina and RPE.

Sasai and colleagues determined that the earliest morphological changes in the vesicle appear to arise from pulling force generated by 'motor proteins' acting on microscopic filaments within the indenting epithelial cells. These motor proteins become less important as invagination proceeds in earnest; later stages instead appear to be powered by a pushing force generated by the physical expansion and active proliferation of cells within the developing retinal tissue.

All part of the program

To investigate the further subspecialization of neural retina layer cells, the researchers dissected their ESC-derived optic cups and cultured this cell layer independently. Within two weeks, these tissues had developed into highly stratified structures composed of a diverse array of extremely specialized cell types, each of which was spatially restricted to the appropriate level within the overall neural retina structure (Fig. 2). Aside from some minor differences, such as a relatively reduced population of color-sensing 'cone' photoreceptors, these *in vitro*-derived tissues were largely indistinguishable from a postnatal mouse retina.

In spite of Spemann's historic predictions of retinal self-organization, Sasai admits that he and his colleagues were surprised by the extent to which such a complex organ can form in the absence of external guidance. "This means that the cells fated to become the retina have a latent, intrinsic order that allows them to form such an elaborate tissue structure by following an internal program," he says.

Further studies will be required to determine whether these ESC-derived retinas retain full functional capacity in terms of light-sensing and signal transmission, and whether they might be used to repair retinal damage in animal models. If they pass these and other tests, such engineered tissues could prove invaluable as material for transplantation in the treatment of a variety of eye diseases.

This work may also offer useful starting points for investigating similar 'internal programs' that might underlie the autonomous development of stem cell populations into other complex tissues, including the elaborate and heterogeneous structures that comprise the brain. "We are now analyzing the mechanism of self-driven morphogenesis at the cellular and molecular level," says Sasai, "and we ultimately hope to incorporate these pieces of information into a three-dimensional computer simulation."

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2. Eiraku, M., Watanabe, K., Matsuo-Takasaki, M., Kawada, M., Yonemura, S., Matsumura, M., Wataya, T., Nishiyama, A., Muguruma, K. & Sasai, Y. Self-organized formation of polarized cortical tissues from ESCs and its active manipulation by extrinsic signals. *Cell Stem Cell* **3**, 519–532 (2008).

ABOUT THE RESEARCHER

Yoshiki Sasai was born in Hyogo prefecture, Japan, in 1962. He graduated with a medical degree from the Kyoto University School of Medicine in 1986. After an internship in internal medicine, he studied molecular neurobiology in the laboratory of Shigetada Nakanishi, where he obtained his PhD degree (1993) for work on the identification of the mammalian HES gene family. He subsequently worked with Eddy De Robertis at the University of California, Los Angeles in the USA on isolation of the neural inducer Chordin in *Xenopus*. He became an associate professor of Kyoto University in 1996, and full professor in 1998. He moved to the RIKEN Center for Developmental Biology in 2003 as group director and is currently working on early neural patterning of vertebrate embryos and *in vitro* recapitulation of neural and retinal development using three-dimensional embryonic stem cell culture. He is an editorial board member for journals including *Neuron* and *Developmental Dynamics*.

Making holograms look more real

A full-color three-dimensional hologram has been created by harnessing electron density waves in thin metal films

Although human vision is capable of perceiving objects in three dimensions (3D), we spend much of our day looking at two-dimensional screens. The latest televisions and monitors can trick us into perceiving depth, by presenting different images to our left and right eyes, but they require special-purpose glasses, or specialized large-area lenses applied directly to the screen.

Holographic 3D imaging, on the other hand, presents a 'true' representation of an object by exactly reconstructing the light rays that would come from that object if it were present. However, integrating color into 3D holograms has proved a challenge. Consequently, holograms are usually either monochromatic, or—as in the case of credit card holograms—colored in a way that does not correspond to the real object. Now, creating true, 3D color holograms has become possible using a technique developed by Satoshi Kawata and colleagues at the RIKEN Advanced Science Institute in Wako[1].

The researchers' hologram consists of a periodic grating, which is encoded with an interference pattern and covered with a thin film of silver. As with other holograms, when properly illuminated at a later time, the hologram can recreate the light rays that would result from the original object if it were present. The innovation comes in how this grating interacts with the silver film, whose electrons can be excited into density waves called surface plasmon polaritons (SPPs). SPPs are associated with a short-range, non-radiative electromagnetic field. When this field interacts with the grating, it is converted into visible light that can be observed by a viewer at a distance.

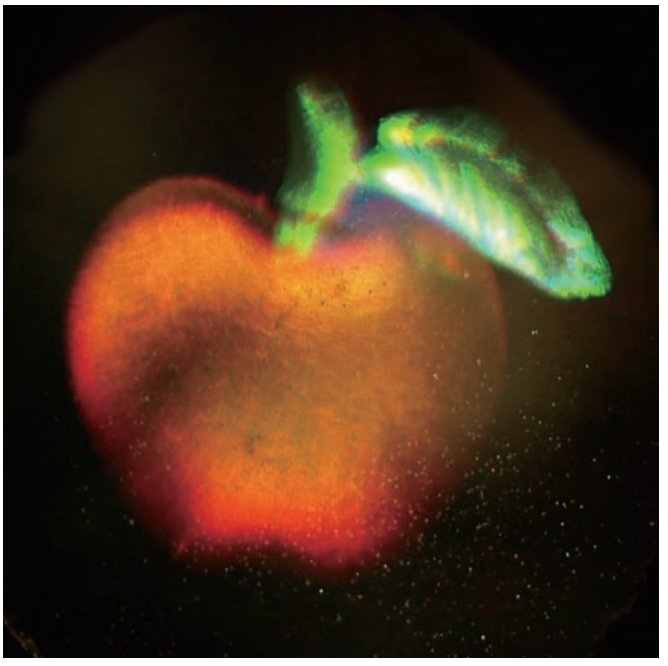

Figure 1: A full-color holographic apple achieved using surface plasmon polaritons in a silver film.

Critically, the nature of the SPPs excited in the silver depends on the angle of light that excites them. Therefore a particular type of SPP can be created by illuminating the film at a particular angle, and this in turn leads to a particular image being observed by the viewer. By encoding red, green and blue images into their grating, and then illuminating the grating and silver film simultaneously with three light beams at different angles, Kawata and colleagues produced a full-color hologram (Fig. 1).

To make the hologram easier to operate, the researchers also coated their silver film with a layer of silicon dioxide. This increased the separation between the angles of the incoming beams, and reduced the angular precision required. The team notes that the hologram works with beams of white light, and does not suffer from the 'ghosting' that is apparent with credit card holograms. ■

1. Ozaki, M., Kato, J. & Kawata, S. Surface-plasmon holography with white-light illumination. *Science* **332**, 218–220 (2011).

Unfazed by imperfections

The strong coupling between electrical currents and magnetization in topological insulator materials is surprisingly unaffected by impurities

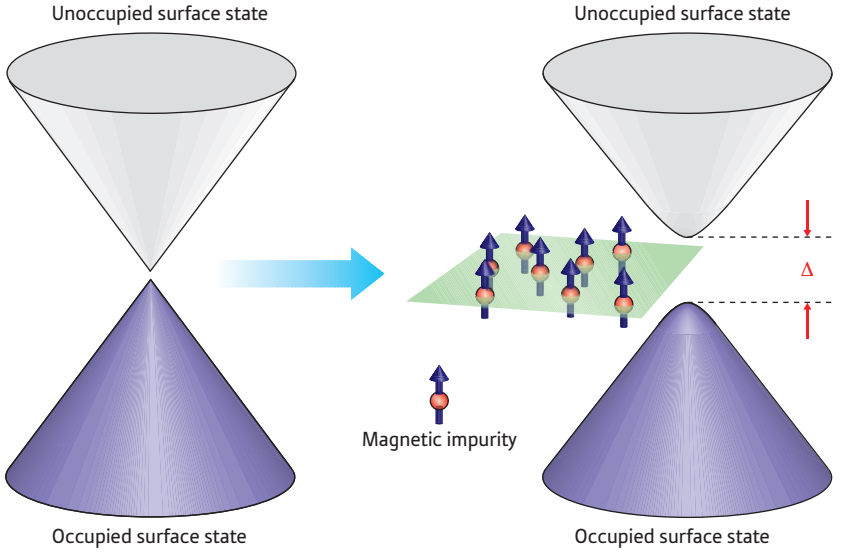

Figure 1: The introduction of magnetic impurities into a topological insulator causes a gap to open in the characteristic double-cone energy structure of the material (the x-axis shows electron momentum, the y-axis shows the energy).

While insulating against electrical currents in their interior, the surface of materials called topological insulators permits the flow of electron spins relatively unhindered. The almost lossless flow of spin information makes topological insulators a promising new class of materials for electronic applications: the electron spins could be harnessed to transmit information in the same way that electrical charges are used in conventional electronics. Electron spins are also susceptible to magnetic fields, so electrical control of magnetization of these materials would offer further control over the properties of electronic devices. Magnetic impurities in these materials, however, have thwarted attempts by experimental physicists to fabricate topological insulators, because they destroy the characteristic energy structure of a topological insulator (Fig. 1).

In a theoretical study, Kentaro Nomura and Naoto Nagaosa from the RIKEN Advanced Science Institute, Wako, have unexpectedly discovered that the electrical control of magnetization in topological insulators is actually enhanced by the presence of magnetic impurities[1]. It may be possible, therefore, to develop novel devices from topological insulators by creating magnetization with electrical fields.

Topological insulators owe their unique properties to time-reversal symmetry: if the flow of time were reversed, the material would behave in the same way. Magnetic impurities break this symmetry, as magnetism is sensitive to time reversal; electrical currents flowing forward and backward in time create magnetic fields pointing in opposite directions. Physicists therefore expected that magnetic impurities would disrupt the magnetization generated by electrical currents on the surface of a topological insulator.

Nomura and Nagaosa's calculations, however, showed that randomly distributed magnetic impurities do not influence the strong coupling between electrical currents and magnetization. Electrical currents at the surface are quantized, which means that they change only in steps. Therefore, a change in the energy structure of the material would not affect the electric current and magnetization. The randomness of the impurities increases the usable energy range, says Nomura. "Usually impurities and disorder smear desired effects. In this case, imperfections enhance them."

This finding is welcome news for experimental physicists working on topological insulators. All samples fabricated to date contain so many impurities that observing spin currents at their surface is almost impossible. The discovery that magnetic impurities should have no detrimental effect improves the likelihood of observing the proposed control of magnetization. Consequently, says Nomura, "a number of experimental groups are already working on this issue. I think this effect will be observed, hopefully soon." ■

1. Nomura, K. & Nagaosa, N. Surface-quantized anomalous hall current and the magneto-electric effect in magnetically disordered topological insulators. *Physical Review Letters* **106**, 166802 (2011).
2. Chen, Y.L., Chu, J.-H., Analytis, J.G., Liu, Z.K., Igarashi, K., Kuo, H.-H., Qi, X.L., Mo, S.K., Moore, R.G., Lu, D.H., *et al*. Massive Dirac fermion on the surface of a magnetically doped topological insulator. *Science* **329**, 659–662 (2010).

Superconductivity's third side unmasked

A previously unknown and unexpected mechanism gives rise to superconductivity in specific types of materials

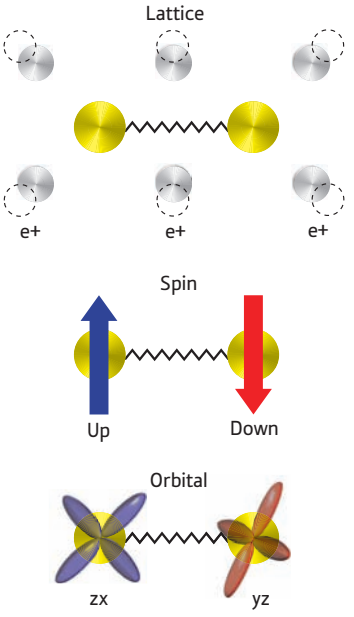

Figure 1: The three types of glue for superconducting electrons: lattice vibrations (top), electron spin (middle), and fluctuations between two electron orbitals (zx and yz) (bottom). The yellow spheres represent Cooper pairs of electrons.

The debate over the mechanism that causes superconductivity in a class of materials called the pnictides has been settled by a research team from Japan and China[1]. Superconductivity was discovered in the pnictides only recently, and they belong to the class of so-called 'high-temperature superconductors'. Despite their name, the temperature at which they function as superconductors is still well below room temperature. Realizing superconductivity at room temperature remains a key challenge in physics; it would revolutionize electronics since electrical devices could operate without losing energy.

Superconductivity in a material arises when two electrons bind together into so-called Cooper pairs. This pairing leads to a gap in the energy spectrum of the superconducting material, which makes the electrons insensitive to the mechanisms causing electrical resistance. Electrons can bind into Cooper pairs in different ways, leading to different categories of superconductors.

Until the work of Takahiro Shimojima from The University of Tokyo and his colleagues, including researchers from the RIKEN SPring-8 Center in Harima, superconducting materials were classified into two broad categories. In classical superconductors, which function at very low temperatures, vibrations of atoms in the crystal lattice of the material provide the necessary glue for the pairing. In cuprates, the original high-temperature superconductor compounds, magnetic interactions based on an electron's spin generate the superconductive pairing (Fig. 1). In the pnictide high-temperature superconductors, physicists assumed that the underlying mechanism was similar to that for the cuprates, but conflicting experimental results meant that the precise mechanism was controversial.

To investigate this debated pairing mechanism of pnictides, the researchers studied the properties of the material's electronic gap. Thanks to a unique set of high-energy lasers based on very rare laser crystals available to only a few laboratories, their experiments resolved these states with unprecedented detail.

Shimojima and colleagues were surprised to discover that interactions between electron spins do not cause the electrons to form Cooper pairs in the pnictides. Instead, the coupling is mediated by the electron clouds surrounding the atomic cores. Some of these so-called orbitals have the same energy, which causes interactions and electron fluctuations that are sufficiently strong to mediate superconductivity.

This could spur the discovery of new superconductors based on this mechanism. "Our work establishes the electron orbitals as a third kind of pairing glue for electron pairs in superconductors, next to lattice vibrations and electron spins," explains Shimojima. "We believe that this finding is a step towards the dream of achieving room-temperature superconductivity," he concludes. ■

1. Shimojima, T., Shin, S., *et al*. Orbital-independent superconducting gaps in iron-pnictides. *Science* published online 7 April 2011 (doi: 10.1126/science.1202150).

Another handy role for pockets

Tiny membrane pockets that gain catalytic activity upon self-assembly in water shed light on biological enzymatic processes

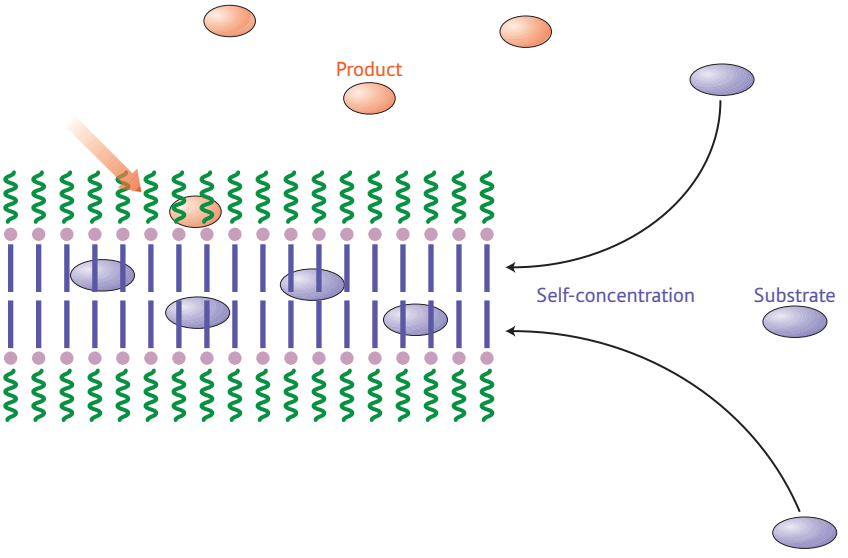

Figure 1: Schematic illustration showing the water-mediated catalysis of organic reactions within the self-assembled vesicle membrane.

Biological membranes play key roles in the body. They determine, for example, how molecules enter and exit cells, and the architecture of their lipid bilayer allows them to host enzymes and enhance their catalytic performance under natural conditions. To clarify the mechanisms that govern these processes, a team of chemists in Japan has generated in water tiny, catalytically active, free-standing membrane pockets, called vesicles, using a self-assembly method based on a small palladium complex[1]. The team was led by Yasuhiro Uozumi from the RIKEN Advanced Science Institute in Wako and the Institute for Molecular Science in Okazaki.

Many researchers have already used ultra-small self-assembled pockets to perform reactions in solution while protecting the reagents from their potentially destructive surroundings. However, unlike Uozumi's vesicles, few of these reaction vessels were 'architecture-based' catalysts; that is, structures that exhibit activity only when self-assembled.

The team's palladium complex is a rigid, planar, pincer-like structure with hydrophilic 'arms' and hydrophobic 'legs'. The different affinity for water and orientation of these functional groups directs vesicle assembly in water. Furthermore, these properties allow the complex to gain unique catalytic activity for specific chemical reactions. "This, conceivably, would approach an artificial enzymatic system," notes Uozumi.

"The vesicle, which bears a hydrophobic inner region, was self-constructed in water, and this inner region served as a reservoir for the substrate," says Uozumi. He explains that the entire reaction system—including the medium, molecular structure of the palladium complex, and substrate—cooperatively governs a 'self-concentration' process. During this process, substrate molecules penetrate the hydrophilic outer shell and accumulate in the hydrophobic reservoir where the reaction takes place (Fig. 1). After a quick catalytic transformation, the product exits the vesicle.

The researchers conducted a series of carbon–carbon bond-forming reactions, which are central to chemical synthesis, in the presence of the vesicles. They found that the vesicles stimulated the transformations in high yields at room temperature in water. The palladium complex was also recoverable in its original, disassembled form after the reaction. When they ran the same experiment in hydrophobic organic solvents, which hinder vesicle formation, no catalysis occurred—proof that water-mediated self-assembly is crucial for the catalytic activity of the complex.

The team is currently developing new catalysts by changing the hydrophilic and hydrophobic groups and substituting palladium for other metal species. It is also applying these catalysts to other organic reactions. These water-enabled transformations will lead to greener and safer approaches to organic chemistry, Uozumi concludes. ■

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Embracing superficial imperfections

Numerical simulations reveal that deliberately engineering defects into ultrathin oxide films enhances catalytic water-splitting reactions

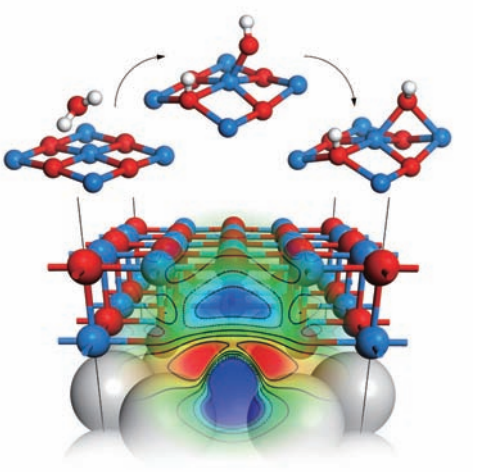

Figure 1: Defects inside an ultrathin magnesium oxide film (red and blue spheres, bottom) accumulate electronic charges (red and blue contour map) and enhance the catalytic dissociation of water molecules (top).

Chemists normally work rigorously to exclude impurities from their reactions. This is especially true for scanning tunneling microscopy (STM) experiments that can produce atomic-scale images of surfaces. Using STM to investigate processes such as catalysis usually requires pristine substrates—any flaws or foreign particles in the surface can critically interfere with the test study. Preconceptions about interface defects and catalysis are about to change, however, thanks to recently published research led by Yousoo Kim and Maki Kawai at the RIKEN Advanced Science Institute in Wako[1].

Through a series of high-level computer simulations, the researchers found that the catalytic splitting of water molecules occurs faster on an ultrathin insulating film containing misplaced atoms than on a non-defective surface. Because water splitting reactions are one of the easiest ways to generate hydrogen fuel, this finding could be a boon to future fleets of hybrid vehicles.

Recently, Kim, Kawai, and colleagues discovered that depositing insulating magnesium oxide (MgO) onto a silver (Ag) substrate enabled extraordinary control over water dissociation reactions[2]. By injecting electrons into the MgO/Ag surface with an STM tip, they were able to excite absorbed water molecules and cause them to sever hydrogen and hydroxide ions. Optimizing the MgO film thickness was a key part of this approach; only ultrathin layers could direct water splitting owing to its enhanced electronic interaction strength[3].

This relationship between insulator thickness and chemical reactivity suggested to the researchers that the oxide–metal interface plays a crucial role in directing catalytic reactions. Engineering specific flaws into the ultrathin interface could be one way to heighten the electronic control over the water-splitting process. However, since artificially manipulating oxide atoms is a difficult experimental procedure, they used density functional theory simulations, based on quantum mechanics, to analyze the role of structural imperfections in MgO.

Surprisingly, the researchers found that three different types of defects—oxygen and magnesium impurities, as well as an oxygen vacancy—improved water adsorption and substantially lowered dissociation energy barriers compared to an ideal MgO film. Further analysis revealed that the oxide defects accumulate charges injected into the substrate (Fig. 1), creating an electronic environment that speeds up the catalytic water splitting. "In the presence of these defects, the film's chemical reactivity can be greatly enhanced," says Kim.

The next goal for the researchers is to find systematic techniques to control interface imperfections on these novel catalytic films—an objective best achieved by the team's unique combined experimental–theoretical approach, notes Kim. ■

1. Jung, J., Shin, H.-J., Kim, Y. & Kawai, M. Activation of ultrathin oxide films for chemical reaction by interface defects. *Journal of the American Chemical Society* **133**, 6142–6145 (2011).
2. Shin, H.-J., Jung, J., Motobayashi, K., Yanagisawa, S., Morikawa, Y., Kim, Y. & Kawai, M. State-selective dissociation of a single water molecule on an ultrathin MgO film. *Nature Materials* **9**, 442–447 (2010).
3. Jung, J., Shin, H.-J., Kim, Y. & Kawai, M. Controlling water dissociation on an ultrathin MgO film by tuning film thickness. *Physical Review B* **82**, 085413 (2010).

Stopping malignancy in its tracks

A newly discovered natural product produced by a fungus prevents cancer cells from becoming malignant

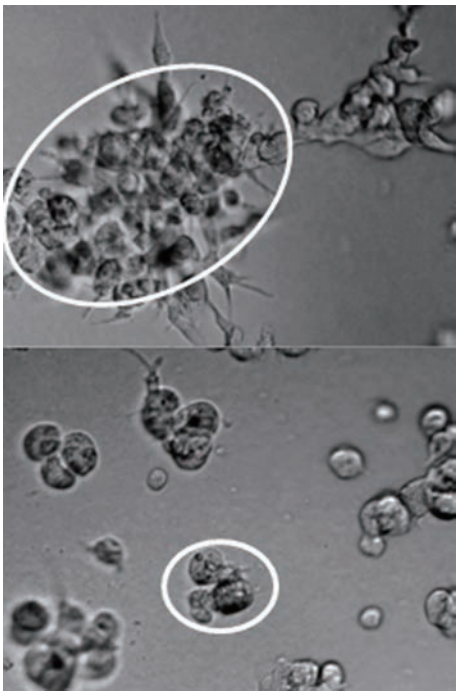

Figure 1: Cancer cells normally form colonies (top), but not when newly discovered natural product fusarisetin A is added to the culture (bottom).

An unusual chemical compound isolated from a mud-dwelling fungus found in a soil sample collected in Daejeon, South Korea, could lead to a new family of antitumor drugs. Discovered by teams led by Jong Seog Ahn at the Korea Research Institute of Bioscience and Biotechnology (KRIBB), Ochang, and Hiroyuki Osada at the RIKEN Advanced Science Institute, Wako, the compound prevents cancerous cells from forming mobile colonies—the point at which cancers become malignant and spread through the body[1]. The teams began collaborating after Yukihiro Asami from RIKEN joined KRIBB.

The researchers spotted the compound while searching extracts of the fungus for candidate drug compounds using a recently developed screen called a 3D epithelial culture system. To date, this kind of biological assay has rarely been used to search for natural products with novel bioactivity, says Ahn. It was during the 3D screen, which they spiked with cancerous cells, that the researchers realized that a compound produced by the fungus was inhibiting the cancer cells from clumping together to form colonies (Fig. 1). This type of screen is difficult using a conventional two-dimensional cell culture.

The researchers isolated the bioactive compound and named it fusarisetin A. They then investigated its structure using an array of chemical characterization techniques, including nuclear magnetic resonance (NMR) and X-ray crystallography. They showed that fusarisetin A was a previously undescribed compound. Being able to grow crystals of the compound for X-ray studies was a breakthrough, says Osada. "It is very important for exact structural elucidation to get crystal analysis," he says.

Having established that fusarisetin A is a new compound, the researchers probed its bioactivity in more detail. They showed that it simply blocks colony formation rather than killing cancer cells. They then compared the compound to others known to inhibit this process, and showed that it works differently to other compounds capable of blocking clumping. This suggests to the researchers that it could offer a new way to treat tumors.

The team is already working to discover how fusarisetin A inhibits the clumping of cancerous cells by looking for its molecular target. "We have already got candidate target proteins," Osada adds.

Fusarisetin A itself is not bioactive enough to become a drug. However, it may be possible to fine-tune the structure to improve its activity, from which new drugs could be developed. "If we can get higher biological activity derivatives [of fusarisetin A], it may be possible," says Ahn. ■

1. Jang, J.-H., Asami, Y., Jang, J.-P., Kim, S.-O., Moon, D.O., Shin, K.-S., Hashizume, D., Muroi, M., Saito, T., Oh, H., Kim, B.-Y., Osada, H. & Ahn, J.-S. Fusarisetin A, an acinar morphogenesis inhibitor from a soil fungus, *Fusarium* sp. FN080326. *Journal of the American Chemical Society* **133**, 6865–6867 (2011).

Preventing overreactions

Identification of the transcription factor that regulates a protein that dampens immune responses could aid the fight against autoimmune disease

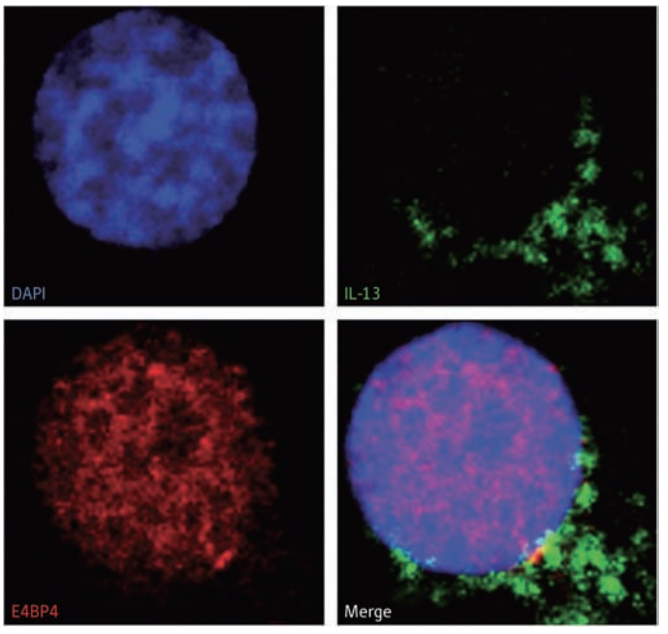

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Figure 1: In T cells stained blue (top left), the transcription factor E4BP4 (red) regulates that production of IL-13 (green) and IL-10 (not shown).

Interleukin-10 (IL-10) is an anti-inflammatory cytokine protein that reduces immune responses and staves off autoimmune disease. Now, a research team led by Masato Kubo at the RIKEN Research Center for Allergy and Immunology, Yokohama, has identified a transcription factor called E4 promoter-binding protein (E4BP4) that is responsible for driving the expression of IL-10 in multiple types of immune cells[1].

The researchers investigated E4BP4 because of a unique property of a subset of immune cells called T helper type 1 (T_H1) cells, which generally enhance immune responses by secreting pro-inflammatory cytokines. However, under chronic stimulation with foreign antigens—that occur during chronic infection—T_H1 cells can also produce cytokines, such as IL-10 and IL-13, which are normally made only by other immune-cell types. While the immune system is fighting the infection, IL-13 modulates allergic responses, and IL-10 prevents the immune system from attacking the body.

Kubo and colleagues compared genes expressed in T_H1 cells with and without chronic antigen stimulation, and found that E4BP4 was expressed only in instances of chronic antigen stimulation. When they expressed E4BP4 in T_H1 cells that had not been chronically infected, it induced production of IL-10 and IL-13 in conditions in which those cytokines would not normally occur (Fig. 1). E4BP4-deficient T_H1 cells could not increase expression of IL-10 and IL-13 after chronic antigen stimulation. The researchers found that other T cell subsets also required E4BP4 to modulate the expression of IL-10, but not IL-13.

Transcription factors can control the expression of genes by binding to a region on the genomic DNA called the promoter. Kubo and colleagues observed that E4BP4 bound to the IL-13 promoter in T_H1 cells that had been chronically stimulated with antigen. No binding occurred with T_H1 cells lacking chronic stimulation. Kubo explains, however, that: "E4BP4 seems to regulate the expression of IL-10 in a totally different way—by altering the chromosomal structure in the region of that gene."

Mice lacking IL-10 can spontaneously develop intestinal autoimmune disease. Interestingly, Kubo and his team found that E4BP4-deficient mice produced lower levels of IL-10 than control mice, and showed some symptoms of gastrointestinal inflammation along with diarrhea. The mice lacking E4BP4 also developed more severe symptoms of a neurological autoimmune disease caused by exposure to brain antigens. E4BP4 is therefore a key factor in preventing the immune system from attacking the body's own organs, and "induction of expression of E4BP4 may cure many types of autoimmune inflammatory diseases," says Kubo. ■

1. Motomura, Y., Kitamura, H., Hijikata, A., Matsunaga, Y., Matsumoto, K., Inoue, H., Atarashi, K., Hori, S., Watarai, H., Zhu, J., Taniguchi, M. & Kubo, M. The transcription factor E4BP4 regulates the production of IL-10 and IL-13 in $CD4^+$ T cells. *Nature Immunology* **12**, 450–459 (2011).

Awaiting orders to retaliate

Signaling proteins that help immune cells develop also enable those cells to mount an effective counterattack against infections

When immune system B cells are alerted to the presence of a threat within the body, they form structures called germinal centers, which serve as ad hoc headquarters for marshaling a targeted immune response. These cells subsequently differentiate into plasma cells, which produce antibodies directed against foreign entities, or memory cells, which retain the capacity to become plasma cells if the same threat reappears in the future.

The extracellular signal-regulated kinase proteins (ERK1/2) are integrally involved in the early stages of this process, making it a challenge to assess their subsequent contributions. "If we delete both ERK genes entirely, differentiation of B cells is impaired and we cannot analyze the function of ERKs during the immune response," explains Kohei Kometani, a researcher in Tomohiro Kurosaki's group at the RIKEN Research Center for Allergy and Immunology, Yokohama.

To address this challenge, Tomoharu Yasuda and Kometani developed transgenic mice in which ERK expression is lost only after the initial differentiation of B cells[1]. Their initial results were striking; following vaccination with a highly immunogenic antigen, ERK-deficient mice showed a 10- to 40-fold reduction in antibody production (Fig. 1). This selective deletion of the two ERK genes led to a sharp decrease in the number of antigen-specific plasma cells but had little effect on memory cell counts. The researchers determined that these signaling factors appear to directly facilitate plasma cell maturation. "It was surprising that ERKs regulate differentiation but do not affect cell proliferation, because many people think of the ERKs as important molecules for cell growth," explains Kometani.

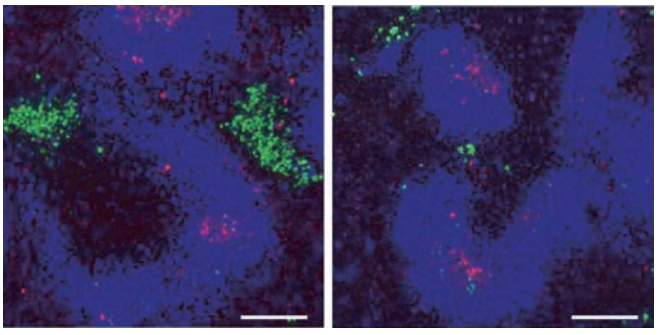

Figure 1: Fluorescently labeled spleen tissue from wild-type (left) and ERK-deficient (right) mice reveals a dramatic difference in the production of antibodies (green) in response to an antigen challenge. Blue indicates expression of B220, a B cell specific marker, while red indicates labeling for a marker commonly found in germinal centers. Scale bars, 200 µm.

Several proteins known as transcription factors contribute to the maturation of plasma cells by turning key genes on or off. Blimp-1 is among the most important of these, as it also helps to inhibit transcription factors that maintain germinal center B cells. Yasuda and Kometani determined that the gene encoding Blimp-1 is a primary target of ERK signaling. They also identified another protein, Elk-1, which appears to be an important intermediary in this process.

As their findings also indicate that other signaling pathways are likely to intersect with ERK signaling in this developmental process, Yasuda and colleagues hope to explore this complexity in the future. "Harmful or excess antibody production are sometimes the cause of autoimmunity and allergy," he says, "and from this point of view, it may be interesting to check the involvement of not only the ERKs, but also the molecules upstream and downstream." ■

1. Yasuda, T., Kometani, K., Takahashi, N., Imai, Y., Aiba, Y. & Kurosaki, T. ERKs induce expression of the transcriptional repressor Blimp-1 and subsequent plasma cell differentiation. *Science Signaling* **4**, ra25 (2011).

Pinpointing a tell-tale mark of liver cancer

A newly identified gene variant could lead to predictive tests for a major cause of cancer-related deaths

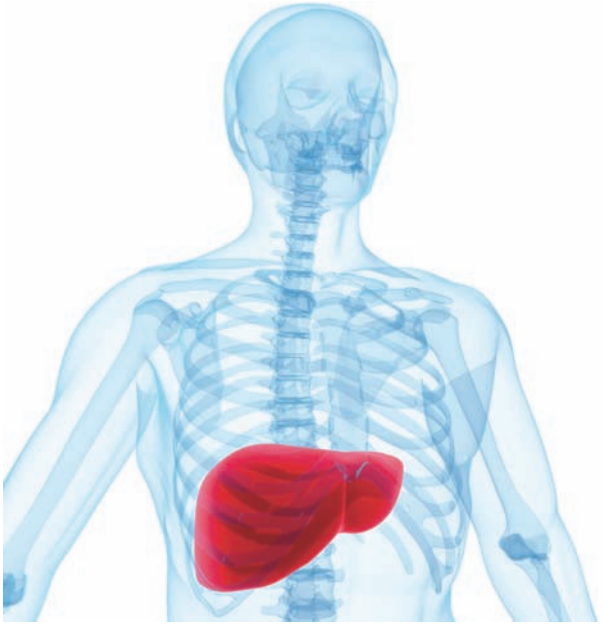

Figure 1: Individuals who carry a specific genetic variant and become infected with the hepatitis C virus could be susceptible to developing liver cancer.

Persistent hepatitis C virus (HCV) infection can lead to chronic hepatitis C and then progress to fatal liver diseases including liver cirrhosis and liver cancer, the third most common cause of cancer-related deaths. Worldwide, more than 170 million people are infected with HCV, and the virus accounts for 30–70% of liver cancer cases. The recent identification of a genetic variant associated with increased susceptibility to hepatitis C virus-induced liver cancer could have major implications for global healthcare, as it may lead to tests that predict liver cancer susceptibility.

Michiaki Kubo of the RIKEN Center for Genomic Medicine and colleagues from RIKEN and The University of Tokyo discovered the variant by analyzing the entire genomes of 721 Japanese individuals with HCV-induced liver cancer and comparing them with those of 2,890 HCV-negative controls[1]. This allowed them to identify variants potentially associated HCV-induced liver cancer (Fig. 1). They confirmed the association of one variant by replicating the study in another 673 liver cancer patients and 2,596 controls.

This variant was located within a region on chromosome 6, which contains many genes that are critical for immune system function. It lies between the genes encoding MICA, a membrane protein that activates the anti-tumor effects of white blood cells, and the *HLA-B* gene, which encodes a peptide that enables the immune system to distinguish between the body's own proteins and those produced by invading microbes.

The researchers checked the variant in another 1,730 individuals with chronic hepatitis C who had not developed liver cirrhosis or liver cancer, and revealed that it was significantly associated with progression from chronic hepatitis C to liver cancer, but not with susceptibility to chronic hepatitis C.

Finally, Kubo and colleagues examined MICA protein levels in patients with chronic hepatitis C and HCV-induced liver cirrhosis, and found that the level of MICA in blood samples was elevated during early stages of the disease compared to healthy controls. They also found that the identified variant was correlated with low MICA levels in patients with chronic hepatitis C.

These findings suggest that individuals carrying the genetic variant would express low levels of MICA. This in turn would lead to reduced response by white blood cells to cells infected with viruses, increasing the likelihood of progression from chronic hepatitis C to liver cancer.

"Our results suggest that low serum MICA levels are a marker for higher susceptibility to progression of liver cancer in the patients with chronic hepatitis C," says Kubo. ■

1. Kumar, V., Kato, N., Urabe, Y., Takahashi, A., Muroyama, R., Hosono, N., Otsuka, M., Tateishi, R., Omata, M., Nakagawa, H. *et al*. Genome-wide association study identifies a susceptibility locus for HCV-induced hepatocellular carcinoma. *Nature Genetics* **43**, 455–458 (2011).

Unraveling the ins and outs of brain development

Brain structure is maintained during development by two distinct mechanisms that regulate the production and movement of cells

The embryonic nervous system is a hollow tube consisting of elongated neural progenitor cells, which extend from the inner to the outer surface of the tube. In a section inside the tube called the ventricular zone (VZ), these cells divide and produce immature neurons that migrate outwards. This involves well-characterized movements that are coupled to cell division. After a cell divides at the inner-most VZ region, the nuclei migrate to the outer region, where they synthesize new DNA before returning.

To determine how the direction of movement is coupled to the cell division cycle, Yoichi Kosodo and colleagues in Matsuzaki's group labeled nuclei in the embryonic mouse brain with green fluorescent protein[1]. This enabled them to not only track their movements in cultured brain slices using a video-imaging system, but also correlate their positions with phases of the cell cycle. They found that outward nuclear migration involves back and forth 'ratcheting' motions and occurs more slowly than inward migration.

Importantly, they discovered that blocking the cell cycle before DNA synthesis caused nuclei to accumulate at the outer VZ surface (Fig.1), and reduced outward migration. Nuclei migrating back inwards normally crowd out those just finished dividing, thus pushing them away from the inner VZ surface.

Examining their results further, the researchers computationally modeled nuclear migration, and incorporated fluorescent magnetic beads into the inner VZ surface. They observed the beads moving away from the inner VZ surface, and remaining at its outer region.

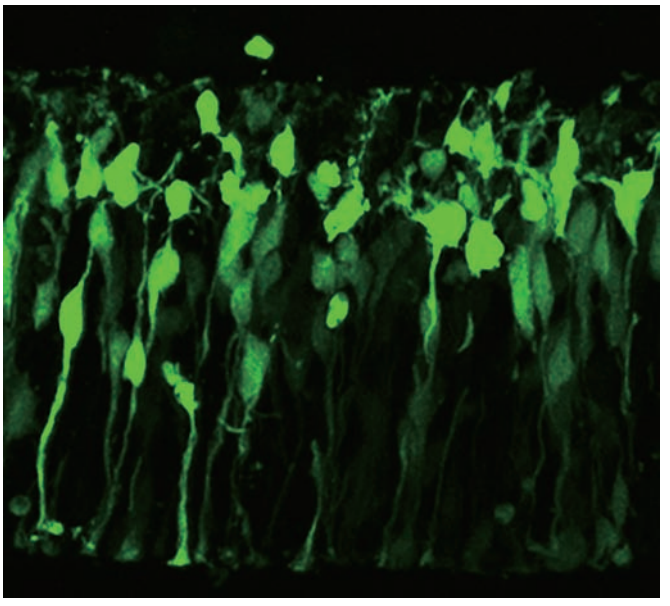

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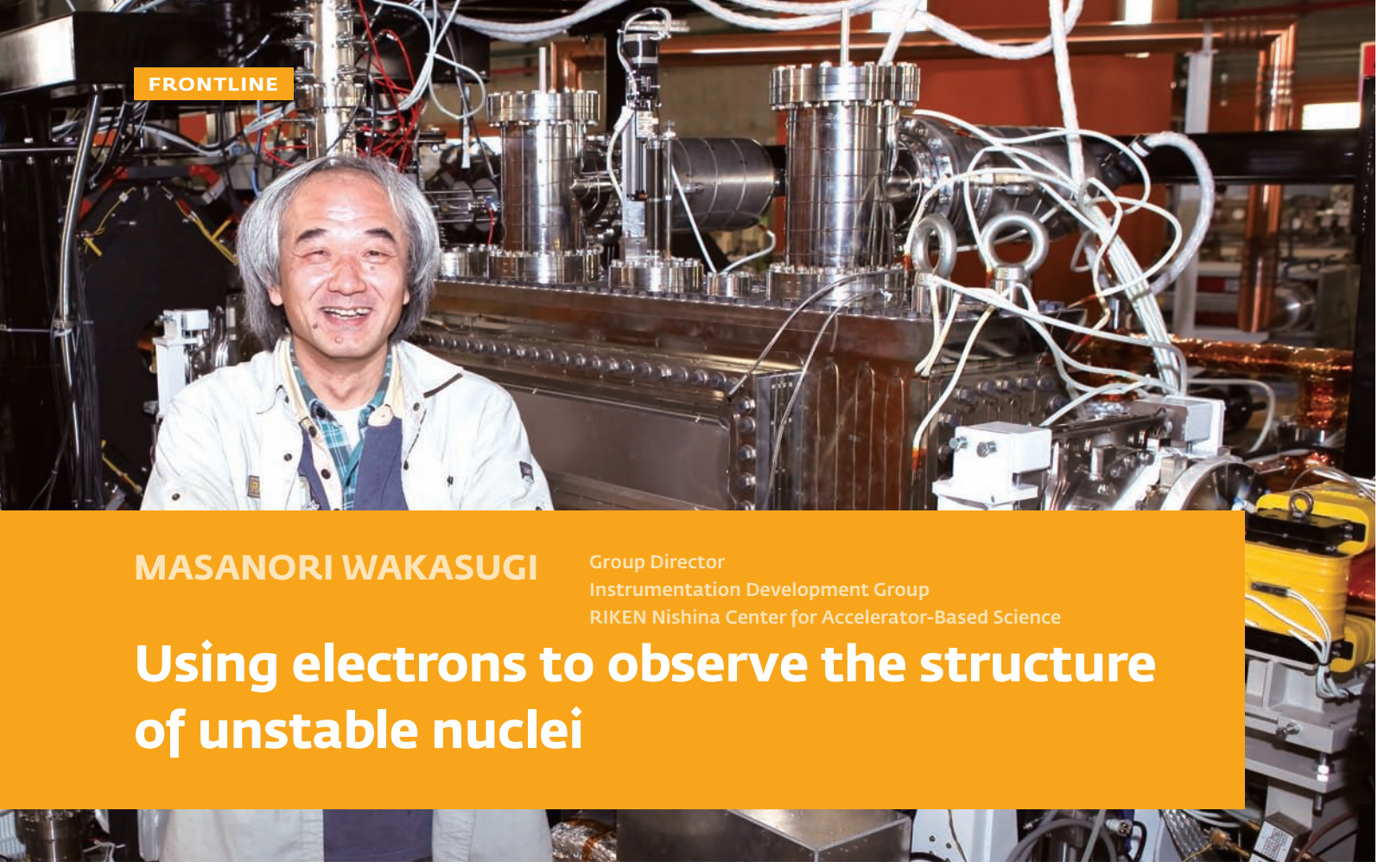
Figure 1: Cell nuclei of brain cells accumulate at the outer surface of the ventricular zone when the cell cycle is blocked.

The researchers also showed that inward migration is closely linked to microtubule reorganization orchestrated by a protein called Tpx2, which is initially expressed in the nuclei of progenitors before moving to the mitotic spindle. This separates newly duplicated chromosomes. Translocation of Tpx2 to the cell region nearest the inner VZ surface promotes migration of the nucleus in that direction by microtubule re-organization. Reducing Tpx2 activity lowered the velocity of inward migration, but introducing the human Tpx2 gene into the cells lacking Tpx2 restored normal speed.

The researchers conclude that two mechanisms maintain brain structure during development. One couples cell migration to the cell cycle, and occurs independently of other cells, with Tpx2 providing an active driving force; and the other involves interactions between the nuclei in the VZ. ■

1. Kosodo, Y., Suetsugu, T., Suda, M., Mimori-Kiyosue, Y., Toida, K., Baba, S. A., Kimura, A. & Matsuzaki, F. Regulation of interkinetic nuclear migration by cell cycle-coupled active and passive mechanisms in the developing brain. *The EMBO Journal* **30**, 1690–1704 (2011).



MASANORI WAKASUGI

Group Director
Instrumentation Development Group
RIKEN Nishina Center for Accelerator-Based Science

Using electrons to observe the structure of unstable nuclei

Observing the structure of collapsing unstable atomic nuclei using electrons is an experimental goal that has not been achieved anywhere in the world. Masanori Wakasugi, director of the Instrumentation Development Group at the RIKEN Nishina Center for Accelerator-Based Science (RNC), is working on this challenging issue. The current theoretical model of the atomic nucleus has been constructed with major contributions from electron-scattering experiments, in which electrons are collided with stable atomic nuclei to visualize the nuclear structure. In recent years, however, a wide range of experiments on the properties of unstable atomic nuclei has revealed a number of phenomena that are inconsistent with the current model of the atomic nucleus. Radioisotope–electron scattering experiments in which electrons collide with unstable nuclei are indispensible in establishing the ultimate model of the atomic nucleus, which will yield a comprehensive understanding of both stable and unstable nuclei. Wakasugi and his colleagues are taking unique approaches to achieve this world-first experiment.

Using fast electrons to visualize atomic nuclei

Use of an electron microscope to fire accelerated electrons at a sample and capture how they are scattered allows us to 'see' the atoms. However, the atomic nucleus is invisible even under electron microscopy, since it is only about one 10-thousandth of the diameter of an atom (Fig. 1). "We cannot see the atomic nucleus without accelerating the electrons and firing them at the nucleus, and this requires a particle accelerator," says Wakasugi.

In the 1950s, US physicist Robert Hofstadter succeeded in visualizing the atomic nucleus and determining the distribution of protons in his electron scattering experiment, in which electrons were accelerated using a particle accelerator and fired at stable nuclei. By subtracting the proton distribution from the entire atomic nucleus, the distribution of neutrons was also inferred. Hofstadter became the joint winner of the 1961 Nobel Prize in Physics for his contributions to clarifying the structure of the atomic nucleus.

Wakasugi continues, "Another approach is available for watching and characterizing a particular atomic nucleus, which involves making it collide with other atomic nuclei. However, this method unavoidably involves making assumptions in the interpretation of the resulting experimental data, as the strong interactions between different atomic nuclei have not yet been fully elucidated. On the other hand, the theory of electromagnetic interaction between electrons and atomic nuclei is well-established, so the data obtained by electron scattering experiments is absolutely undisputable. In this way, a theoretical model of the atomic nucleus was developed to explain the data from electron scattering experiments using stable nuclei."

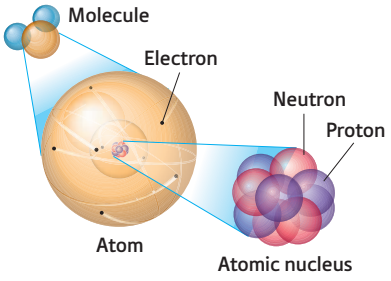


Figure 1: The atom and the atomic nucleus.
Consisting of a nucleus and electrons moving in various orbits around it, an atom measures approximately 0.1 nanometers across. An atomic nucleus consists of protons and neutrons, each measuring approximately 0.1 picometer in diameter.

Firing electrons at unstable nuclei

The 1980s saw the development of technology for generating a beam of unstable nuclei (radioactive isotopes) using a particle accelerator, and researchers began experimentally investigating the properties of unstable nuclei, leading to the discovery of phenomena undermining the current model of the atomic nucleus.

A brief explanation of the term 'radioisotope' is needed here. Ninety different elements, from hydrogen to uranium, have been found in nature. Taking into account the 'isotopes' of each element, which contain differing numbers of neutrons, about 300 kinds of stable nuclei occur naturally. These share an identical number of protons and nearly the same number of neutrons, both of which constitute the atomic nucleus. In theory, however, about 10,000 kinds of atomic nuclei can occur, depending on the combination of the numbers of protons and neutrons. Most of these have an excess number of either protons or neutrons, soon collapsing into other kinds of atomic nuclei. An isotope with a nucleus that is prone to collapse is a radioactive isotope.

"The current theoretical model of the atomic nucleus has been built on the basis of experimental data on stable nuclei, which account for only 3% or so of the about 10,000 different atomic nuclei. To achieve a comprehensive understanding of all atomic nuclei, experiments must be conducted to elucidate the properties of radioisotopes, which account for the majority of the possible nuclei."

Currently, the properties of radioisotopes are being investigated through experiments in which a radioisotope beam is fired at well-characterized stable nuclei. However, no-one in the world has succeeded in a radioisotope-electron scattering experiment, firing electrons at radioisotopes. "For a radioisotope-electron scattering experiment to be conducted using conventional methods, estimates have suggested that a vast number of radioisotopes would be required; specifically more than 10^{20}, that is, more than one hundred million trillion radioisotopes. In addition, that number of radioisotopes could not be produced even if a state-of-the-art particle accelerator were in operation continuously for a hundred years, so radioisotope-electron scattering experiments have generally been accepted as unfeasible, despite being essential for building the ultimate model of the atomic nucleus."

In the 1990s, researchers at RIKEN were working on drawing up a project to construct the Radioactive Isotope Beam Factory (RIBF), an accelerator facility to produce a wide variety of new radioisotopes for extensive examination. These researchers started to wonder of radioisotope-electron scattering experiments might be performed by producing a large number of radioisotopes using the RIBF, and improving the method for achieving collisions with electrons.

Since radioisotope beams from the RIBF are dispersed and thick and produce radioisotopes that move with a very diverse range of speeds and directions, use of beam as it is results in a very low probability of collision with electrons. "As a solution, researchers considered circulating a radioisotope beam in an accumulation ring to obtain a uniform speed and direction of movement, and to focus the beam. By arranging for an electron beam to collide with the radioisotope beam head-on, the probability of collision can be increased." The collider that was used to achieve this was named the Multi-USe Experimental Storage Rings (MUSES), and Wakasugi joined the developmental team.

"Initially, the construction cost for MUSES was estimated at about 40 billion yen. In addition, the experiments require sophisticated techniques, and it was recognized that adjustments would be required for many years before starting actual experiments even after completion of the facility. I began to look for a method that would allow us to collide radioisotopes and electrons at lower cost with simpler procedures."

In 2000, Wakasugi began investigating a new method for radioisotope-electron scattering experiments in cooperation with Yasushige Yano, senior advisor at RNC (the first director of RNC). "The RIBF produces fast radioisotope beams. Circulating such a beam requires a huge accumulation ring, which costs a vast amount of money. With this in mind, we considered a system based on a "slow radioisotope beam." The accumulation ring would be dramatically smaller, allowing a major cost reduction. However, getting the electron beam to collide with the radioisotope beam head-on is still difficult. Since both the electrons and radioisotopes are moving in beams, controlling both is quite tricky. So we proceeded to investigate a method in which the radioisotopes were allowed to stand, and only the electrons were fired as a beam to collide with the radioisotopes."

To achieve this, the radioisotopes must be introduced into the orbit in which the electron beam is running, and allowed to accumulate. "One day, while discussing with Yano, I concluded that if radioisotopes were ionized to form cations and cast into the electron beam axis, they would be attracted by the negative charge of the electron beam and would accumulate in the electron beam orbit. In reality, the suspected phenomenon proved to be "ion trapping," a commonly known phenomenon in the field involving the use of electron beams."

The accumulation ring for circulating an electron beam has to be maintained in a vacuum so as to prevent the electrons from colliding with other particles. However, a complete vacuum is difficult to achieve; gases leaking from the inner wall of the system are stripped of their electrons by the electron beam and form cations, which are then attracted by the negative charge of the electron beam, resulting in the accumulation of non-radioisotope cations in the orbit.

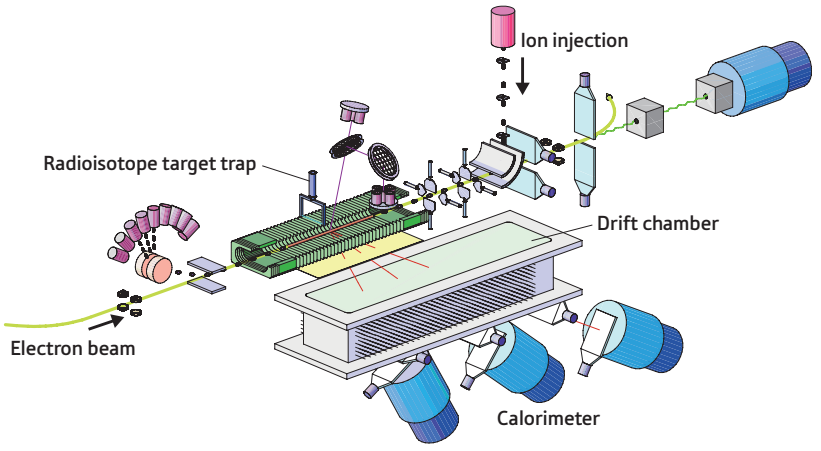


Figure 2: Configuration of the SCRIT-based radioisotope-electron scattering system.
Ionized radioisotopes are injected, then an electric field is applied using the radioisotope target trap to force the ions to enter and accumulate in the electron beam orbit. Electrons scattered by collision with the radioisotope ions are measured using a drift chamber and calorimeter to determine the precise distribution of radioisotope protons.

"These non-radioisotope cations that accumulate in the orbit are a nuisance because they collide with the electron beam and interfere with its stable circulation. In the electron accumulation ring, such interference is avoided by applying an electric field to remove the cations. By ionizing the radioisotopes into cations, it should be possible to guide them into the electron beam orbit and let them accumulate, applying the same electric field as the one used to eliminate the radioisotopes, and following the reverse process to elimination."

No success for more than three years

Wakasugi and his colleagues named the new method 'Self-Confining Radioactive Isotope ion Target (SCRIT)', and filed a patent application in 2002. RIKEN subsequently changed their method for radioisotope-electron scattering experiments from MUSES to SCRIT. Wakasugi and his colleagues performed extensive simulation calculations for SCRIT. They published a paper presenting theoretical evidence for the feasibility of SCRIT in 2004, and decided to conduct demonstration experiments (Fig. 2). "However, no budget was available to build the new electron accumulation ring that is indispensible for the demonstration experiments, so we had to borrow an existing facility. We visited many facilities throughout Japan, but most of the electron accumulation rings in actual operation in Japan are designed to utilize the radiation from electron beams. Since the demonstration experiments for SCRIT require the electron accumulation ring to be reformed, we were initially unable to find a lender. Luckily, however, we were allowed to use the Kaken Storage Ring, an electron storage ring, thanks to Prof. Akira Noda at the Kyoto University Institute for Chemical Research."

Thus in 2004, Wakasugi started demonstration experiments for SCRIT using stable nuclei. "If an electric field were successfully applied, an ion beam could be introduced into the electron beam to allow radioisotopes to accumulate in the electron beam orbit. In reality, however, repeated attempts for more than three years did not produce a single successful result. I used to go to Kyoto several times a year, and on my way back here after no success, I was always thinking of what excuse I could give to Yano. Despite these discouraging results, Yano never said, "Stop doing it." This was because Yano and I were certain that we were on the right track."

At last, in April 2007, the momentous day came. "It was the night before I had to return to Tokyo. I had tried many methods, but all had failed. There was nothing more I could do. Helplessly, and with no expectations of success, I halved the energy of the incident ions. The ions entered the electron beam orbit, and a signal indicating a collision with the electrons appeared on the monitor. For just an instant I was astonished, and spluttered 'What?' Then I realized 'So that's how it was!' and understood the reason for my success."

As the incident energy was decreased, the relationship between the potential gradient applied to create the ion incidence orbit and the potential produced by the electron beam changed to an extent that allowed the ions to enter the electron beam orbit and accumulate (Fig. 3B).

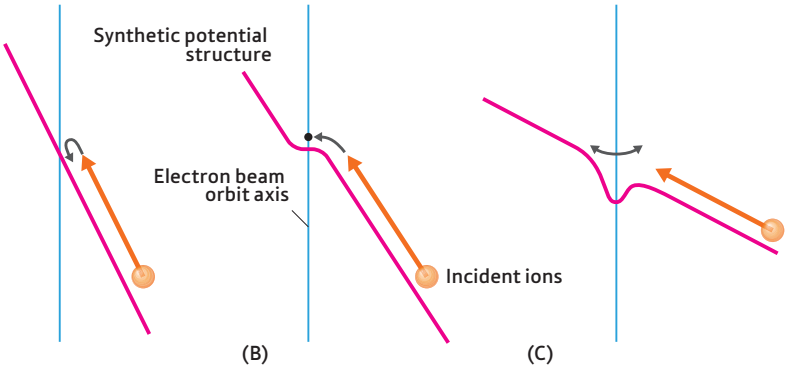


Figure 3: How to accumulate ions in the electron beam orbit.
Ion incident energy alters the relationship between the potential gradient for ion injection and the potential from the electron beam (synthetic potential structure). If the incident energy is appropriate, the ions will move into the synthetic potential structure and accumulate in the electron beam orbit.

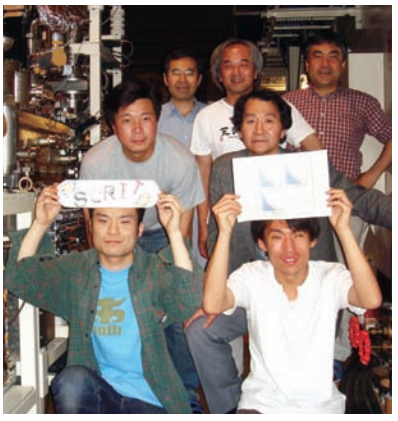


Figure 4: Lab members celebrating the success of the SCRIT demonstration experiment.

April 28, 2007, midnight. A world-first in the development of a key component for the precise determination of proton distributions in unstable atomic nuclei.

"I immediately emailed Yano, and he replied, "Bravo! Three cheers!" (Fig. 4).

Wakasugi and his colleagues then performed further experiments. "In December of that same year, we found that radioisotope-electron scattering experiments could be successful with only 10^6, or one million, radioisotopes available."

The project for building the actual machine for SCRIT-based radioisotope-electron scattering system then started. "The next problem was securing a budget. Just at the right time, Sumitomo Heavy Industries offered RIKEN their electron accumulation ring (AURORA-2S), which was due to be closed down, at no cost. In the summer of 2008, we dismantled the AURORA-2S and transferred it to RIKEN. Even so, we had no budget for assembling the ring. The Lehman shock occurred in the fall of that year, and the Government of Japan drew up a second extra budget as an economic measure, which luckily included an allocation for assembling the ring."

Establishing standards for radioisotope-electron scattering experiments

In 2009, assembly of the electron accumulation ring was completed, and the ring's performance was verified. Installation work on the radioisotope target trap for accumulating radioisotopes in the electron beam orbit was completed in 2010 (Fig. 5). Wakasugi and his colleagues are currently working on preliminary experiments and adjustments in preparation for performing a radioisotope-electron scattering experiment.

Meanwhile, how will the slow radioisotope beam needed for SCRIT be generated? "Gamma rays will be fired at uranium to produce radioisotopes and generate a slow radioisotope beam. We are planning to start construction work on the necessary system in fiscal 2012, with the aim of becoming the world's first research establishment to conduct a radioisotope-electron scattering experiment."

The Institute for Heavy Ion Research (Gesellschaft für Schwerionenforschung) in Germany is planning a project for radioisotope-electron scattering experiments using a particle collider like MUSES, but construction work on the facility has not been scheduled. For a time, RIKEN's SCRIT looks like being the world's only facility able to perform radioisotope-electron scattering experiments.

"The estimated construction cost and space for our SCRIT-based RI-electron scattering system are less than one-tenth of those required for a collider, and existing electron accumulation rings can be utilized after modification. As we obtained an accumulation ring at no cost, the construction costs are likely to be less than one billion yen. In addition, SCRIT is technically straightforward. If our experiments prove successful, SCRIT-based radioisotope-electron scattering experiments could begin all over the world. Our mission is to demonstrate the feasibility of SCRIT-based radioisotope-electron scattering experiments to the world, establish standards for how to carry out radioisotope-electron scattering experiments and analyze the resulting data by making extensive measurements using tin-132 as the reference isotope."

Figure 5: The SCRIT-based radioisotope-electron scattering system under development and preliminary operation.

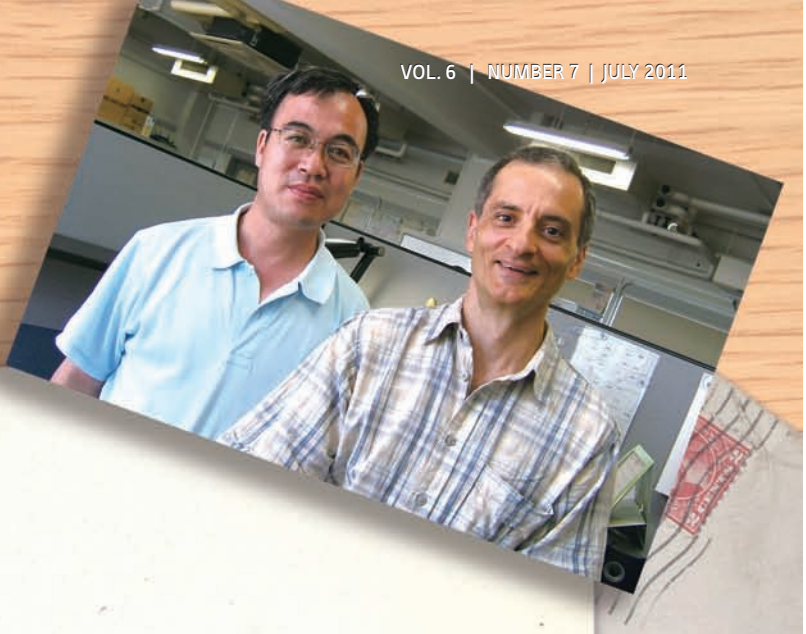
The world's first radioisotope-electron scattering experiment is aimed at colliding radioisotope ions and electrons by accumulating radioisotope ions within the electron beam orbit.

The method involving firing gamma rays at uranium enables the creation of several hundred kinds of slow radioisotope beams. On the other hand, at the RIBF, which went into full operation in 2007, researchers are planning to create 4,000 kinds of radioisotopes. In the future, it will be possible to conduct radioisotope–electron scattering experiments by decelerating a fast radioisotope beam generated at the RIBF and introducing it into the SCRIT system. Based on direct measurement of the proton distribution (proton wave function) by performing electron scattering experiments for a wide variety of radioisotopes, the ultimate model of the atomic nucleus will be constructed, allowing a comprehensive explanation of all kinds of atomic nuclei.

"In the development of SCRIT, fortune smiled on us at important turning points with perfect timing. As Yano said to me, "History is moving in our direction!" ■

ABOUT THE RESEARCHER

Masanori Wakasugi was born in Oita, Japan, in 1961. He graduated from the Faculty of Sciences at Hokkaido University in 1984, and obtained his PhD from Hiroshima University in 1990. After one year in a postdoctoral position at the Cyclotron Laboratory in RIKEN, he became a research scientist at RIKEN. He moved to a position as a research scientist at the RIKEN Nishina Center for Accelerator-Based Science in 2006, and was later promoted to group director of the Instrumentation Development Division. His research focuses on the development of experimental systems for electron scattering using short-lived unstable nuclei.



Dr Franco Nori
Team Leader
Digital Materials Team
RIKEN Advanced Science Institite
Wako-shi, Saitama, Japan

Dear Prof. Nori,

Although I left your research group about two years ago, I still feel that I am a member of your group and of the great RIKEN family. Indeed, my wife is also a RIKEN researcher, so RIKEN is like a family to me. I still pay close attention to all publications from your group, and when I see new results I always feel very proud of the exciting science being conducted there. The quiet and beautiful RIKEN campus is always in my mind—the scenery and green areas there are quite pretty.

RIKEN is a great place to do scientific work and it attracts many high-level scientists. I feel privileged to have had the opportunity to grow professionally there. The quality of my research work grew enormously after spending several years in your group. Your input and feedback on our projects was very helpful to me and to all members of the group. I still remember the many long hours you were working, including nights, weekends and holidays...all the time. Several discussion sessions lasted many hours. Even our seminars often lasted several hours, with very many questions.

I am now creating my own group in Tsinghua University, one of the best universities in China. I have to start my own group here, and the experience I gained by seeing how you started your group at RIKEN, from scratch, helps considerably in guiding me on how to start my group here.
I was very fortunate to have worked with you in the past and hope that we can continue our collaboration and make more contributions to science.

Best wishes,

Yu-xi Liu
Professor, Institute of Microelectronics
Tsinghua University
Beijing 100084, China

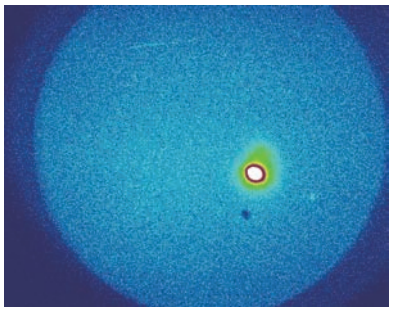
First X-ray lasing of SACLA

The world's second X-ray Free Electron Laser (XFEL) recently went online in Japan, hot on the heels of the first, the Linac Coherent Light Source (LCLS) at the SLAC National Accelerator Laboratory in the US, which began operating in the hard X-ray region in 2009.

The advent of lasers in 1960 led to a fundamental change in photon science and technology due to the unprecedented intensity, high degree of coherence and narrow pulse width of the light that lasers can emit. Since then, tremendous efforts have been made toward creating shorter-wavelength lasers in the hard X-ray region, with expectations for fundamental changes in X-ray science and technologies similar to those seen in the infrared, visible and ultraviolet spectral regions. As the spatial resolution of observations using light is directly related to the wavelength of light used, one of the biggest advantages of using shorter wavelength, X-ray light is the significant resolution enhancement is provides—allowing observation of subnanometer-scale structures such as atoms and molecules.

X-ray lasers cannot be built with the same technologies used to create conventional, longer-wavelength lasers. Accelerator-based free-electron lasers, however, using a self-amplified spontaneous emission (SASE) scheme, are able to generate coherent electromagnetic radiation in the X-ray region. The SASE X-ray Free Electron Laser consists of a high-performance electron linear accelerator (LINAC) and a long undulator, in which high-energy, high-density, low-emittance electron bunches are alternatively deflected in a periodic magnetic field, causing them to emit quasi-monochromatic X-rays at an energy determined by the electron energy, the magnetic field strength and the magnetic period. The interaction between the electromagnetic field of the emitted X-rays and the electron bunch as it travels through the long undulator eventually aligns the electrons in the bunch with the period of the X-ray wavelength. The principle of SASE is that the aligned electrons move coherently in the magnetic field of the undulator to emit coherent X-rays.

The SPring-8 synchrotron radiation and SACLA XFEL facilities at the RIKEN Harima Institute



The first laser light produced by the SACLA X-ray laser facility

Construction projects for SASE XFEL facilities began to be discussed in the US and Europe around 2000, and later materialized as the LCLS and Euro-XFEL projects. At that time, an 8 GeV electron storage ring for synchrotron radiation facility, SPring-8, was being commissioned in Japan. SPring-8 was one of three large-scale synchrotron radiation sources in the world at the time, alongside European Synchrotron Radiation Facility (ESRF) in France and the Advanced Photon Source (APS) in the US. As the technologies developed for the construction of SPring-8 are very similar to those necessary for an XFEL, the concept of the SPring-8 Compact SASE Source (SCSS), a prototype of an SASE XFEL, emerged. To support this prototype, an in-vacuum undulator technology, higher-frequency accelerator tubes in the C-band (5,712 MHz) and a combination of a classical thermionic electric gun with a CeB6 single crystal as cathode were adopted. Based on this concept, an SASE XFEL with an 8 GeV LINAC capable of emitting coherent electromagnetic radiation at a wavelength 0.06 nm was designed. This combination of technologies allowed the facility to be constructed at a third of the length of the LCLS or Euro-XFEL facilities, measuring just 700 m.

Following the prototype SCSS project, the construction project for the XFEL was launched in FY2006 as one of the Japanese government's 'Key Technologies of National Importance'. At the end of FY2010, all the hardware was in place, and the facility was named the SPring-8 Angstrom Compact Free Electron Laser, or SACLA.

One of the unique features of the facility compared with the LCLS and Euro-XFEL, is its co-location with the SPring-8 synchrotron radiation facility. A new building was set up to allow the XFEL and SPring-8 X-ray beams to intersect at a sample, which will make it possible to use SPring-8 rays to observe how materials relax following an instantaneous impact from the XFEL beam. An electron beam transport from the XFEL LINAC to the SPring-8 storage ring was also built to allow the XFEL LINAC to be used as an injector for SPring-8. Electron beam commissioning for this brand-new SACLA facility began in March 2011.

On June 7, the first SASE lasing was observed at SACLA. Plans now call for reaching SASE saturation and for the initiation of X-ray optics commissioning and end-station commissioning in FY2011. In March 2012, the facility will be opened to public users from around the world. ■

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