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RESEARCH HIGHLIGHTS

30 YEARS OF HISTORY : 1991 – 2021

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NAIST Research Highlights

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Aims & Scope

NAIST Research Highlights showcases promising and important research achievements at NAIST and presents its current research and core technologies to the public. The publication distills highly technical research papers into short, easy-to-understand articles that appeal to a global audience of both specialists and non-specialists. NAIST Research Highlights aims to inform readers of the latest developments in NAIST's pioneering research and to stimulate new and existing international collaborations.

NAURA

NAURA (NAIST URA team) publishes NAIST Research Highlights under the auspices of the "Program for Promoting the Enhancement of Research Universities", which is funded by Japan's Ministry of Education, Culture, Sports, Science and Technology (MEXT). Through this program, NAIST further supports its cutting-edge research while expanding into new interdisciplinary fields in science and technology toward becoming a globally recognized education and research center.

About NAIST

Nara Institute of Science and Technology (NAIST) was founded in 1991 as a Japanese national university with the aim of conducting advanced research and educating scientists and technologists to support the development of society. NAIST consisted of graduate schools in the three areas of information science, biological sciences and materials science. In 2018, NAIST underwent an organizational transformation to continue research in these areas while promoting interdisciplinary research and education across traditional fields. At present, over 1,000 students – roughly 25% from overseas – are supervised by roughly 200 faculty members.

With its cutting-edge facilities and a 5 to 1 student-to-faculty ratio, NAIST's world-leading research and education are a direct result of its rich, global environment and supportive infrastructure. The outstanding achievements of NAIST's faculty and students are shared worldwide through publications, patents, licenses and active exchange with overseas partners.

History

1991 Nara Institute of Science and Technology established
Graduate School of Information Science established

1992 Graduate School of Biological Sciences established
Information Technology Center established

1993 Research and Education Center for Genetic Information established

1996 Graduate School of Materials Science established

September 1992

May 1995

April 1999

1998 Research and Education Center for Materials Science established

2004 Nara Institute of Science and Technology National University Corporation established

2017 Data Science Center established

2018 Graduate School of Science and Technology established (Graduate Schools of Information Science, Biological Sciences and Materials Science merged together)

2021 Center for Digital Green-innovation established

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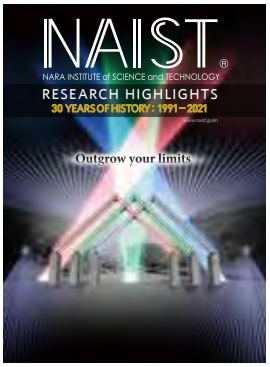
RESEARCH HIGHLIGHTS

30 YEARS OF HISTORY:
1991–2021

Nurturing discovery

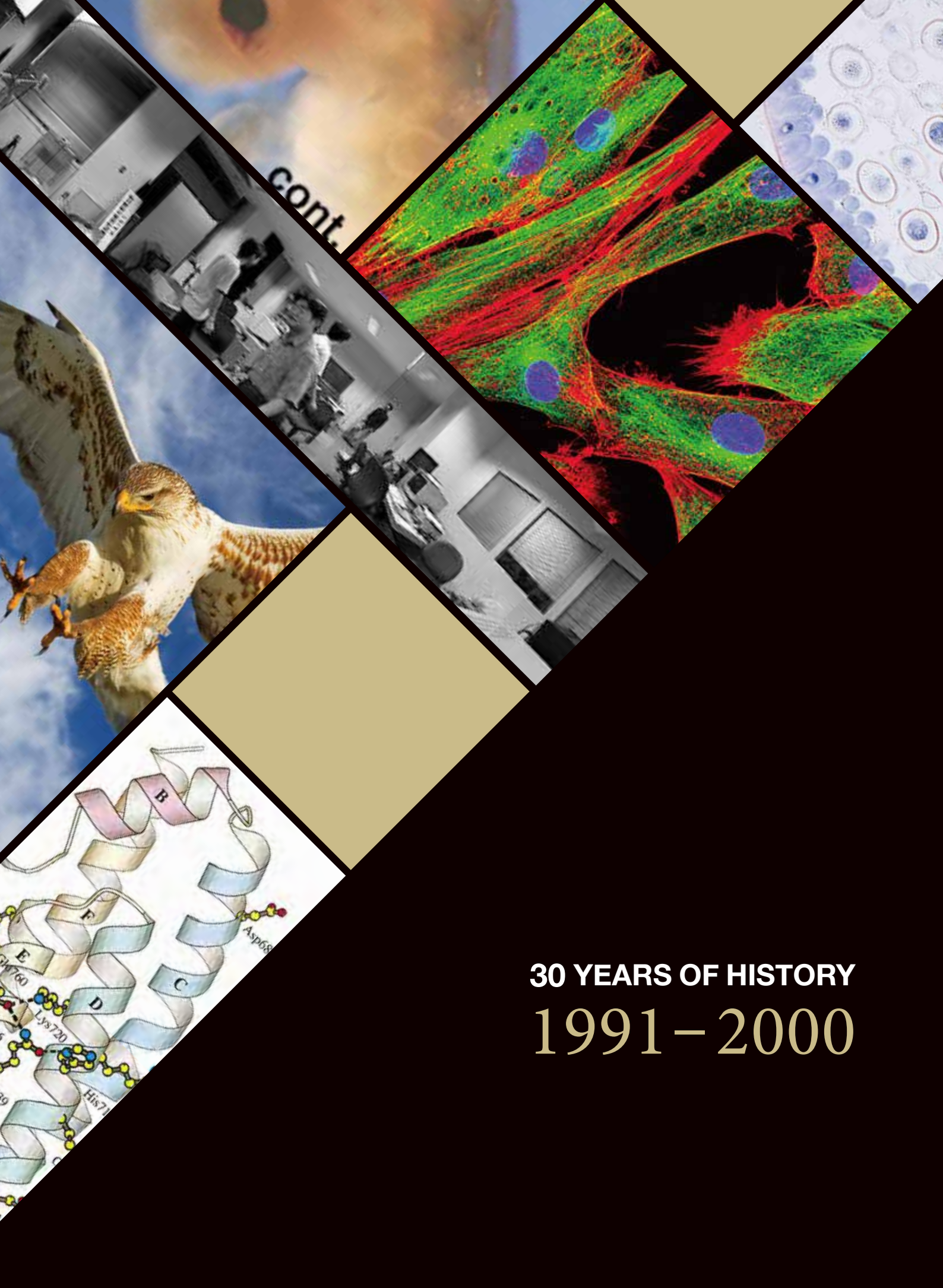
Welcome to NAIST RESEARCH HIGHLIGHTS – 30 YEARS OF HISTORY: 1991–2021. This publication provides an overview of highly impactful work conducted by researchers at Nara Institute of Science and Technology in the past 30 years since its establishment. In these pages we present short features of 44 selected topics.

Of course, at the heart of such outcomes are our researchers. Each year NAIST attracts more leaders in scientific fields. We look forward to nurturing their important work and the careers of all our researchers in the atmosphere of cutting-edge discovery that will continue to be fostered at NAIST.



On the cover

The main gate of the NAIST campus. Three triangles represent the three mountains of Yamato (Kagu-yama, Unebi-yama and Miminashi-yama) described in Manyoshu, the oldest anthology of poems in Japan. They also symbolize NAIST's three research areas (information science, Biological Science and materials science), the mainstay of today's advanced science and technology.



30 YEARS OF HISTORY

1991–2000

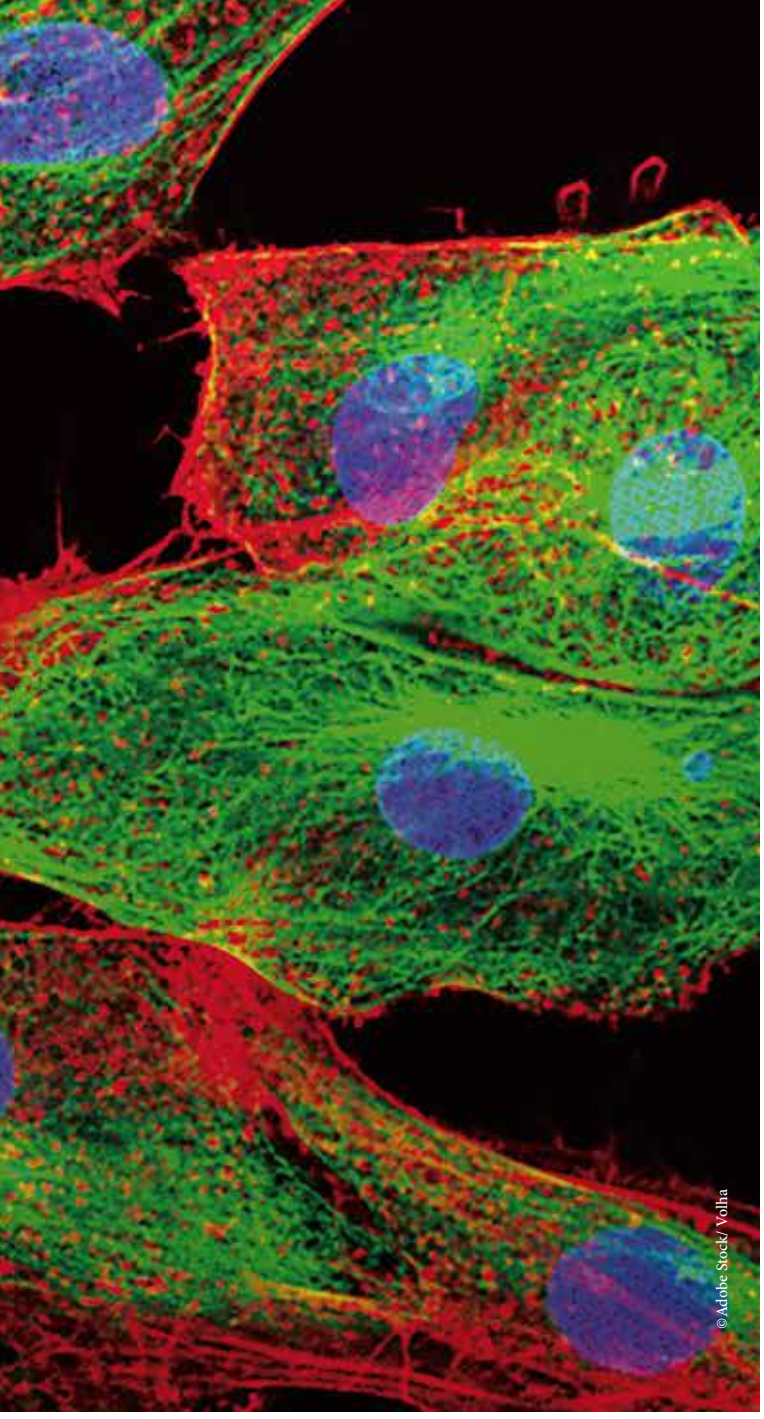
Kozo Kaibuchi

Right on target: Identification of the first known targets of Rho

Rho, a small guanosine triphosphate, binds to protein kinase N and Rho-kinase, and activates Rho-kinase to enhance the formation of actin stress fibers and focal adhesions

Signaling cascades are crucial for transmitting messages from the extracellular environment to the interior of the cell, as well as regulating and modifying processes within cells. However, these cascades are often very complex, involving multiple key players and affecting a broad range of targets. Thus, the discovery of an important signaling molecule is often the first step in a journey to find interacting partners for that molecule and understand what they do and how they do it. One key intracellular signaling protein, the Ras-like small guanosine triphosphate Rho, was known to help regulate processes as diverse as stress fiber formation, cell morphology, cell aggregation and cytokinesis. However, despite the involvement of Rho in so many crucial cellular activities, its downstream effectors remained unknown. In 1995 and 1996, a research team led by Kozo Kaibuchi at NAIST published a series of papers in *Science* identifying two targets of Rho and demonstrating how one of these targets mediates an important biological effect of Rho.

In a study published in *Science* in February of 1996, the research team sought to identify proteins that could mediate RhoA's effects using an affinity column approach, in which different forms of RhoA were immobilized on a column and extracts from cow brain membranes were then run over the columns. All proteins that bound to the RhoA-saturated column were then retrieved and analyzed. This strategy yielded a protein that was determined to be protein kinase N (PKN), which had already been characterized in humans. The researchers revealed that not only does PKN bind directly to RhoA, but RhoA also induces PKN phosphorylation and stimulates PKN kinase activity, indicating that this interaction is functionally significant. Importantly, RhoA and PKN also interacted directly in live cells, showing that this relation-



ship is not restricted to artificial laboratory conditions, but functions in real life.

A subsequent paper published in *Science* in July of 1996 reported the identification of additional targets of Rho that could function in other signaling pathways. In this study, the research team focused specifically on the stimulation of smooth muscle contraction, which was known to involve phosphorylation of a protein called MLC and was thought to be regulated by a guanosine triphosphate (GTP)-binding protein. Given that Rho is a GTP-binding protein that had been implicated in smooth muscle contraction, it seemed reasonable that Rho would help regulate this process through an as-yet-unknown target protein. Using the same column approach described earlier, the group identified the myosin-binding subunit (MBS) of myosin phosphatase. The tail end of MBS interacts directly with RhoA; intriguingly, this part of the protein has a very similar structure to that of PKN, suggesting that the specific shape of these proteins mediates their binding to RhoA. Somewhat unexpectedly, however, RhoA did not appear to modulate MBS activity, suggesting that there may be an intermediate factor required for this step of the signaling pathway. Given that a separate study from the same research group had identified a serine-threonine kinase (that is, an enzyme that phosphorylates other proteins) known as Rho-kinase as an additional target of RhoA, and that MBS thiophosphorylation is associated with its activity, the researchers suspected that Rho-kinase was this intermediate factor. This indeed turned out to be the case: Rho-kinase thiophosphorylated MBS, and this process was enhanced by the presence of RhoA. Thus, this study identified a novel Rho target and showed that this interaction is functionally important, because it helps regulate smooth muscle contraction.

In a later paper published in *Science* in February of 1997, the research team explored the role of Rho in the formation of stress fibers and focal adhesions, and specifically the function of Rho-kinase in this process. To address this, they investigated the effect of different domains of the Rho-kinase protein on phosphorylation of myosin light chain, which is a component of stress fibers. The catalytic domain of Rho-kinase showed activity equivalent to that of the full protein, and this activity was inhibited by treatment with a protein kinase inhibitor, suggesting that the catalytic domain is crucial for myosin light chain phosphorylation. Furthermore, injecting live cells with the catalytic domain induced the formation of both stress fibers and focal adhesions, and treatment with the same inhibitors prevented these effects. Thus, activation of Rho leads to Rho-kinase–mediated regulation of stress fiber and focal adhesion formation.

Taken together, these three papers reported the identification of the first known targets of Rho, a central signaling molecule involved in multiple cellular processes, and revealed the mechanism by which Rho signals through one of these targets, Rho-kinase, to induce functionally important structural changes within cells. This groundbreaking work established the basis for much of what is known about Rho signaling today and provided crucial insight into a fundamental signaling network that regulates a host of essential cellular processes. The findings from these three studies substantially changed the landscape of the cell signaling field and set the stage for much of the subsequent work that has been done in the field since their publication 25 years ago.

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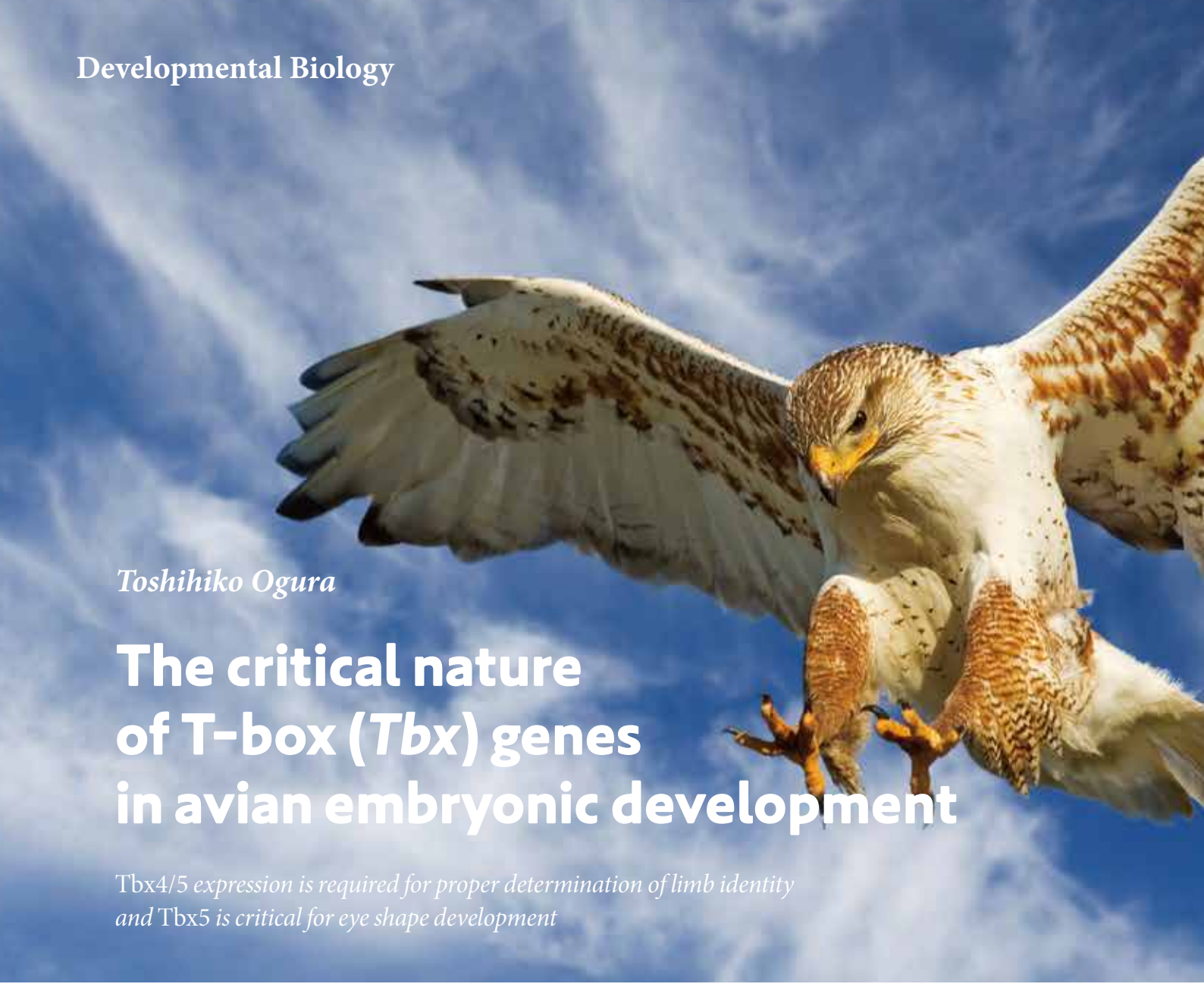


Toshihiko Ogura

The critical nature of T-box (*Tbx*) genes in avian embryonic development

Tbx4/5 expression is required for proper determination of limb identity and Tbx5 is critical for eye shape development

Embryonic development is a detailed and precise process that is facilitated by specific changes in gene expression in different parts of the embryo. This allows for the formation of various organs and structures at defined times, but also helps in the determination of anatomical directionality and identity. This is of particular importance for limb development, where the fore- and hindlimbs begin to form similarly but ultimately become different structures. This phenomenon can be seen in many species, such as arms and legs in humans and wings and legs in birds. Understanding these processes helps provide insight into the genetic components of development to better appreciate how and why abnormalities can occur.

One relevant family of genes is the T-box (*Tbx*) genes, which code for transcription factors. Previous research demonstrated that *Tbx4* is expressed in the leg buds and *Tbx5* is expressed in the wing buds of developing chickens. In addition, the Tbx genes are part of the same family of transcription factors as the *Drosophila optomoter blind* gene, which is involved in *Drosophila* limb and optic lobe development. Combined with the expression pattern of *Tbx4/5*, this suggested that these genes are involved in limb identity in chickens, but it was not clear how significant their roles were in development.

In a study published in *Nature* in 1999, a research team led by Toshihiko Ogura at NAIST used a system called *in ovo* electroporation where they injected a retroviral plasmid into chick embryonic cells to "misexpress" *Tbx4* in the wing bud and *Tbx5* in the leg bud. Interestingly, expressing *Tbx5* in the leg resulted in partial wing skeletal morphology development and the growth of feathers where scales should be present. *Tbx4* expression in the wing showed opposite effects, including leg-like formation with clawed digits and scale growth. Expression of control genes, such as green fluorescent protein, did not cause any of these changes, suggesting they are *Tbx*-specific.

Mechanistically, the researchers showed that these *Tbx* genes can regulate leg and wing developmental identity by inducing the expression of limb-specific genes, including *Hoxc9* in the leg and *Hoxd9* in the wing. Plasmid-driven

directions. Expression levels of ventral-specific genes like *Pax2* and *Vax* were repressed. However, expression of *EphrinB1/2*, which is typically restricted to the dorsal side, was observed in the ventral retina following *Tbx5* expression.

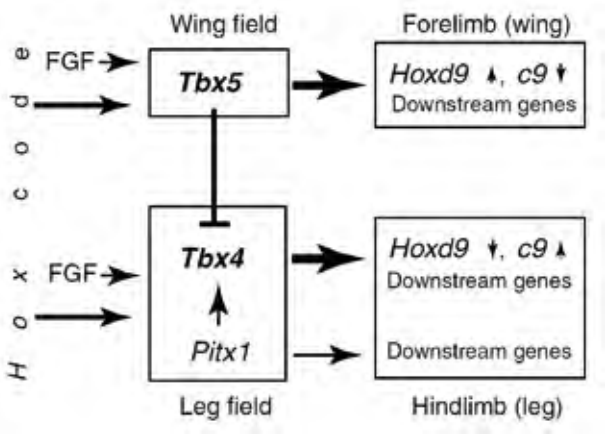
Furthermore, the researchers examined the axons of the retinal ganglion cells, because these normally enter the optic tectum. When *Tbx5* was misexpressed in the ventral retina, some but not all of these axons showed abnormal trajectories into the tectum. They also examined the role of bone morphogenetic proteins (BMPs), as they have been implicated in dorsal-ventral axis determination. Interestingly, misexpression of mouse *BMP4* in the ventral eye cups resulted in upregulated *Tbx5* and lower levels of *Pax2* and *Vax*. Overall, these findings suggest that formation of the eye dorsal-ventral axis involves a pathway where *BMP4* can induce *Tbx5*, which then induces or represses specific downstream genes.

These two highly impactful publications from the research team demonstrated key findings in chicken limb and eye development. They both centered on *Tbx5*, a critical gene for wing formation and eye dorsal-ventral axis determination. Their study also identified specific downstream genes involved in signaling cascades that mediate these phenotypic effects. Importantly, these two articles collectively showed that the same gene is involved in two crucial, yet very different, developmental processes. Their findings therefore highlight the indispensable nature of *Tbx5* in chicken developmental biology. Additionally, these mechanistic results are also useful for understanding the genetic components of abnormal development, possibly opening the door for therapeutic intervention or tissue regeneration and transplantation in humans.

expression of *Tbx4* induced *Hoxc9* and suppressed *Hoxd9*, while the opposite was observed with *Tbx5*. Additionally, they found that *Tbx4* or *Tbx5* could be specifically selected given certain genetic factors. Expression of a gene called *Pitx1* could induce *Tbx4*, while *Tbx5* expression could inhibit *Tbx4*. This allows for specific gene expression repertoires in both the wing and leg, facilitating limb determination.

Because of its importance in the limbs, the research team then became interested in examining the developmental role of *Tbx5* in other parts of the body. They investigated this gene's function in the chick eye, publishing their findings in *Science* in 2000. Previous research showed that *Tbx5* is expressed in the dorsal side of the eye during development, leading to the hypothesis that this gene is critical for dorsal-ventral axis determination.

Tbx5 expression was observed in the dorsal retina during early developmental stages. Similar to their approach in chick limbs, the team used *in ovo* electroporation to misexpress *Tbx5* in the ventral eye. After doing this in stage 8 embryos, oval-shaped eyes developed, suggesting the eye cup was elongated in both



Interactions regulating the wing/leg identity of limb buds (Takeuchi *et al.*, 1999, *Nature*).

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Hiroshi Yoshikawa

Determination of key proteins in cellular mechanisms for controlling the initiation of DNA replication

Two proteins are shown to be critical for regulating progression through the cell cycle after DNA replication and effecting the cell's response to DNA damage

DNA replication is a fundamental biological process that is key for cell division and growth. It takes place during the 'S phase' of the cell cycle, and is tightly regulated to preserve the fidelity of the genetic information. Cell-cycle dysregulation is thought to occur in a range of illnesses such as inflammation, neurodegenerative disorders, heart disease and cancer; therefore, research into the molecules controlling DNA replication have wide potential clinical application. The mechanisms by which the cell regulates the timing of the various steps of the process remained shrouded in mystery until 1998, when a research group led by Hiroshi Yoshikawa at NAIST obtained new insights into this process. Using the model organism *Saccharomyces cerevisiae,* known commonly as budding yeast, they identified the role of two specific checkpoint proteins, Rad53 and Orc2, in the timing and surveillance of DNA replication.

Chromosome VI of budding yeast contains multiple places at which DNA replication begins, known as the Origin of Replication (ORI). In wild-type cells these can be seen to fire at different times in a defined sequence throughout S phase. To investigate the temporal control of the various ORIs of chromosome VI, the group used an agent that damages DNA, called MMS (methyl methane sulphonate). MMS halts the cell cycle at the S phase because detection of DNA damage is a key cellular checkpoint that prevents the cell cycle from progressing until the damage is resolved. The protein Rad53 was thought to be involved in the mechanism by which the cell monitors progression through S phase, but the detailed mechanism was unknown. Another important protein in this process is Orc2. This protein is part of the complex that recognizes and binds the replication origins, which is crucial for replication initiation.

When wild-type yeast were treated with MMS, they stalled in the S phase and the initiation of DNA replication from the late-initiating ORIs was blocked. The S phase surveillance mechanism was still in effect, because the cells were still viable. In contrast, when *rad53* and *orc2* mutant yeast were treated with MMS, they progressed through S phase into the next phase of the cell cycle, known as G2 phase, albeit with a concomitant loss of viability. Because entry into the S phase naturally occurs at different times within a population of cells, the researchers confirmed these results using cells with cycles that were artificially synchronized to enter the S phase at the same time. When MMS was present, wild-type yeast showed a clear reduction in the numbers of cells that progressed to S phase. However, yeast lacking Rad53 exhibited a slower than usual progression through the S phase and were still able to reach the G2 phase.

The researchers then went on to look at the sequential firing of the ORIs identified on the right arm of chromosome VI. When treated with MMS, wild-type cells showed replication initiation at the early ORI, but late and weak ORIs showed no replication initiation. Cells lacking Rad53 or Orc2, however, exhibited no such block of the initiation of replication at the late or weak ORIs. Under normal conditions, in the absence of MMS, cells lacking Rad53 or Orc2 initiated replication at the late ORIs more frequently than wild-type cells did.

To determine how general these results were, the researchers then studied another late ORI located on chromosome V and observed the same results for this origin: MMS blocked initiation in wild-type cells but not in cells lacking either Rad53 or Orc2. This confirmed that both Rad53 and Orc2 are involved in blocking initiation from late and weak ORIs as a response to DNA damage.

Further investigation using Rad53 and Orc2 mutants revealed the related but distinct roles of these checkpoint proteins at the different ORIs. The researchers found that Rad53 mutation affected both the timing of initiation at late ORIs and the movement of the replication fork at the end of the chromosome, whereas mutation of Orc2 affected the timing of initiation of both late and early ORIs but did not affect the movement of the replication fork. Under normal conditions, in the absence of MMS, Rad53 and Orc2 mutation increased the efficiency of replication initiation at the late ORI and both early and late ORIs, respectively. They also saw that Rad53 mutation accelerated the timing of initiation of late ORIs.

Taken together, these results suggested that Rad53 acts by suppressing activation of late ORIs to regulate the efficiency and timing of replication initiation from them, whereas Orc2 could regulate the activation of early ORIs as well as suppressing late ones.

The cellular response to DNA damage and proper management of the cell cycle is essential for the maintenance of genomic stability and prevention of cell death. In higher organisms, dysregulation of these processes can lead to the propagation of damaged DNA and precursor cell mechanisms in the development of cancer. The results of this study may be useful for greater understanding of conditions thought to be related to cell-cycle dysregulation, such as neurodegenerative disorders, inflammation and cardiovascular disease.

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Kunio Yasuda

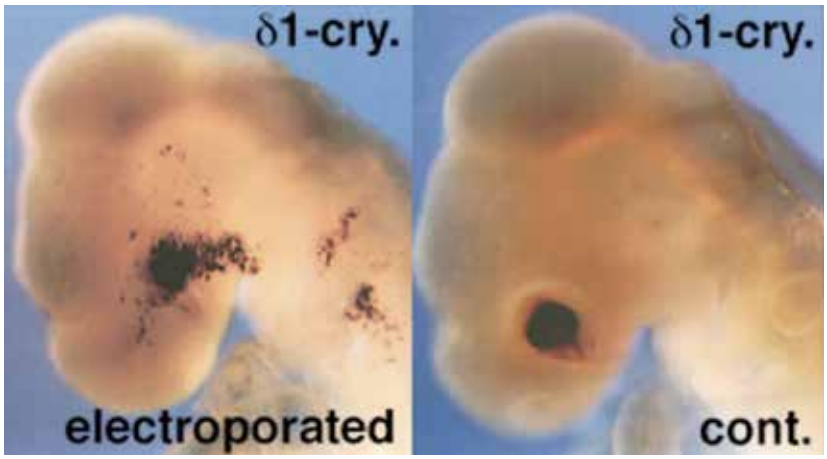
A novel transcription factor for the precise control of lens development

The L-Maf protein discovered in vertebrates regulates genes that control cell differentiation in the lens, which may provide insights for the development of regenerative medicine technologies

The process by which a single cell develops into a fully formed organism is one of the enduring mysteries of science. It is posited that the complex interplay between gene expression, external signals and cell differentiation must play a crucial role. By studying the emergence of specific structures, such as the lens of the eye, scientists can start to form a better understanding of the intricacies of this process. The development of the lens is an intriguing model system for this purpose because it contains specialized crystallin proteins that provide transparency and are not found in other parts of the body. In addition, the cellular differentiation required for development in vertebrates is known to be precisely controlled

by intercellular signaling and transcription factors. An improved understanding of the molecular mechanisms governing this process could be applied more widely to the development of artificial organs and tissues for use in transplantation or regenerative medicine. As a result, this area of research has the potential to provide crucial insights that could revolutionize human health and longevity.

A series of studies in chickens demonstrated that the gene expression of αA-crystallin is controlled by a lens-specific enhancer element, named αCE2, which is a portion of DNA surrounding the gene. This enhancer is located about 100 base pairs upstream of the gene's transcription start site, and is conserved in other crystallin genes. These findings indicate that this sequence plays an important regulatory role, and a research group led by Kunio Yasuda at NAIST worked to identify protein factors that could bind to αCE2 to better understand their role in lens formation.



Ectopic expression of L-Maf induces lens differentiation in chick embryos (Ogino & Yasuda, 1998, *Science*).

The researchers first screened an embryonic lens cDNA library with an αCE2 oligonucleotide probe and mapped gene expression in the lens tissues of chicken embryos. They found that an important event in development occurred when cells from the head ectoderm tissue layer made contact with the optic vesicle. The mRNA expression remained confined to the lens throughout subsequent stages of development, and αA-crystallin genetic transcripts, a newly detected mRNA type, were seen even before δ1-crystallin mRNA, an early marker of lens development.When these transcripts were further characterized, they were found to encode a protein with 286 amino acids, predicted to be a transcription factor similar to those of the *maf* family. This novel factor was named "lens-specific Maf" (L-Maf).

The researchers next performed transfection experiments to examine the effects of specific DNA sequences by adding synthetic versions directly to cells. Using a bioluminescent detection system based on the enzyme luciferase, the αA-crystallin promoter was found to produce significantly more light when placed into chicken lens primary cells with a plasmid expressing L-Maf, compared with other sequences. These observations suggest that L-Maf can transactivate αA-crystallin expression via the αCE2 sequence in its promoter.

To further test the role of L-Maf in lens development, the researchers conducted gel mobility shift DNA binding assays. Overexpression of L-Maf could transcriptionally activate any of the crystallin elements tested, including several from chickens and two from mice. However, activation was not observed in a gene without a crystallin element, nor in the chicken αA reporter with a mutated αCE2 sequence.

The researchers then examined the role of L-Maf in lens cell differentiation by transiently transfecting the L-Maf plasmid into chicken embryo neural retina cells. These cells were found to express both αA- and δ1-crystallin. Furthermore, the cells displayed morphology similar to lens fiber cells. This retina-to-lens cell conversion was not seen with cells without L-Maf transfection.

Finally, a retrovirus expressing L-Maf was generated, and allowed to infect retinal cell cultures. Compared with uninfected cells, the L-Maf retrovirus could induce expression of αA-, βB1- and δ1-crystallin expression at both the protein and RNA levels. The researchers also found that the RNA expression of filensin, a marker of terminally differentiated lens fiber cells, was significantly higher in the L-Maf retrovirus-infected cells.

Overall, this study demonstrated that L-Maf, a previously unidentified transcription factor, has the ability to activate genes by binding to the αCE2 element in their promoter regions. Moreover, these genes were shown to be specific to the lens, and activating their transcription could induce other cell types to differentiate into lens cells. While eye development is very complex and many other genes are also involved, such as *Pax-6* and *Six3*, L-Maf is the gene most strictly localized to the developing lens. This leads to the conclusion that L-Maf is likely directly targeted by signals coming from the optic vesicle, especially because of its specificity to the lens and rapid expression.

Obtaining an enhanced understanding of the genetic cascade involved in lens development is a significant achievement for the overarching objective of being able to predict or even control organ development. For example, replacement lenses might be grown for patients based on their own cells. Similarly, while the need for other organs like hearts and kidneys today far outstrips the number available for transplant, in the future they may also be generated by exposing adult stem cells to the correct sequence of signaling factors. This would represent a breakthrough for tissue engineering and regenerative medicine.

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Toshio Hakoshima

Resolving the crystal structure of the bacterial histidine phosphotransfer domain

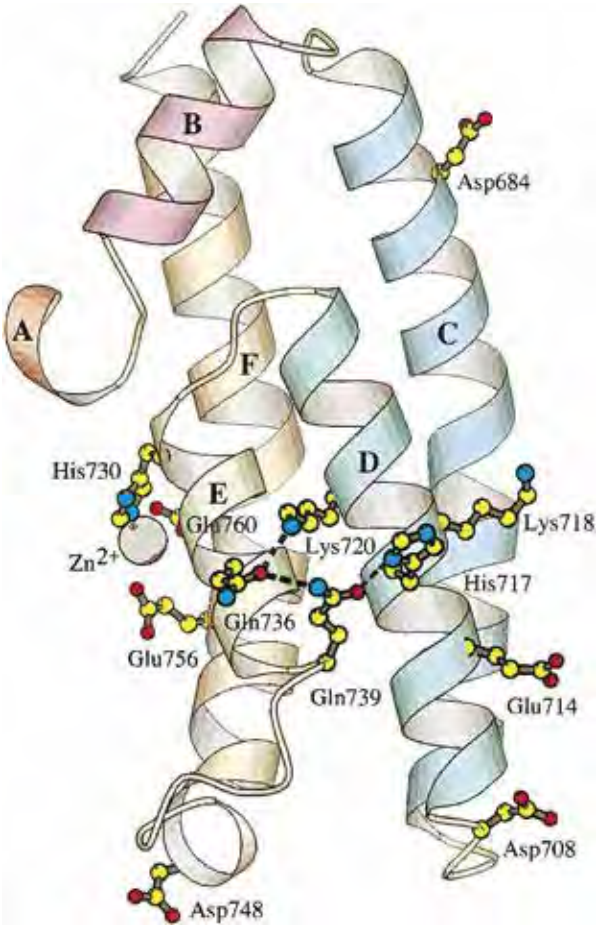
X-ray diffraction analysis reveals the 3D structure of the histidine phosphotransfer domain of anaerobic sensor kinase ArcB, providing insights into an important signaling system for environmental responses in bacteria

The ability to detect and respond to external stimuli are essential features of living systems. Many organisms have evolved highly specialized signaling circuits that are able to transmit information regarding the outside environment from where it is sensed, at the exterior of the cell, to other locations where it can be used to modulate activity. In particular, some cells use a specific two-component regulatory system in which a membrane-bound histidine kinase protein can be triggered by external conditions to transfer a phosphoryl group to an internal response-regulating domain. By employing this system, changes in the environmental oxygen level or osmotic concentration, for example, can trigger alterations in behavior. However, while these two-component regulatory systems are known to be very common in bacteria, the extent of structural similarity between the signaling proteins used by prokaryotes and eukaryotes was not well established.

To shed light on this question, a research team led by Toshio Hakoshima at NAIST used X-ray crystallography to determine the structure of the histidine phosphotransfer (HPt) domain of the bacterial signaling protein ArcB. This segment of the protein is made up of six alpha helices that combine to form a tightly packed core. The scientists focused on the phosphorelay system, in which an active histidine amino acid acts as part of the chain that sends a phosphoryl group to an aspartate on a receiver protein. These "hybrid" histidine kinases are more complex compared with other signaling systems, because the HPt histidine is just the final part of a longer relay that receives the phosphoryl group before transferring it to the response regulator protein.

Even though HPt domains vary significantly between bacterial species – so much so that the genetic sequences share only about 20% similarity with each other – the researchers found conserved structural features linked to the phosphorelay function. This indicated that these features were essential for the correct operation of the system, which was especially true for the His-717 active site, a common aspect of all protein domain instances studied. Moreover, similarities in structural motif were seen with eukaryotic sensor kinases, including those used by yeasts.

Understanding which structural features of signaling domains are conserved across species can be very useful when designing new antibiotic drugs. This is because certain HPt domains are known to be involved in the process of activating the virulence of certain bacteria. Developing inhibitory drugs that target these conserved regions would be useful across a wider range of pathogens, and it would be much harder for these critical domains to mutate to confer resistance.



Structure of the histidine phosphotransfer domain of ArcB (Kato *et al.*, 1997, *Cell*).

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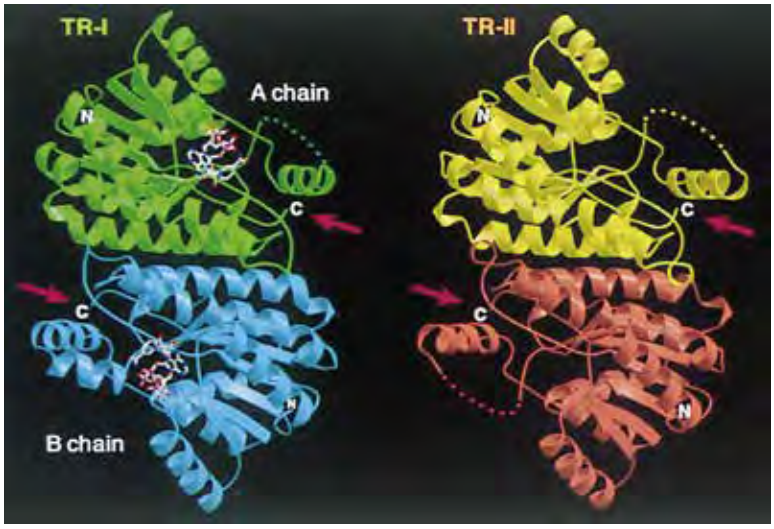
Yasuyuki Yamada

The same but different: Structures of a pair of stereospecific tropinone reductases revealed

The crystal structures of two tropinone reductases are solved to determine the features that influence the stereochemistry of medicinally important tropane alkaloid products

Natural products often exist as stereoisomers – molecules with the same structural formula, but different orientations in 3D space. The reactions that lead to these different products are catalyzed by pairs of enzymes that each give a specific isomer. Identifying the differences within pairs of enzymes that lead to their specificity, contributes to the continued understanding of how complex molecules within the cells of living beings are produced during biosynthesis.

Tropinone reductases (TRs) catalyze the reduction of tropinones to give tropane alkaloids. However, the structural differences between TR-I, which produces the tropine stereoisomer, and TR-II, which produces the pseudotropine stereoisomer, were not understood until a research group led by Yasuyuki Yamada at NAIST published their crystal structures in 1998.



Structures of TR dimers (Nakajima *et al.*, 1998, *PNAS*).

Tropane alkaloids are natural molecules known to elicit effects as stimulants and anticholinergics, with cocaine and atropine being widely known examples. The reduction of tropinones is therefore an important reaction in the research and development of new physiologically active compounds.

Two pairs of TRs have been identified from the species *Datura stramonium* and *Hyoscyamus niger*. And both TR-I/TR-II pairs are known to share 64% of their amino acid residues, indicating that they diverged from a common protein relatively recently. The researchers therefore focused on determining the structural features that result in the diastereomeric configurations of the reaction products.

The crystal structures of the two *D. stramonium* derived enzymes were determined with 2.4- and 2.3-Å resolution and the 120-amino acid sequence at the C-terminal of the enzymes – which represents most of the substrate binding site – was investigated to determine how structural differences in that region influence the reaction products.

53 residues in the sequence were found to differ between TR-I and TR-II. Determining whether these variations result in differences in folding, or whether only a small number of the differences influence the activities, was an important focus of the work. The overall folding of the proteins was found to be almost identical for both enzymes. In addition, the cofactor binding sites, and the positions of the active site residues were almost the same in both cases. And the hydrophobic nature of many of the residues at the active site was common to both enzymes.

However, differences in the charged residues between the two TRs resulted in variations in the electrostatic environments. Modeling of these differences showed that they influenced the orientation of the tropinone binding to the enzyme, which ultimately led to opposing stereochemistry in the reaction products.

Not all stereospecific enzyme pairs are as structurally similar as the TRs pairs were found to be, with some enzymes differing almost entirely except for at the active sites, which have evolved to be mirror images of each other. Stereospecific enzymes are crucial for the manufacture of stereospecific chemicals and compounds such as pharmaceuticals, flavorings and fragrances. Gaining a full understanding of the structural similarities of TRs provided an important piece of the overall picture of stereospecific enzyme evolution.

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Jun-ya Kato

Releasing inhibitions: Jab1 counteracts inhibition of cell cycle progression by $p27^{Kip1}$

Jab1 translocates the cyclin-dependent-kinase inhibitor $p27^{Kip1}$ from the nucleus to the cytoplasm, where its proteasome-mediated degradation enables cell cycle progression

The process of mammalian cell proliferation is carefully regulated to ensure that cells do not grow out of control. Multiple cell cycle checkpoints and other regulatory mechanisms exist to prevent proliferation of damaged cells and appropriately time the proliferation of healthy cells. Disruption of these systems is a common feature of cancers, which typically involve unregulated or inappropriate cell proliferation. One component of the cell cycle regulatory system is the cyclin-dependent-kinase inhibitory protein $p27^{Kip1}$. Reduced levels of $p27^{Kip1}$ expression are associated with poor survival in patients with breast or colon cancer, and $p27^{Kip1}$ abundance is regulated by a variety of mechanisms. A research group led by Jun-ya Kato at NAIST found a novel cellular mechanism that decreases $p27^{Kip1}$ levels.

To identify factors involved in the regulation of $p27^{Kip1}$ abundance, the researchers performed a yeast two-hybrid screen designed to detect proteins that interact directly with $p27^{Kip1}$. One of the candidates retrieved from this screen was a protein called Jab1 that was originally identified as a co-activator of c-Jun and JunD. Different portions of Jab1 were then fused to a variety of tags and reporters to determine how it interacts with $p27^{Kip1}$ and where it is active within the cell.

The results showed that Jab1 binds to amino acids 97 to 151 of $p27^{Kip1}$, and that phosphorylation of a threonine residue at position 187 of $p27^{Kip1}$ may reduce the stability of this interaction. Overexpressing Jab1 in mammalian cells significantly decreased the half-life of $p27^{Kip1}$ in a proteasome-dependent manner, suggesting that it promotes $p27^{Kip1}$ proteasome-mediated degradation. Indeed, further investigation showed that, while $p27^{Kip1}$ was primarily located in the nucleus, coexpression of Jab1 resulted in a shift of both proteins to the cytoplasm. Simply moving $p27^{Kip1}$ to the cytoplasm by fusing it to a nuclear export signal did not result in its degradation, suggesting that Jab1 may accelerate $p27^{Kip1}$ degradation by specifically bringing it into proximity with degradation machinery in the cytoplasm. Importantly, at a functional level, expressing $p27^{Kip1}$ in mouse fibroblasts inhibited cell cycle progression, and coexpression of Jab1 rescued this effect.

Overall, the findings from this study showed that Jab1 negatively regulates $p27^{Kip1}$ by causing it to translocate from the nucleus to the cytoplasm and promoting its degradation by the proteasome. This process results in decreased levels of $p27^{Kip1}$, thereby releasing inhibition of cell cycle progression and allowing cells to exit the G1 phase and enter the S phase of the cell cycle. Given the importance of $p27^{Kip1}$ abundance in predicting survival in breast and colon cancer patients, the findings from this study provide important insight into how regulation of $p27^{Kip1}$ levels may be involved in cancer progression. More broadly, the interaction between Jab1 and $p27^{Kip1}$ sheds new light on cell cycle regulation in general.

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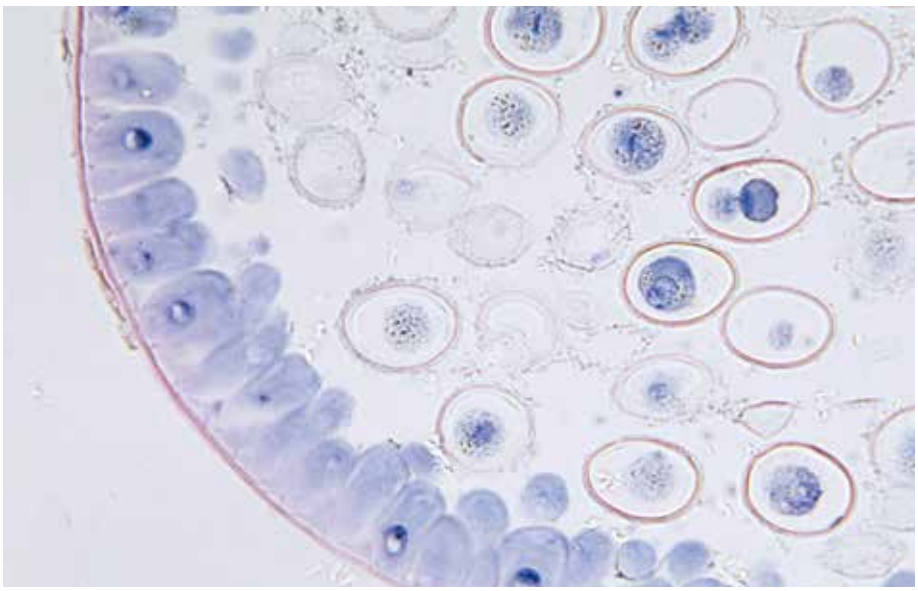

Naokazu Yokoya

Improving virtual reality: Design of a stereoscopic omnidirectional video capture system

A 12-camera omnidirectional video capture system is created that can produce high-resolution video with stereoscopic perspective, which may allow for greater immersive, panoramic experiences

Technological advances in computing power and graphics rendering capabilities have made virtual reality (in which a user interacts with an immersive simulated environment) and mixed reality (in which computer-generated graphics are overlaid on live video) more practical than ever before. However, conventional video capture methods lack the high resolution and dynamic wide-angle scope to create believable scenes. In addition, panoramic approaches often create extreme image distortion, as with a fisheye lens or paraboloid mirror. As a result, an omnidirectional video system that can simultaneously capture images at video-rate in all directions would represent a significant advance for this area.

To address this need, a research team led by Naokazu Yokoya at NAIST developed an image capture system that produces stereoscopic, omnidirectional images from a single viewpoint using two sets of six cameras. Precisely aligned mirrors in the form of equilateral hexagonal pyramids help combine the camera shots resulting in real-time video in all directions simultaneously. Each camera must have a wide viewing angle to enable an omnidirectional image to be obtained from a single viewpoint; however, the wide viewing angle led to undesirable distortion in the recorded images.

To overcome the problem of radial image distortion, a correction process known as Tsai's calibration method was implemented. Tsai's method for camera calibration has often been utilized with stereoscopic systems to determine the interior and exterior orientation parameters in the form of a power series of distortion coefficients. Combined with an image scaling factor, the recorded image coordinates can be mapped to known calibration points. This process can be done quickly with open-source image software and a calibration grid of black dots. The raw images also have to be transformed to compensate for the hexagonal shape of the mirrors. This is done by digitally projecting the data onto a virtual cylindrical surface with parameters that are found heuristically based on continuity of the image and consistency between the two sensor arrays. The data are recorded as 12 still images for each "frame" of video, which can then be recombined into an omnidirectional stereo movie.

The system the researchers developed is among the first to achieve all four of the requirements for complete scene capture – namely, it is omnidirectional, stereoscopic, high-resolution, and records with a video-speed frame rate. On the basis of these results, the use of mirrored camera arrays can be applied more broadly to other situations that require real-time panoramic imaging. Future versions of this imaging system could be mounted on vehicles for dynamic video capture of wide areas or used to achieve seamless integration of real and virtual elements in simulated environments for urban planning of smart cities.

An omnidirectional stereo panoramic image (Kawanishi *et al.*, 1998, *ICPR*).

Reference

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Yukio Imanishi

Light-responsive polymer serves as a reversible on-off switch for enzyme solubility and activity in organic solvents

Modifying an enzyme with a polymer that undergoes a photo-reversible structural change allows for control over its solubility in organic media, and thus its catalytic activity, potentially enabling improved biological catalyst recovery and efficiency

Enzymes are known to catalyze many important biochemical reactions which, harnessed and controlled appropriately, could be extremely valuable for industrial applications involving chemical synthesis. However, enzymes (which are polar) are largely insoluble in the nonpolar organic solvents that are usually used in industrial synthesis. Thus, solubility in nonpolar organic media is highly beneficial because this would increase the enzyme's efficiency by removing the barrier of diffusion, thereby facilitating the substrate's access to the catalytic active site.

To modulate their solubility in nonpolar solvents, enzymes are often chemically modified, but it is difficult or costly to recover or reuse the modified enzymes after the reactions. In 1999, a research team led by Yukio Imanishi at NAIST demonstrated that attaching a photo-responsive polymer to an enzyme enabled control over its solubility in organic media via ultraviolet (UV) and visible light treatment; furthermore, the solubilized enzyme showed enhanced reactivity relative to the native enzyme suspended in nonpolar organic media (toluene).

Subtilisin (Sub) is an enzyme that catalyzes industrially applicable transesterification reactions, which involve exchanging the organic R-groups between an ester and an alcohol. The photo-sensitive polymer (PSP) used in this study contained spiropyran, which is an organic group that undergoes a nonpolar-to-polar structural change under UV irradiation. Importantly, this photo-induced reaction is reversible under visible light irradiation. The researchers successfully coupled these two components to generate PSP-Sub, and chromatographic characterization confirmed that the product contained several PSP chains per Sub enzyme. This indicated the potential for reversibly controlling the modified enzyme's solubility.

Importantly, although the native Sub is insoluble in toluene, the enzyme modified with the nonpolar PSP was highly soluble. After irradiation with UV light, the modified enzyme precipitated from the organic solution because the photo-induced structural change in the PSP rendered it polar. When the resulting suspension was irradiated with visible light, the PSP was converted back to its nonpolar form, and all of the precipitated enzyme was resolubilized. The UV/visible irradiation-induced solubility changes could be repeated multiple times, and, at any point, precipitation via UV irradiation could lead to complete recovery of the coupled PSP-Sub.

To confirm that the enzyme's solubility in organic media enhanced its activity, the catalytic transesterification reactivity of the native Sub was compared with that of the PSP-Sub, in terms of the initial reaction rate in toluene. Remarkably, the catalytic activity of PSP-Sub (soluble) was more than 100 times that of native Sub (insoluble), and the modified enzyme system retained this high level of reactivity even after several precipitation/solubilization cycles. As a proof-of-concept, the PSP-Sub also exhibited photo-reversible solubility in chloroform, as well as enhanced catalytic activity relative to native Sub (insoluble in chloroform), although not as high as in toluene.

This work highlights an effective approach for expanding the use of enzymes in organic synthesis by controlling their solubility in organic medial through coupling with photo-responsive polymers. This solubility control could enhance the industrial application of biological enzymes in the production of chemicals with increased efficiency and sustainability, owing to improved catalyst recovery.

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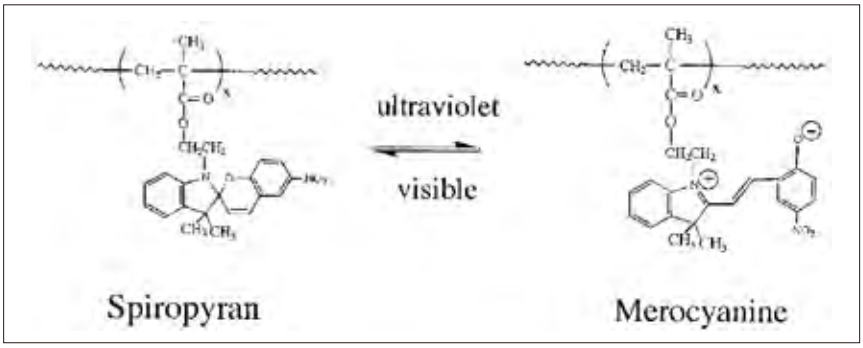
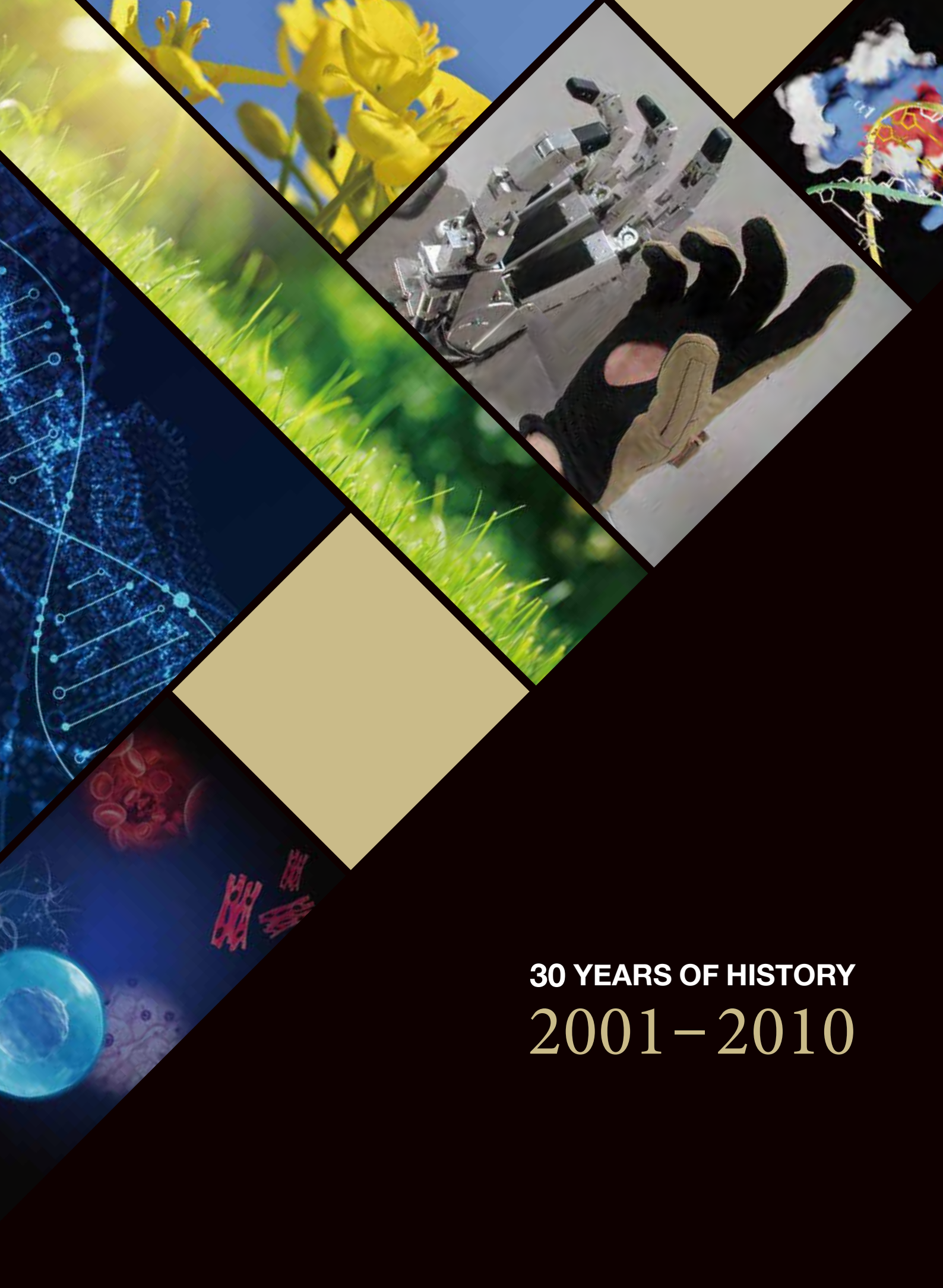


Photoisomerization of spiropyran in response to UV light (Ito *et al.*, 1999, *Nat Biotechnol*).



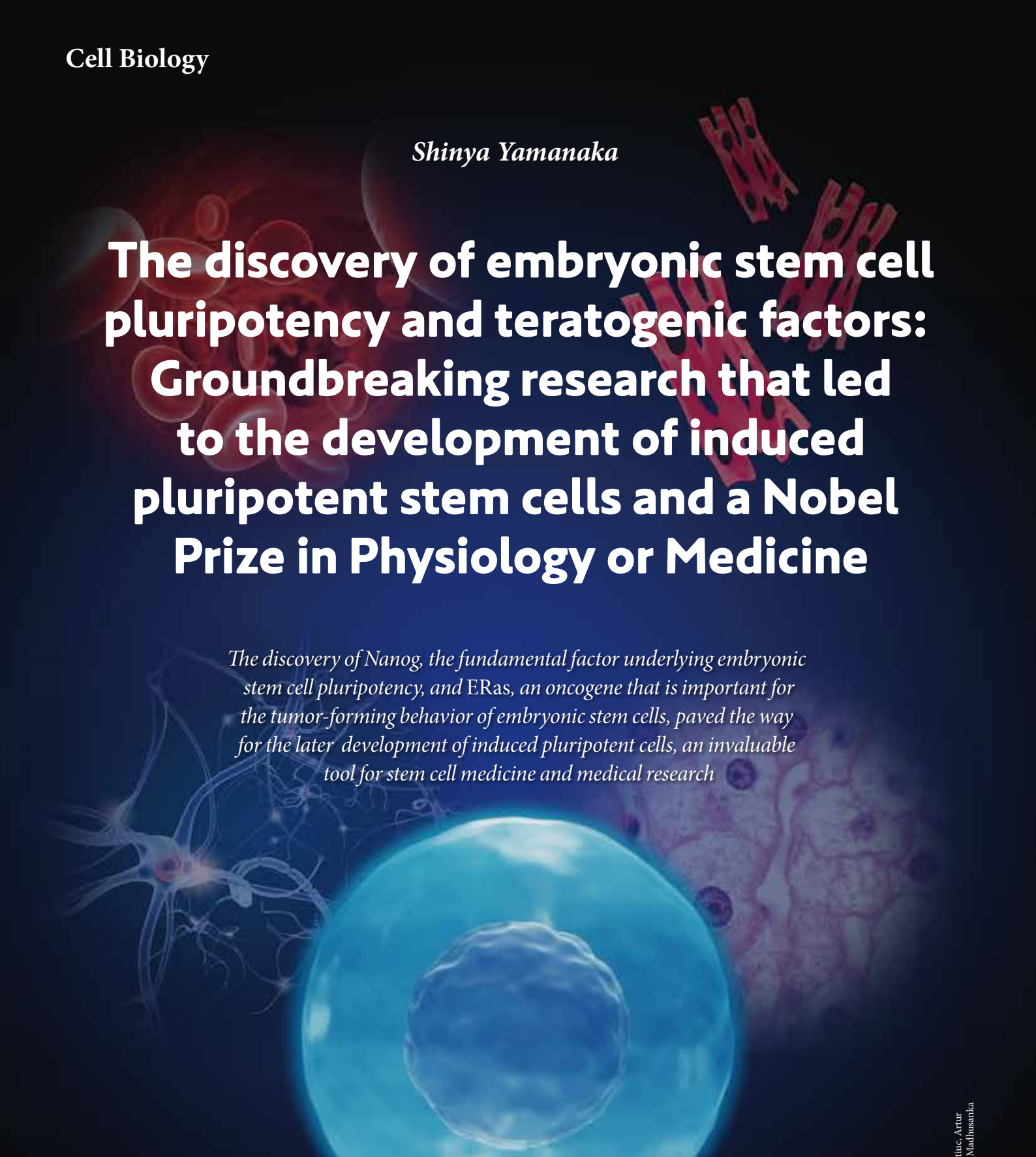
30 YEARS OF HISTORY

2001–2010

Shinya Yamanaka

The discovery of embryonic stem cell pluripotency and teratogenic factors: Groundbreaking research that led to the development of induced pluripotent stem cells and a Nobel Prize in Physiology or Medicine

The discovery of Nanog, the fundamental factor underlying embryonic stem cell pluripotency, and ERas*, an oncogene that is important for the tumor-forming behavior of embryonic stem cells, paved the way for the later development of induced pluripotent cells, an invaluable tool for stem cell medicine and medical research*



Embryonic stem cells are a special cell type in early mammalian embryos that have a unique feature called pluripotency, which means that they are capable of differentiating into every cell type that will comprise the adult organism. The field of stem cell medicine is based on the idea that pluripotent embryonic stem cells could be used to regrow mature cell populations, thereby restoring the function lost with the loss of the original cells. However, this approach faces some challenges. First, embryonic stem cells can only be isolated from embryos, which raises substantial ethical concerns. Second, implanted embryonic stem cells can produce tumors. In 2003, two seminal papers were published by a research group led by Shinya Yamanaka at NAIST, recipient of the Nobel Prize in Physiology or Medicine 2012, that explored the molecular basis of embryonic stem cell pluripotency and teratogenic behavior to discover solutions to these challenges.

Prior to these investigations of embryonic stem cell pluripotency, it was known that leukemia inhibitory factor (LIF) signaling through Stat3 could maintain symmetrical self-renewal – the process by which cells divide into two identical daughter cells – of mouse embryonic stem cells. However, LIF/Stat3 signaling is not required for maintenance of the inner cell mass of developing embryos, where these cells are located, nor is it necessary for human embryonic stem cell self-renewal. Therefore, the aim was to identify the factor, or factors, that are crucial for pluripotency by detecting genes that are expressed specifically in embryonic stem cells.

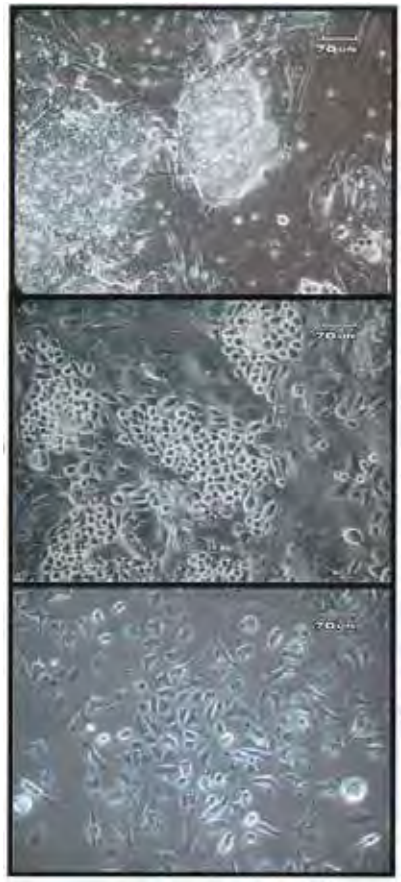
The researchers initially identified 20 candidate genes, some of which had already been experimentally identified as embryonic stem cell–specific markers. Nine uncharacterized genes were expressed specifically in embryonic stem cells, but only one, when expressed constitutively in embryonic stem cells without LIF, resulted in retention of their stem cell morphology. This gene was named *nanog*, for Tir Na Nog, which in Irish mythology is "the land of the ever young"; *nanog* was expressed in four independent embryonic stem cell lines, but not in twelve somatic organs, confirming that it is embryonic stem cell–specific. In addition, when *nanog* was deleted, the cells grew more slowly, changed shape, and expressed markers of endodermal cell lineages, suggesting that they had lost pluripotency and were committed to an endoderm cell fate. Furthermore, *nanog*-deficient mouse embryos exhibited a disrupted tissue structure, and cells retrieved from the inner cell mass of these embryos differentiated into endoderm-like cells, again reflecting a loss of pluripotency.

The groundbreaking work reported in this paper published in *Cell* showed for the first time that *nanog* is the fundamental factor underlying pluripotency in the inner cell mass and in embryonic stem cells. The identification of this critical molecular regulator of cell fate provided crucial insight into early embryogenesis, and broke new ground in stem cell medicine by delineating one way in which cells could be induced to adopt and/or maintain a pluripotent state for differentiation into clinically relevant cell types for therapeutic applications.

In the second study published in *Nature*, Yamanaka's research group sought to identify the factor(s) responsible for the tumor-like properties of embryonic stem cells. They identified a gene expressed specifically in embryonic stem cells in mice that exhibited a high level of homology, or similarity, to known *Ras* genes, and was therefore named *ERas*. Comparison with human genes showed considerable similarities between *ERas* and *HRasp*, which was previously thought to be a pseudogene but in fact is the human ortholog of *ERas*, and was therefore renamed human *ERas*. Importantly, both mouse and human *ERas* transformed cells *in vitro* and induced tumor formation in nude mice, confirming that these are teratogenic factors. When *ERas* was deleted from mouse embryonic stem cells, the mice grew and developed normally, although the cells themselves grew slowly and produced smaller tumors than wild-type cells in nude mice, suggesting that *ERas* is required for the tumor-like properties of embryonic stem cells, but not for embryonic stem cell pluripotency.

The findings from this study show that *ERas* is a transforming oncogene that is at least partly responsible for the tumor-like behavior of embryonic stem cells. This has important implications for stem cell therapeutics, because it could help engineer cells that are pluripotent but not tumor-forming. The identification of *ERas* therefore provided a crucial new avenue for adapting embryonic stem cells for clinical use.

Taken together, these two papers from Yamanaka's laboratory represented significant advances in our understanding of stem cell biology. Crucially, this groundbreaking work later led to the development of induced pluripotent stem cells, which are mature, adult cells that have been induced to dedifferentiate into an embryonic stem cell-like state by the application of only a few factors, one of which is Nanog. The ability to create these cells from adult cells circumvents the ethical dilemmas associated with using stem cells from embryos, and they are thus an invaluable tool for stem cell medicine. Yamanaka's seminal contributions to the development of induced pluripotent stem cells led to him being awarded the Nobel Prize in Physiology or Medicine in 2012.



Morphology of wild type RF8 cells grown on STO feeder cells (top) and *nanog* null cells grown with or without STO feeder cells (middle or bottom, respectively) (Mitsui *et al*., 2003, *Cell*).

References

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Seiji Takayama

How *Brassica* plants recognize their own pollen

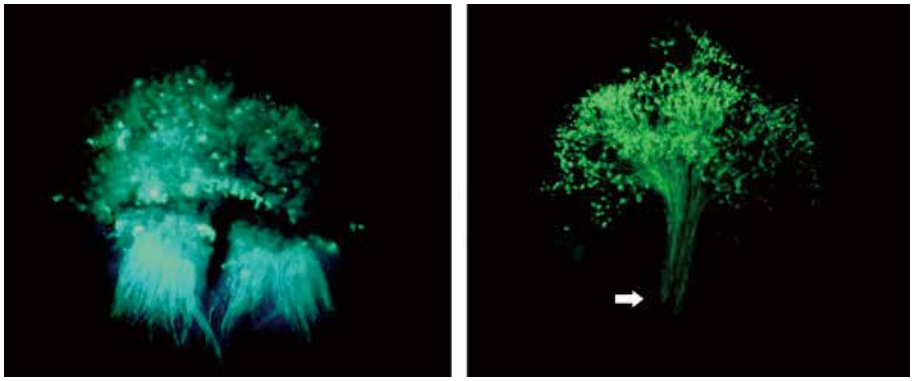
The identification of a novel signaling protein and small non-coding RNA revolutionizes our understanding of the self-incompatibility response in plants

Many flowering plants have evolved to develop a self-incompatibility (SI) response, avoiding self-fertilization from its own pollen and the potentially negative genetic consequences resulting from this. This is the case in *Brassica* plants, which have SI regulated by a series of genes called *S* haplotypes. Together, the pistil determinant gene and pollen determinant gene from the *S* haplotype help initiate the molecular signaling cascade associated with the SI response. A thorough investigation of the specific details of this cascade was therefore necessary to fully understand this complicated but crucial plant process.

Previous work in the field demonstrated that the *S* locus contains three genes vital for SI in *Brassica*: *S*-receptor kinase (*SRK*), *S*-locus protein 11 (*SP11*) and *S*-locus glycoprotein (*SLG*). The SRK protein is a membrane-bound kinase that determines the *S*-haplotype specificity of the stigma, the portion of the pistil that receives pollen, while the SP11 protein is the pollen *S*-haplotype determinant and is part of the pollen coat. SP11 can be secreted by the pollen and bind to the respective SRK, initiating the SI response in the stigmatic papilla cells. However, the role of SLG in this process was a point of contention. This gap in the knowledge interested Seiji Takayama at NAIST, who then spent years researching the nuances of the SI signaling cascade in *Brassica* and ultimately made significant contributions to the field.

In a study published in *Nature* in 2001, a research team led by Takayama investigated previous observations that SLG – a protein secreted by the stigma and very similar to a portion of SRK – could enhance the SI response. Here, they studied the S_8 haplotype. They first isolated and purified the S_8-SP11 protein from the pollen, and then based on mass spectrometry analysis, they showed that its structure is stabilized by four intramolecular disulfide bonds. Interestingly, after performing crosslinking experiments to identify proteins that S_8-SP11 can interact with, they found that it binds to both S_8-SRK and S_8-SLG to form a larger complex. Formation of this complex resulted in autophosphorylation of S_8-SRK and thus the start of the SI cascade. This did not occur with S_8-SP11, emphasizing the *S*-haplotype specificity.

Though it was clear that a signaling cascade is initiated when self-fertilization occurs, the



Brassica mm **plants (right) show defective self-incompatibility response with penetrated pollen tubes (arrow) (Murase *et al.*, 2004, *Science*).**

molecule(s) and effects downstream of autophosphorylated SRK were unknown. The research team therefore continued their characterization of this pathway with a study published in *Science* in 2004. The *M* locus became of interest, because plants with a recessive mutation in the *modifier* (*m*) gene had complete absence of the SI response in the stigma with normal activity in the pollen. In fact, the protein encoded by the *M* gene was fully undetermined. The team identified this as a 404-amino acid protein kinase, which they called *M* locus protein kinase (MLPK). They showed that *MLPK* expression was mainly in the stigma and levels increased immediately prior to flowering, similar to the expression pattern of *SRK*. The researchers then pinpointed a single nucleotide difference in the mutant *MLPK* gene (*m*) that resulted in an amino acid substitution preventing the kinase's autophosphorylation activity. Moreover, the MLPK protein was undetectable in mutant plants, suggesting the substitution affected the protein's stability. Transiently expressing wild-type MLPK in mutant papilla cells restored the SI response, demonstrating that functional MLPK is indispensable for proper rejection of self-pollen in these plants.

The researchers provided crucial data that revolutionized our understanding of the molecular signaling governing the SI response in *Brassica*. However, they remained interested in the upstream *S*-haplotype genes and how their dominant/recessive relationship is regulated in these plants. In particular, *SP11* genes are expressed in a monoallelic pattern, meaning the version of the gene from one parent is expressed (dominant allele), while the version from the other parent is not (recessive allele). The molecular details of how this is controlled remained elusive to the team, leading to a set of experiments that were published in *Nature* in 2010. The group previously found that promoter methylation could turn off expression of the recessive allele. Intriguingly, they observed inverted genomic sequences in regions surrounding dominant *SP11* alleles that were similar to sequences in recessive *SP11* promoters. Expression of this sequence led to the production of a 24-nucleotide small non-coding RNA that they named *SP11* methylation inducer (*Smi*). This RNA can work in *trans* to activate methylation of the recessive allele promoter, repressing transcription. This was the first report of a small non-coding RNA working in this manner to affect a dominant/recessive allele relationship.

Through a series of high-impact publications spanning over a decade, the research team revealed significant molecular information regarding the SI response process in *Brassica* plants. This included from *S* locus haplotype gene expression mechanisms to the SI signaling cascade. The team identified two novel factors: MLPK as a crucial SI signaling mediator and *Smi* as a *trans*-acting non-coding RNA in marking the recessive *SP11* allele. Furthermore, they found that the SLG protein is part of a larger SI-associated complex and the mutant *MLPK* gene erases the SI response in the stigma. These results were groundbreaking for a greater understanding of self-recognition in plants.

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Toshiharu Shikanai

Cyclic electron flow is critical for higher plant photosynthesis

Biochemical and genetic studies on cyclic electron flow in photosynthesis uncover crucial proteins involved in this process

Photosynthesis is the process by which plants convert light energy to chemical energy that can be used as fuel for its metabolic reactions. This is performed by photosystem I (PSI) and photosystem II (PSII), protein complexes that operate in the thylakoid membrane of chloroplasts. Elucidating the different molecular mechanisms that govern photosynthesis, particularly under conditions of stress such as extreme light, low temperature and drought, is important to better understand plant biology. This will also lend a greater appreciation of the differences between plant species at the molecular level.

During photosynthesis, light energy is used to generate a proton gradient (ΔpH) across the thylakoid membrane, which is then used to synthesize ATP. This gradient can be generated via at least two routes: linear electron flow and cyclic electron flow. Linear electron flow involves both PSI and PSII, where electrons produced by the splitting of water are used to form reduced ferredoxin or NADPH in the chloroplast stroma. In contrast, cyclic electron flow is driven solely by PSI – electrons are recycled around PSI to plastoquinone from either reduced ferredoxin or NADPH.

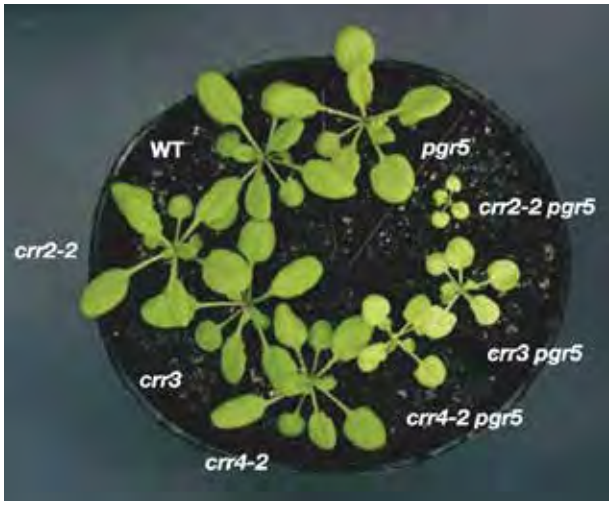
Cyclic electron flow and the generation of ΔpH are central to energy production in plants. However, the specific details of these processes and how they are maintained under various stress conditions were poorly understood. A series of studies led by Toshiharu Shikanai at NAIST provided key insights into the mechanisms of cyclic electron flow in photosystems.

Under excessive light conditions, PSII is downregulated to dissipate heat to avoid production of dangerous byproducts. However, electron transport is often limited when the plant is under stress, bringing into question how ΔpH is maintained in these cases. In a study published in *Cell* in 2002, the research team hypothesized that cyclic electron flow is induced to facilitate this heat dissipation. They examined the role of the *proton gradient regulation* (*PGR5*) gene in cyclic electron flow around PSI by isolating an *Arabidopsis thaliana* mutant, *pgr5*, that exhibited impairment of PSII photochemistry downregulation in response to intense light. *pgr5* mutants are therefore defective in cyclic flow. The team revealed that *PGR5* encodes a previously unidentified thylakoid membrane protein that plays a role in electron transfer from ferredoxin to plastoquinone. These results suggested that the PGR5 pathway contributes to the generation of a ΔpH that triggers heat loss when Calvin cycle activity is reduced and also prevents PSI photoinhibition by limiting overreduction of its acceptor side. By identifying PGR5, these findings helped characterize the molecular nature of the alternative electron transfer pathway for the first time.

Following this increased understanding of cyclic flow, the team aimed to investigate its effects on photosynthesis efficiency. Their results were published in *Nature* in 2004. Double mutants of *A. thaliana* were constructed in which both PSI cyclic pathways were impaired by crossing *pgr5* mutant plants with *crr* plants (impaired NA(P)DH dehydrogenase (NDH) activity). Severe phenotypes were observed in these double mutants, including slowed growth and reduced chlorophyll content. These results suggested that the significance of cyclic flow had been underestimated, and that linear flow cannot sustain the required ratio of ATP/NADPH production in the absence of cyclic flow. This ultimately results in excessive accumulation of NADPH in the stroma, and consequently its overreduction. These findings showed that cyclic flow is essential for the prevention of stroma overreduction, and therefore key to efficient photosynthesis. These data also further

expanded upon their earlier demonstration of the importance of PGR5 in this process.

After appreciating the importance of cyclic electron flow in photosynthesis, the researchers examined the genetic components that govern this machinery and how they are regulated, which was published in *Nature* in 2005. The chloroplast genome of higher plants encodes 11 subunits (*ndhA*–*ndhK*) of the NDH complex. Interestingly, when examining the *A. thaliana crr4* mutants (impaired NDH activity) they investigated previously, the team identified *crr4* transcripts with deficient RNA editing. RNA editing is a process of RNA maturation involved in nucleotide modification, deletion or insertion. In higher plant organelle transcripts, editing often leads to restoration of conserved amino acid residues, an essential process for protein function in plastids. Further experiments indicated that a pentatricopeptide repeat (PPR) protein acts as a factor for recognizing the target RNA in these plants and enables the access of a general factor that contains cytidine deaminase activity. The genetic approach used by the team was not previously done in higher plants. Their reported discovery of PPR as crucial to RNA editing in chloroplasts provided significant insight into the machinery controlling this process in these plants.



Visible phenotype of *Arabidopsis thaliana* mutants (Munekage *et al.*, 2004, *Nature*).

The results of the extensive work done by the research team demonstrated key details about cyclic electron flow in photosynthetic pathways in certain plants, and provided molecular characterization of the genes and factors involved in regulating these processes. The team identified PGR5 as the thylakoid membrane protein involved with cyclic electron flow in plants that helps dissipate heat. They also showed that this cyclic electron flow is essential for efficient photosynthesis and prevention of chloroplast stroma overreduction. Furthermore, the team discovered PPR as a core part of the RNA editing machinery in these plants' chloroplasts. These findings revolutionized our molecular understanding of how the photosynthesis process responds to excess light.

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Ko Shimamoto

Blooming brilliant: Discovering the master regulator of flowering timing in rice plants

Hd3a is a mobile signal in rice plants that travels from the leaves to the shoots, where it forms a functional protein complex that induces flowering

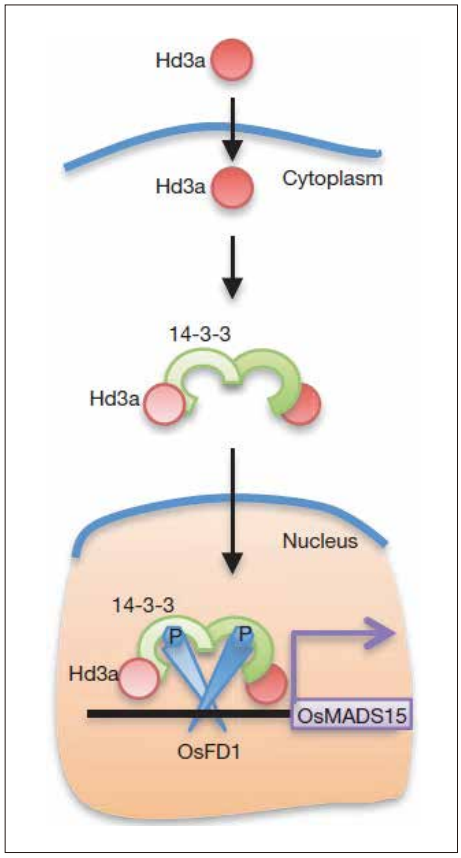
Plants have a complex lifecycle that is highly regulated by many environmental factors, such as the availability of water, nutrients and sunlight. In addition to daily variations in these conditions, plants are also subject to seasonal changes that affect their growth and development. A key moment in a plant's life that is central to its reproduction, and therefore survival, is when it flowers, and the timing of flowering is very carefully regulated at a genetic level in response to internal and external cues. Day length in particular is a crucial determinant of plant flowering, and all plants can be classified as long-day plants or short-day plants based on how day length promotes their flowering. *Arabidopsis*, which is a common plant model, is a long-day plant, and the genetic regulation of its flowering cycle had been well-characterized.

However, the flowering process in short-day plants was considerably less well understood.

To address this, a research team led by Ko Shimamoto at NAIST explored the regulation of flowering in rice, an important short-day plant. The *Arabidopsis GIGANTEA* gene was known to promote flowering, so the research team began by overexpressing the rice equivalent of this gene, *OsGI*, in rice, publishing their findings in *Nature* in 2003. Unexpectedly, this resulted in later flowering, indicating that *OsGI* has the opposite role of *GIGANTEA* and suppresses flowering in rice. Overexpression of *OsGI* also led to decreased expression of *Hd1*(*Se1*) and *Hd3a*, rice equivalents of *Arabidopsis* genes that are also involved in the regulation of flowering time. Interestingly, in wild-type rice plants both *OsGI* and *Hd1*(*Se1*) showed similar expression patterns when the plants were exposed to short-day or long-day conditions, while *Hd3a* was strongly suppressed under long-day conditions but showed a daily pattern of expression changes under short-day conditions. Taken together, these findings suggested that, similar to *Arabidopsis*, in rice *OsGI* activates *Hd1*(*Se1*) to promote flowering, except under long-day conditions, when *Hd1*(*Se1*) suppresses *Hd3a* expression, thereby suppressing flowering. This study not only highlighted the conservation of an important regulatory network among plants, but also underlined the central role that *Hd3a* plays in regulating the timing of flowering in rice.

In a study published in *Science* in 2007, the research group explored the possibility that *Hd3a* could be the elusive molecule referred to as 'florigen,' a mobile flowering signal that was known to move from plant leaves to the shoot apex to induce flowering, but whose identify remained unknown for many years. Earlier studies had suggested that the *FLOWERING LOCUS T* (*FT*) gene in *Arabidopsis* could encode the factor known as florigen, as the FT protein is only expressed in leaves but interacts with a transcription factor that is only expressed in the shoot apex. Therefore, the research team investigated the expression of rice *Hd3a*, the *FT* equivalent, in different tissues and found that this gene too is expressed almost exclusively in the leaves. Importantly, Hd3a protein was detected in the shoot apex, suggesting that it migrates through the plant like FT to reach the area where it can actively induce flowering. This groundbreaking finding strongly suggested that Hd3a is therefore florigen, the mobile flowering signal that had remained unidentified for so long.

While this study clearly revealed the identity of florigen, the mechanism by which this factor functions to effectively promote flowering still remained unknown. Therefore, the researchers next sought to identify the molecular mechanism by which *Hd3a* induces flowering in rice, publishing their findings in *Nature* in 2011. The team identified proteins that bound directly to Hd3a and found that one (GF14c) was a known 14-3-3 protein, while the others contained a SAP motif, which is similar to the 14-3-3 recognition motif. Further analysis showed that Hd3a interacts with GF14c in the shoot apex of rice plants, and that the active, stable complex also includes a protein called OsFD1. This three-protein complex was named the florigen activation complex (FAC) and was shown to directly bind the promoter region of *AP1*, a gene that leads to flowering. Analysis of where these proteins are located at different points suggested a model in which Hd3a travels from the leaves to the shoot apex, where it binds to 14-3-3 proteins in the cytoplasm; this two-protein complex then enters the nucleus, where it binds to OsFD1 to create the complete FAC that activates transcription of *AP1*, thereby inducing flowering.



14-3-3 interaction is required for *OsMADS15* activation by Hd3a and OsFD1 (Taoka *et al.*, 2011, *Nature*).

Taken together, this groundbreaking series of studies identified Hd3a as a key regulator of flowering time in rice, a short-day plant, that behaves in an opposite manner to its equivalent protein in *Arabidopsis*, a long-day plant. Importantly, Hd3a was then confirmed to be the factor previously known as florigen, a mobile flowering signal that travels from the leaves to the shoot apex to induce flowering. Characterization of the FAC provided crucial insight into how this signal functions on a molecular basis to regulate the central process of flowering during the rice plant's lifecycle. This series of studies not only answered longstanding questions in the field of plant biology, but also represents a substantial advance in our understanding of the conservation and function of central pathways in plant cell biology, as well as providing new tools and conceptual frameworks for future studies.

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Naotake Ogasawara

The minimal gene set required to sustain bacterial life

Systematic inactivation of Bacillus subtilis *genes reveals the minimal set of genes that are indispensable for bacterial life*

The single thread that ties all organisms together, from the largest predators to the smallest microbes, is that they are alive. Given the incredibly variety on the theme of 'life,' a fundamental question in biology is what elements are actually required to sustain life. This question is important, because its answer may illuminate the most fundamental functions required for life. Certain processes, such as the taking in of nutrients and turning them into energy, are clearly common to all living organisms. But without the systematic analyses of research, it is difficult to say whether these obvious functions are all that is really needed to be a living organism; there may be other, less evident, processes that are equally important and shared among all living things. In addition, defining the minimal requirements for life has important implications for biotechnology, in that it could help optimize and streamline laboratory-based manufacturing processes designed to generate clinically and industrially important products using bacteria as living 'factories.' Therefore, an outstanding question in the life sciences is what is the smallest amount of genetic information needed for an organism to be alive.

To explore this question, a research team led by Naotake Ogasawara at NAIST focused on *Bacillus subtilis*, a model organism that is one of the best understood prokaryotes in terms of molecular and cellular biology, and one that is also of biotechnological significance. To obtain a reliable estimate of the minimal protein-encoding gene set required for bacterial life, the researchers systematically inactivated each gene in the *B. subtilis* genome by inserting a nonreplicating plasmid into the target gene. This resulted in complete elimination of expression of that gene and allowed the group to classify each gene as essential or nonessential. A gene was considered essential if it was unable to be inactivated by insertion, because this meant that the bacterium did not survive without it.

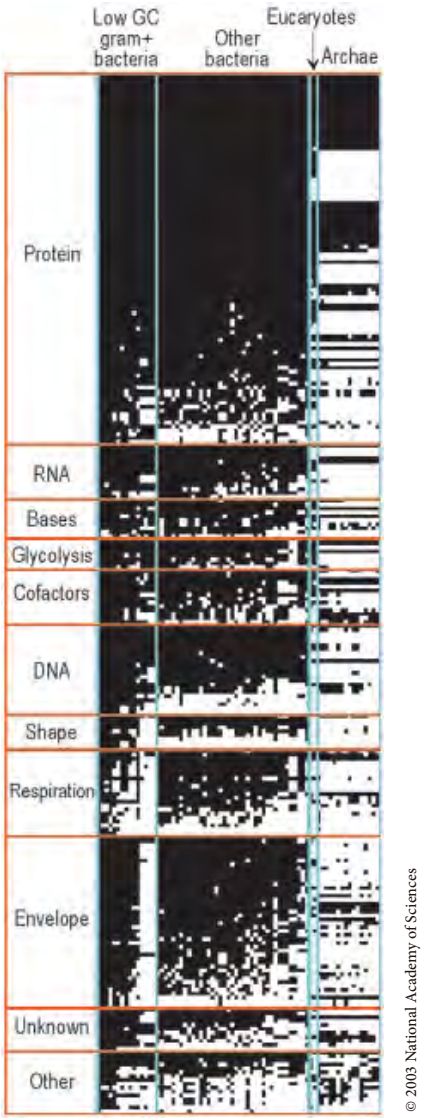
Intriguingly, even though the *B. subtilis* genome comprises approximately 4,100 genes, only 271 genes were identified as being indispensable for growth when inactivated one at a time. Of the essential genes that were identified, most were conserved across a wide range of *Bacteria*, and almost 70% were also found in *Archaea* and *Eucarya*, indicating genetic conservation across kingdoms. Many organisms therefore appear to rely on a similar set of essential functions. The researchers further found that the number of *B. subtilis* essential gene homologues present in an organism depended on two main factors: evolutionary proximity to *B. subtilis* and the size of the genome. Over 80% of essential *B. subtilis* gene homologues were found to be present in all bacteria with genomes over 3 Mb, and even bacteria with the smallest genomes (such as mycoplasma) retained 57% of these gene homologues. This prompted a

rather minimalist view of a bacterial cell, consisting of a compartment (or cell), enclosed by a membrane and a wall, that houses the machinery necessary to synthesize proteins that perform some basic essential functions: (1) duplication and inheritance of genetic information; (2) cell division; and (3) energy production.

The team's study also revealed some unexpected essential genes under the experimental conditions used, including eight genes involved in the EMP glycolytic pathway. The ubiquitous EMP glycolytic pathway is usually necessary for energy generation; however, it was not expected to perform an essential function during growth in nutrient-rich medium (used to create optimal growth conditions in this study) because the medium itself contains various compounds capable of providing the energy and building blocks required for cellular survival. It is therefore possible that the enzymes within the EMP glycolytic pathway that were identified as essential for life in this study may have novel, as yet unidentified roles within the cell.

Intriguingly, the researchers also identified 11 essential genes that at present do not have any known function. Based on comparison with known gene sequences, physiological roles could be suggested for two of these genes, and another five contain recognizable domains or motifs that could provide clues as to what they do. However, the remaining four of these unknown essential genes remain completely uncharacterized, despite their apparently pivotal role in *B. subtilis* survival.

The identification of essential genes in this study provided new insight into the processes that sustain bacterial life. By fully understanding the essential elements required for free living, new possibilities arise for bacterial genomic engineering and synthetic biology approaches with potential therapeutic or industrial applications. Newly identified essential genes that, at present, have no known function will undoubtedly reveal their purpose in future studies that will follow this important study. The benefits of this new knowledge will thus be revealed in the fullness of time.



Phylogenetic profiling of the 271 essential genes (Kobayashi *et al.*, 2003, *PNAS*).

Reference

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Masahiro Shirakawa

Solving the protein structure of a key binding domain sheds light on the neurodevelopmental disorder Rett syndrome

Structural analysis of the methyl-CpG binding domain reveals how proteins containing this domain can interact with methylated DNA, which enables the interpretation of disease-causing mutations in Rett syndrome

DNA methylation is an important biological mechanism for the regulation of gene expression and overall genomic stability. The proper functioning of this process is essential for normal development in mammals. Through methylation, the activity of DNA can be modified via addition of a methyl group, without changing the DNA sequence itself. Erroneous methylation can result in the inappropriate regulation of genes, leading to various diseases such as cancer or cardiovascular disease.

The consequences of DNA methylation are often mediated by proteins that contain methyl-CpG binding domains (MBDs). The structure of the MBD was unknown until 2001, when a research group led by Masahiro Shirakawa at NAIST reported the structure of the MBD in a human protein, MBD1.

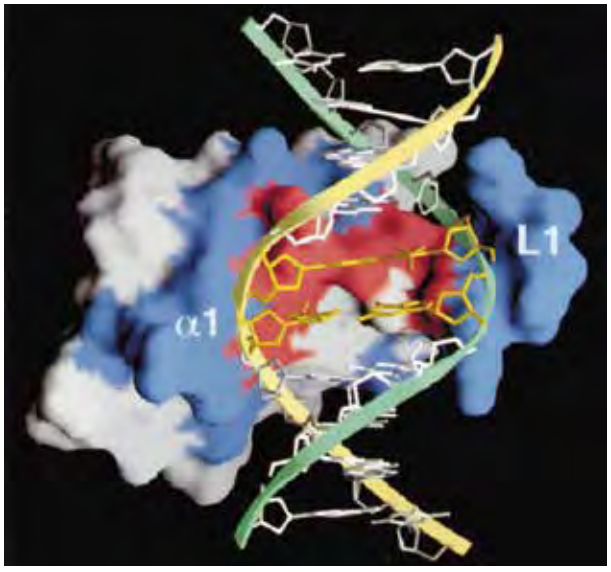
The MBD is highly conserved, meaning that its structure is shared with other MBD-containing proteins in several species. There are five MBD protein family members characterized in mammals, one of which is MeCP2. Seventeen different mutations in MeCP2 have been found to cause Rett syndrome which affects brain development leading to physical and intellectual disability in girls. Eleven of these mutations sit within the MBD, indicating the importance of this domain.

The team overexpressed human MBD1 in *E. coli* and labeled and purified it for nuclear magnetic resonance analysis. This revealed that when MBD binds DNA, structural rearrangements occur to form a loop in the protein structure, causing it to fold into a new DNA binding interface.

They found that five residues contact the major groove of the methylated DNA to identify the two methyl groups that form the methylated CpG group. These five residues form a hydrophobic patch. The lack of symmetry of this region indicates that each of the two methyl groups is recognized distinctly.

A notable feature of the interaction between the MBD and DNA is the small size of the contact area. This small contact area, which sits only on the major groove of the DNA, allows the MBD to access methyl-CpG sites on nucleosome cores without any steric interference from the chromatin structure.

As a result of the findings of this study, residues with known mutations in Rett syndrome could be analyzed. Some of the mutations in MeCP2 may result in failure of the MBD to contact the DNA bases. One reported mutation, in which an alanine is replaced by a valine, likely interferes with the DNA binding affinity, because valine has a bulky side chain. Mutations to other residues probably interfere with MeCP2's activity by causing global structural changes.



The MBD-DNA contacts lie exclusively within the major groove of DNA (Ohki *et al.*, 2001, *Cell*).

This study definitively outlined the protein structure of MBDs, which demonstrated the involvement of several physical interactions between the DNA strand and protein side chains, revealing for the first time how the methyl-CpG sequence can be recognized by the MBD-containing family of proteins. These findings facilitate a greater understanding of the causes of Rett syndrome and may assist research into other disease pathologies in which DNA methylation has been implicated, including vascular tissue changes in atherosclerosis and carcinogenesis.

Reference

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Takashi Hashimoto

The role of microtubules in the handedness of helical growth in plants

Two Arabidopsis thaliana *mutants,* lefty1 *and* lefty2*, are found to exhibit left-handed helical growth and clockwise twisting in elongating organs, and have cortical microtubules with increased sensitivity to microtubule-specific drugs*

Plants usually grow in a linear or circumferential way, but many plants exhibit some type of helical growth in the form of spirals, twists, or coils. Plant parts that show helical growth include the stems or tendrils of twining plants, fan-blade shaped flower petals, coiling roots, and spiral seed pods. Helical growth is relevant to agriculture and horticulture because it underpins plant forms that may be desirable, such as aesthetically pleasing flowers or plant shapes, or plants that are free to explore new environments, like climbing vines. Conversely, this type of growth may be undesirable if it results in plant products that cannot be mechanically processed (e.g., misshapen root vegetables). Understanding the molecular mechanisms that produce helical growth can contribute to the development of new ways of improving this trait to better utilize crops in horticulture and agriculture.

The left–right asymmetry, or handedness, of helical plant forms is often fixed within species, and this phenomenon is found in a broad range of taxa. This indicates that only a few genes underlie such asymmetry. Wild-type forms of the model plant *Arabidopsis thaliana* are symmetrical; however, this symmetry can be disrupted by mutations, including the right-hand twisting mutants, *spiral1* (*spr1*) and *spr2*. In an attempt to discover suppressor mutants of the right-handed helical growth mutant *spr1*, a research group led by Takashi Hashimoto at NAIST identified two independent mutant loci, *lefty1* and *lefty2*.

It was determined that the *lefty1* and *lefty2* mutations were the result of single nucleotide exchanges in the α-tubulin genes *TUA6* and *TUA4*, respectively. It was shown that these dominant negative mutations result in left-handed helical growth and clockwise twisting in *A. thaliana*'s elongating organs. The study demonstrated that the mutant tubulins integrate into microtubule polymers, yielding right-handed cortical arrays in the cells of the root epidermis. The mutants' cortical microtubules had a heightened sensitivity to microtubule-specific drugs; these results indicated that diminished microtubule stability can result in left-handed helical growth in plants.

A number of other helical growth mutants with fixed handedness had been reported in *Arabidopsis* prior to this study; however, the cellular and molecular causes of their asymmetrical growth were not known. The results of this study indicated that oblique orientation of cortical microtubules offered a possible mechanism for producing the left–right asymmetry in elongating plant organs. They also presented a potential molecular explanation for the establishment of handedness in *Arabidopsis* twisting mutants, as well as the natural asymmetry of vines, tendrils, and other twisting plant organs. In addition to contributing to a broadened understanding of helical growth in plants, the results were also relevant to herbicide sensitivity (e.g., dinitroaniline-like herbicides) in plants, as demonstrated by the increased sensitivity to microtubule-specific drugs shown by the mutants' cortical microtubules.

Reference

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Flowers of a wild type (left), a *lefty1* mutant (middle) and a *lefty2* mutant (right) of *Arabidopsis thaliana* (Thitamadee *et al.*, 2002, *Nature*).

Akiho Yokota

RuBisCO-like proteins may be the ancestral enzymes of photosynthetic RuBisCO

Carbon-fixing proteins found in Bacillus *and involved in photosynthesis share a common function that recycles sulfur, indicating a potential ancestral relationship*

Photosynthesis, the metabolic process responsible for fixing atmospheric carbon into organic molecules, dates back to the origins of Earth's biological life. Ribulose bisphosphate carboxylase/oxygenase (RuBisCO) is the predominant CO_2-fixing enzyme in the biosphere. It has both carboxylase and oxygenase activity, and it is the carboxylation and oxygenation of ribulose 1,5-bisphosphate (RuBP) that are the primary events in photosynthesis and photorespiration, respectively. At a high CO_2 concentration, RuBisCO functions only as a carboxylase and participates in the Calvin cycle, whereas, at a high O_2 concentration and a low CO_2 concentration, RuBisCO functions as an oxygenase, as manifested by photorespiration. Photorespiration emerged later than photosynthesis in evolutionary history, along with the increase in the oxygen content of the atmosphere.

All living organisms possess RuBisCO itself or a RuBisCO-like protein (RLP) from the same superfamily. However, not all of these proteins possess carboxylase activity. The functional and evolutionary relationship of RLPs to photosynthetic RuBisCO remained unknown until 2003, when a research team led by Akiho Yokota at NAIST described the functional relationship between a RuBisCO-like protein of *Bacillus subtilis* and photosynthetic RuBisCO.

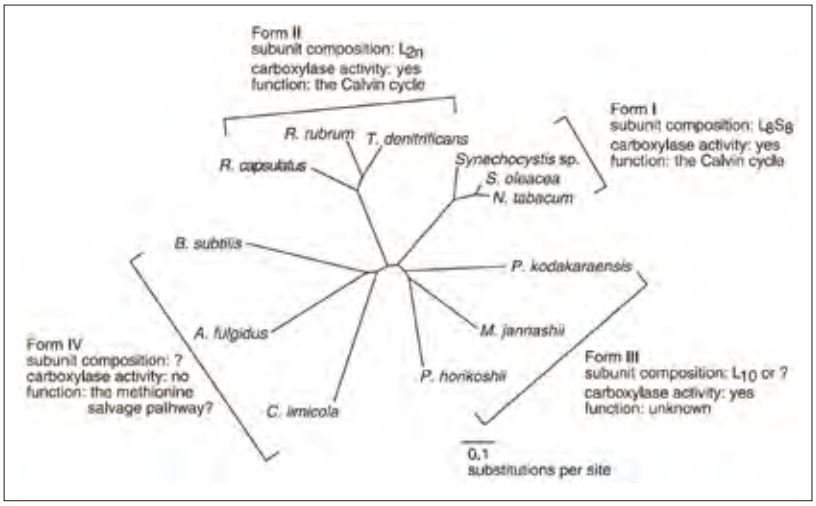
The research group found that the RLP of *B. subtilis* functions as a 2,3-diketo-5-methylthiopentyl-1-phosphate (DK-MTP-1-P) enolase in the methionine salvage pathway – the process by which sulfur is recycled following certain biosytheses – but possesses no RuBP-carboxylation activity. Interestingly, the growth defect resulting from mutation of this RLP-encoding gene in *B. subtilis* was rescued by the RuBisCO gene from the photosynthetic bacterium *Rhodospirillum rubrum*, as demonstrated by *in vivo* experiments. This finding was significant because it proved that the photosynthetic RuBisCO from *R. rubrum* retained the ability to function in the methionine salvage pathway in *B. subtilis*, potentially indicating common ancestry.

Phylogenetically, the researchers found that the RLP from *B. subtilis* did not cluster with RuBisCO enzymes or other RLPs with RuBP-carboxylase activity. Furthermore, the codon usage and GC content of the RLP gene were typical of the organism. On the basis of these findings and previous literature, the group proposed that the genes for RLP and photosynthetic RuBisCo did not originate from lateral transfer of a gene encoding an RuBP-carboxylating enzyme from another unrelated organism, such as an archaeon or photosynthetic bacterium. Instead, the gene for RLP, which in *B. subtilis* is part of the methionine salvage pathway, and the gene for photosynthetic RuBisCO originated from a common ancestral gene.

However, as the group highlighted, the evolutionary origins of bacteria and Archaea that possess RLPs far pre-date the emergence of the Calvin cycle in photosynthetic bacteria. This led to the conclusion that RLPs may be the ancestral enzymes of photosynthetic RuBisCO. An improved understanding of the origin of early RLP-encoding genes may offer insight into how these fundamentally important CO_2-fixing enzymes have overcome changes in global environments throughout history.

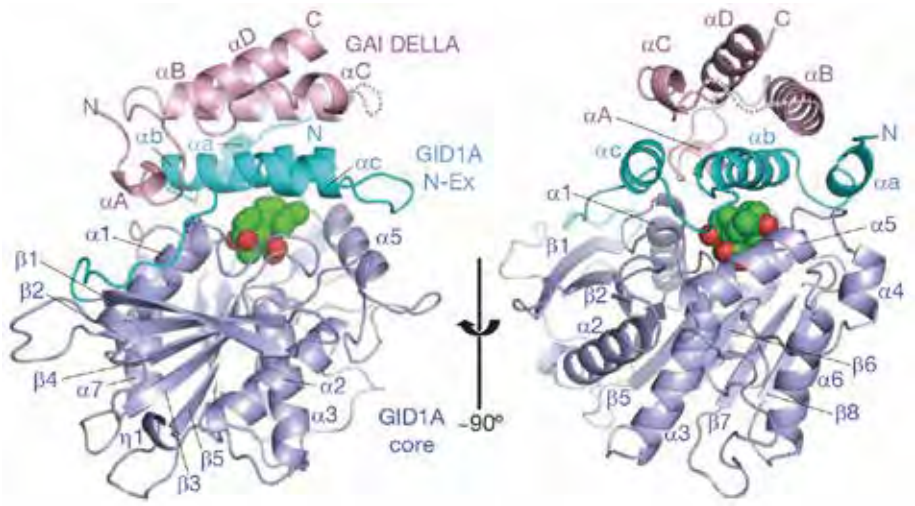
Reference

Ashida, H., Saito, Y., Kojima, C., Kobayashi, K., Ogasawara, N., Yokota, A. 2003. A functional link between RuBisCO-like protein of *Bacillus* and photosynthetic RuBisCO. *Science*, 302, 286–290.



Phylogenetic relationships of the large subunits of RuBisCO (forms I and II) and RLPs (forms III and IV) from various organisms (Ashida *et al.*, 2003, *Science*).

Toshio Hakoshima



Structure of the GA_3–GID1A–DELLA complex (Murase *et al.*, 2008, *Nature*).

How plant cells perceive and decode the gibberellin signal

A structural model of a plant hormone receptor is identified that differs from the previously known underlying mechanism of hormone sensing and effector recognition in auxin receptors

Gibberellins (GAs) are a large, important group of plant hormones that are involved in growth and development in higher plants, including shoot elongation, seed germination, and the development of flowers and fruit. These hormones have a broad range of uses in agriculture, from the production of seedless fruit to the promotion of flowering. GAs regulate gene expression; however, the exact mechanism by which the GA signal is recognized and decoded by plant cells was a key question in plant biology that had remained unanswered for years.

A research group led by Toshio Hakoshima at NAIST addressed this question using the model plant *Arabidopsis thaliana*. GAs regulate gene expression by binding to the receptor GIBBERELLIN INSENSITIVE DWARF1 (GID1), and enabling degradation of DELLA proteins, which negatively regulate GA signaling, including GIBBERELLIN INSENSITIVE (GAI). Because bioactive GAs control various developmental processes during a plant's life cycle, the team specifically examined how bioactive GAs are sensed by and activate GID1 for binding to DELLA proteins. The team set out to determine the structure of GA receptors to understand the detailed relationships between structure and activity regarding these molecules.

Crystallographic and biochemical methods were used to examine how the GA receptor GID1A perceives GA, and to investigate GA-induced binding of GID1A to GAI. The result was the presentation of a crystal structure of a ternary complex comprising GID1A, a bioactive gibberellin, and the amino-terminal DELLA domain of GAI. In this complex, GID1A traps GA in a deep binding pocket, which is covered by its N-terminal region. This region then interacts with the DELLA domain that contains DELLA, VHYNP and LExLE motifs, which are involved in GID1 binding.

The results of the study offered a model for the structure of a plant hormone receptor that differs from the hormone sensing and effector recognition mechanism of the known auxin receptors. Just prior to this study, auxin was revealed to act as the 'molecular glue' that brings a substrate protein together with the receptor, which in the case of the auxin pathway is an F-box protein. Conversely, the GA receptor is a true receptor protein that is activated by GA to serve as a 'ubiquitylation chaperon' that triggers recognition of the substrate by the SCF complex, which is involved in protein degradation. The structural investigation in this study furthered the understanding of cellular function regulation that is dependent on degradation signals, and the results revealed a structural mechanism underpinning the perception of GA and DELLA protein recognition by GID1. The findings also suggested an alternative way forward regarding development of GA derivatives for regulating the growth of ornamental plants and crops.

Reference

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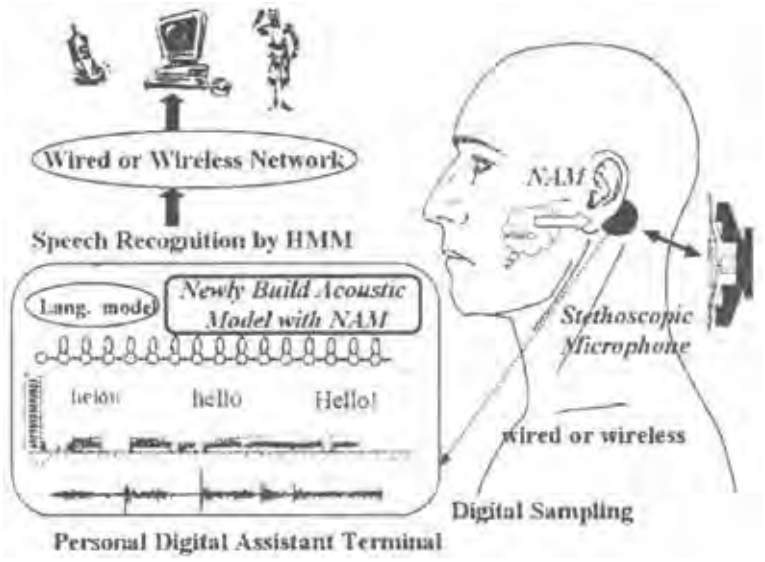
Kiyohiro Shikano



A voice-recognition interface with a stethoscopic microphone attached behind the ear (Nakajima *et al.*, 2003, *ICASSP*).

A non-audible voice interface for enhanced speech recognition

A microphone to be attached directly to a user's skin is designed to register subaudible sounds for voice-recognition software, which may lead to more accurate computer interfaces

As smart devices become increasingly portable, and ever more reliant on voice commands instead of keyboards, robust interfaces that work under a wide range of situations with varying levels of background noise are required. Additionally, as these electronics reach nearly universal adoption, the voice commands themselves begin to add to the interference between users in the same room, potentially limiting use outdoors, in public spaces or inside vehicles. The development of new devices that can overcome these issues contributes greatly to continued technological progress in this field, as well as being more user friendly and secure.

A research team led by Kiyohiro Shikano at NAIST developed a voice-recognition interface that operates by capturing non-audible murmur (NAM) vocalizations via a stethoscopic microphone that can be attached directly behind the ear. Because acoustic vibrations travel more easily through bone and human tissue compared with air, this microphone can decern sounds with intensities much lower than can conventional systems. Normally, once sound energy from the vocal cords leaves a person's mouth, it will propagate in all directions in an unfocused fashion. This requires the user to speak louder, which in turn adds to the room's ambient noise. By contrast, a vocalization with an intensity below the usual threshold of audibility can be directed by the human anatomy to produce vibrations at other locations on the head, to be picked up by the stethoscopic microphone; hence, this system can recognize commands that are too quiet to be heard in the air. This can lead to quieter working environments and promote data privacy. Moreover, this approach is much less susceptible to noise interference and may be suitable for people who are unable to speak loudly owing to health reasons.

The researchers tested the system using the Japanese Dictation Toolkit, which includes voice-recognition software along with acoustic and language modeling capabilities. Accuracy ratings around 90% were achieved, even with background sounds present. The team studied common types of ambient noise, such as a Bach concerto and a television news broadcast. The results are even more impressive given the limitations of voice-recognition software available at the time. The fidelity is expected to be even higher when implemented using contemporary applications.

The results of this work may substantially improve the state of voice-recognition artificial intelligence by providing more accurate commands. In particular, this research may make wearable computers considerably more attractive by providing a reliable, cost-effective hands-free user interface that will work in loud environments.

Reference

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Minoru Ito

A genetic algorithm for optimizing multiple destination itineraries

A personal navigation system that utilizes a genetic algorithm to find optimized routes for trips with multiple destinations, which may assist both tourists and commercial delivery services

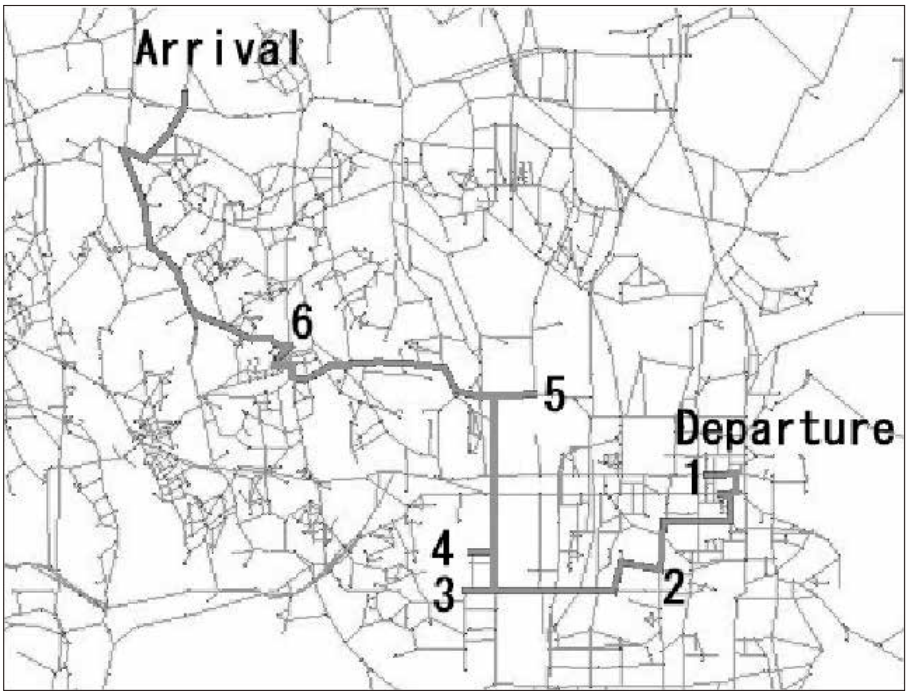
Managing travel itineraries with multiple destinations and limited time has been a challenging task for centuries. One example is the "traveling salesman" problem, which requires finding the shortest path between list of cities, visiting each exactly once. Despite the relative lack of constraints, this problem is known to be "NP-hard", meaning that no algorithm can efficiently find the absolute best solution unless the number of cities is small. Tourists in the real world face even more practical requirements, such as business hours, reservation times, or changes in plans. The potential benefits of a personal navigation system (PNS) that could map out reasonable itineraries was recognized for all kinds of travelers.

To help achieve this goal – in a study that predates both Google Maps and the iPhone – a research group led by Minoru Ito at NAIST created a genetic algorithm that computes "semi-optimal solutions." While previous methods were limited to finding the best way to travel between two given points, the algorithm they made can handle trips with multiple destinations. Genetic algorithms are different from other search optimization methods in that they use a population of candidate solutions. Then, using a process similar to the natural selection of organisms, the solutions that are found to have the highest "fitness" for solving the problem are chosen to be retained. These high-fitness candidate solutions undergo "crossover" and "mutation" operations to produce new possible itineraries.

In the complete program, the well-known A* search algorithm is first used to determine the shortest route between each pair of desired locations. Then, the novel genetic algorithm is used to find the best possible routes. One benefit of this approach is that multiple candidate solutions are considered during each step, so an approximate solution is always available. In addition, the user can be provided a list of possible routes from which to choose.

The researchers conducted a map-based test, selecting up to 13 destinations with specific time restrictions associated with four destinations. Because the route search algorithm was to be executed on a remote server, it could be computed more efficiently compared with being located on the user's device. Future extensions of the algorithm can be made to include multiple transport modalities, such as riding on trains, buses, or walking.

The approach demonstrated by the research can also be applied to more general sets of travel constraints experienced throughout the transportation industry. This may be of significant value specifically for commercial delivery services that would save on fuel and labor costs with shortened routes. Additionally, as factories and warehouses increase in size and automation, the need for indoor itinerary optimization is expected to increase in the future.



The entire route of six destinations obtained by the genetic algorithm (Maruyama *et al.*, 2004, *AINA*).

Reference

Maruyama, A., Shibata, N., Murata, Y., Yasumoto, K., Ito, M. 2004. A personal tourism navigation system to support traveling multiple destinations with time restrictions. *International Conference on Advanced Information Networking and Applications*, 18–21.

Tsukasa Ogasawara

A robotic hand with vision-based feedback control

A robotic hand that mimics human physiology and uses fingertip cameras to measure contact forces leads to prosthetic devices with increased functionality, as well as more robust automated object manipulation

Robotic arms are employed in many automated tasks, such as automotive manufacturing. However, these appendages have typically lacked the dexterity of human hands, and they have been unable to reliably pick up and manipulate objects. In particular, robots have a difficult time calculating and applying the correct amount of force to grasp something without crushing it. Being able to construct a robotic hand that can mimic human motion is an important goal for both prosthetics and industrial applications.

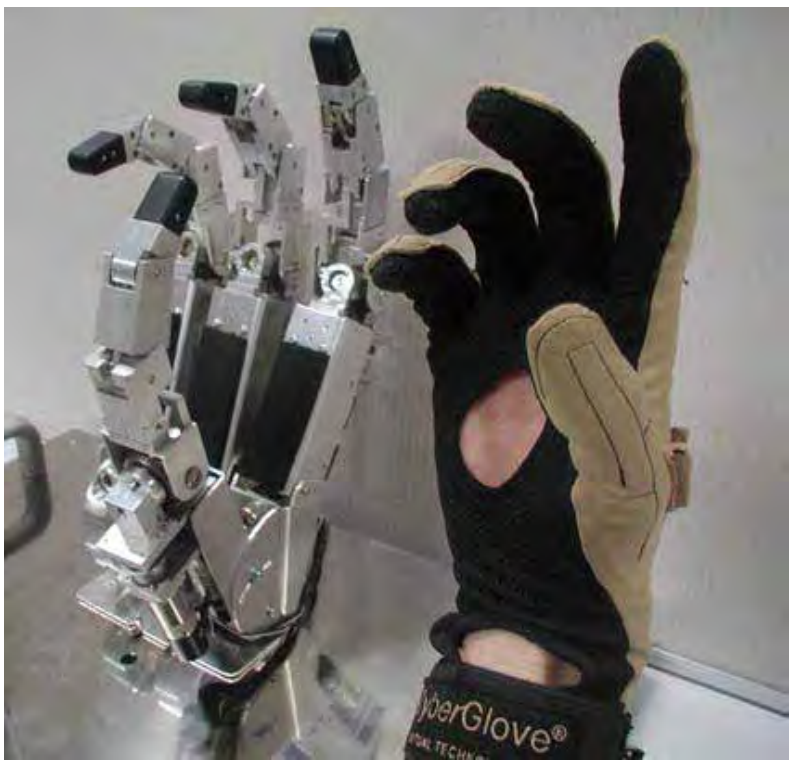
A research team led by Tsukasa Ogasawara at NAIST developed a robotic hand that uses a vision-based tactile sensor in each fingertip. The hand has four fingers, and each finger is capable of three degrees of freedom. The team used a specially designed gear mechanism that placed all three actuators in the palm section, thus avoiding space limitations, as well as the need for a wire-control system. Rotation along each of the three axes is controlled independently by a separate driving mechanism. One of the joints in each finger is capable of adduction/abduction as well as flexion/extension, while a second joint has flexion/extension, similar to human physiology.

To carefully monitor the force being applied by each fingertip, the researchers attached a hemispherical transparent gel with LED light. An embedded camera underneath looked for signs of distortion caused by the compression of the gel and was coupled with a force detector. By measuring the eccentricity of the gel's image, the displacement could be calculated. When this was combined with the radius of the contact area, the "slip margin" could be estimated. While some previous approaches to slip detection used accelerometers, these often have been found to experience too much noise to be practical. Similarly, measuring the strain distribution required a large number of sensors to obtain the necessary spatial resolution. Using a camera-based method had many advantages, including improved accuracy of contact area measurements. In addition, a stable grip could be obtained by using real-time feedback of the slip margin, even when the coefficient of friction was not known. To control the feedback loop, the team used a mathematical model in which the radius of the contact area is proportional to the normal force raised to the ⅓ power. When an incipient slip was detected, the traction force could be adjusted before the object moved too much.

The advances presented by the NAIST Hand provided an important foundation for subsequent improvements in robotic hand technology. Future versions of the robotic hand may be controlled using a motion-capture glove. Operators would be able to move heavy equipment without risk of injury. Additionally, patients requiring a prosthetic hand may be able to experience an improved quality of life by being able to grasp fragile objects, like paper cups, more easily.

Reference

Ueda, J., Ishida, Y., Kondo, M., Ogasawara, T. 2005. Development of the NAIST-Hand with vision-based tactile fingertip sensor. *IEEE International Conference on Robotics and Automation,* 2332–2337.



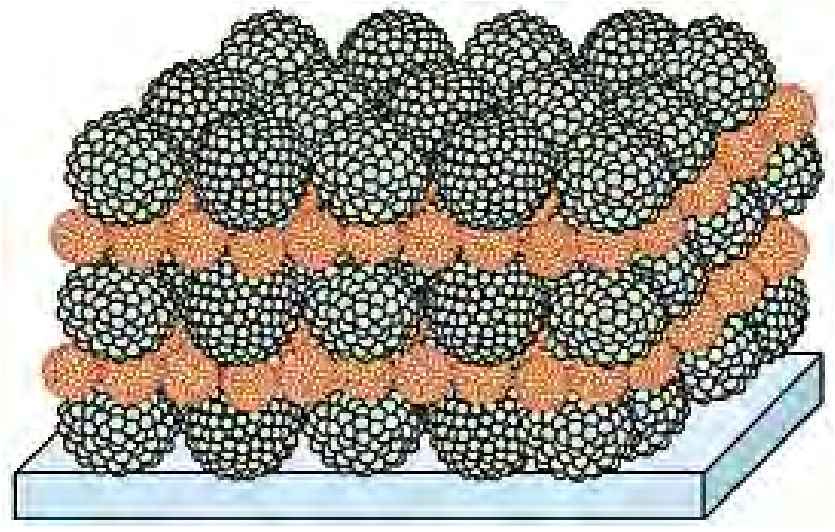
Motion transfer to the NAIST Hand (Ueda *et al.*, 2005, *ICRA*).

Jun-ichi Kikuchi

Paving the way through layer-by-layer assembly of lipid bilayer vesicles

Layer-by-layer assembly of cationic and anionic Cerasomes gives three-dimensional hierarchical bilayer structures on solid substrates

The lipid bilayer membrane that surrounds cells is so effective at its job that it is used as inspiration for synthetic products. For example, vesicles made of a lipid membrane protecting an aqueous environment have been used as carriers for drugs and as artificial cell membranes. The lipid used for the bilayer of these synthetic systems can be purposefully selected, meaning features such as particle size can be controlled. It should therefore follow that different lipids could be combined to achieve multiple functions within one system. However, this advance relies on assembling vesicles that are stable enough to maintain their integrity in a variety of conditions. This was not achieved until a research team led by Jun-ichi Kikuchi at NAIST reported vesicles assembled from organic-inorganic hybrid proamphiphiles in 2002.



Schematic drawing of the three-dimensional assembled structure of the anionic and cationic Cerasomes (Katagiri *et al.*, 2002, *J Am Chem Soc*).

The research team used two different organo-alkoxysilanes to form Cerasomes – organic-inorganic hybrids with a liposomal membrane and a ceramic surface. The ceramic outer layer was expected to act as a protective barrier against the collapse or fusion of the vesicles. Two different types of Cerasome were assembled: one that was cationic at pH 9.0 and one that was anionic. Transmission electron microscopy showed that the vesicles formed were 70–300 nm and 20–100 nm in diameter, for the anionic and cationic Cerasomes, respectively.

In addition, the Cerasomes had properties that would be expected for bilayer vesicles; for example, they showed phase transition behavior from the gel to the liquid crystalline state. The phase transition temperatures were found to be 10.5 and 25.7 °C for the anionic and cationic Cerasomes, respectively, showing that they responded differently to temperature triggers.

The researchers showed that the opposing charges of the Cerasomes could be used to build up layers of vesicles that maintained their discreet morphologies without collapsing, or the membranes fusing. Quartz crystal microbalance measurement showed that alternately exposing a substrate to aqueous solutions of each Cerasome led to sequential increases in mass with each layer. The mass increases were greater for the anionic Cerasome layer, which was anticipated owing to their larger particle diameter. When compared with layer-by-layer exposure to traditional cationic and anionic liposomes, the Cerasomes resulted in much greater mass increases, showing that stable buildup was achieved, unlike for the liposomes.

Atomic force microscopy images were acquired to confirm that the integrity of the Cerasomes in each layer was maintained. The images showed the distinct sizes of the vesicles in each layer, nicely demonstrating the stability of the vesicle morphologies. The packing of the layered structure was likened to a pavement, with the larger anionic Cerasomes acting as paving stones that the smaller cationic structures packed closely around like cement.

The three-dimensional structure reported in this study was the first packed hierarchical structure assembled on a solid substrate using the layer-by-layer technique. The stability of the different vesicle types demonstrated the exciting potential to include different functions within the same system, laying the foundations for many functional systems, such as responsive drug delivery systems that release therapeutics at specific locations in the body and complex artificial cells.

Reference

Katagiri, K., Hamasaki, R., Ariga, K., Kikuchi, J. 2002. Layered paving of vesicular nanoparticles formed with cerasome as a bioinspired organic-inorganic hybrid. *Journal of the American Chemical Society*, 124, 7892–7893.

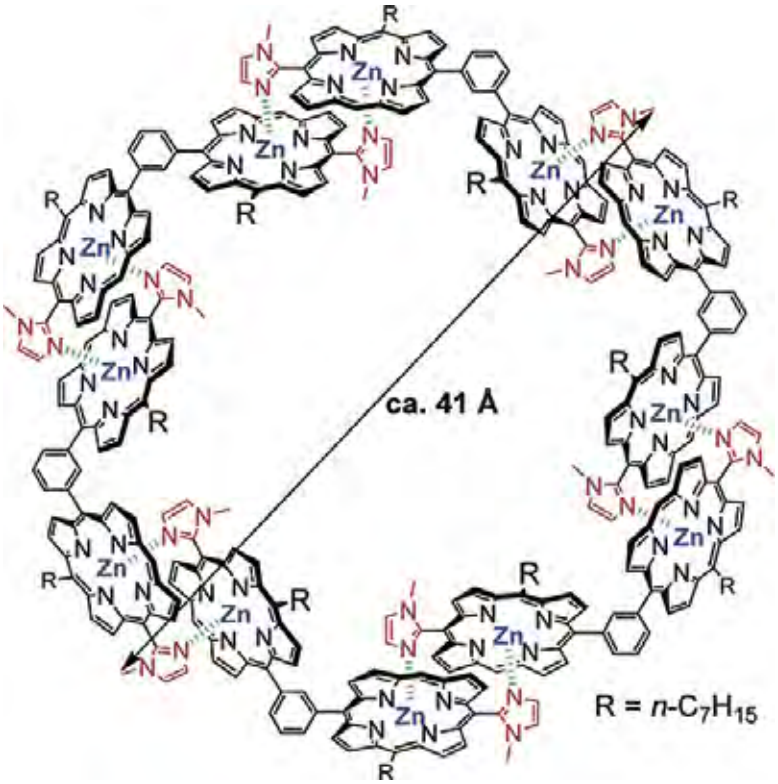
Yoshiaki Kobuke

A synthetic mimic of a biological light-harvesting molecule

A self-assembled, porphyrin-based barrel structure is synthesized, enabling structure–function insights into light harvesting by photosynthetic organisms

Photosynthetic organisms use intricate macromolecular assemblies to absorb solar energy for subsequent conversion into the chemical energy necessary to perform life functions. However, researchers have been unable to fabricate close synthetic mimics of these assemblies, a necessary step to understand how the assemblies work and how to apply them to modern technology. Thus, it can be difficult to optimize light harvesting in artificial constructs, such as in solar cells that may exceed the efficiency of even the most advanced silicon-based designs in use today.

The first step of photosynthesis is absorption of solar energy by light-harvesting complexes that are arranged in a barrel architecture. Artificial analogs of such complexes, based on porphyrin rings, have been synthesized. However, the foundational porphyrin macroring architecture in biology – based on dimers arranged in a slipped-cofacial geometry – has not been synthesized. A research team led by Yoshiaki Kobuke at NAIST published work on the self-assembly of a supramolecule which has intramolecular orientations and distances that reflect the architecture of biological light-harvesting complexes, as confirmed by rigorous experimental characterization.



A structural model of the target cyclic hexamer (Takahashi & Kobuke, 2003, *J Am Chem Soc*).

The macroring design was based on two principles. First, the geometry of the zinc-containing porphyrin-based dimer facilitates light absorption and minimizes energy waste after light absorption. Second, the geometry of the phenylene spacer that links each dimer enables ring closure into a barrel architecture which has center-to-center distances of 6.1 and 11.0 angstroms. This closely mimics the architecture of light-harvesting complexes found in photosynthetic purple bacteria.

Gel permeation chromatography indicated that the intended macroring was synthesized, rather than a linear polymer. Atomic force microscopy indicated that the complex consisted of round particles ~1.5 nanometers in height, consistent with the intended barrel shape. Small-angle X-ray scattering indicated a particle radius of gyration of ~41 angstroms, consistent with the outer diameter predicted by molecular mechanics simulations.

To demonstrate that the macroring was capable of light harvesting, the researchers used ultraviolet-visible spectroscopy to quantitate its light absorption. Bands characteristic of the expected porphyrin absorption were present. Furthermore, the fluorescence quantum yield of the macroring relative to that of the porphyrin dimer subunits was 0.51, which is consistent with analogous observations in bacterial light-harvesting complexes.

These results provide a means of experimentally testing light-harvesting structure–function relationships that are otherwise intractable except by theoretical calculations. Ongoing questions on the molecular-level mechanism of light harvesting can be addressed systematically and quantitatively through this research. Such knowledge could help researchers optimize light harvesting in technology that does not depend on biology, such as organic solar cells.

Reference

Takahashi, R., Kobuke, Y. 2003. Hexameric macroring of gable-porphyrins as a light-harvesting antenna mimic. *Journal of the American Chemical Society*, 125, 2372–2373.

Takashi Fuyuki

Rapid imaging of polycrystalline silicon solar cell defects

Quantitating the main parameter that indicates the efficiency of solar cells with routine instrumentation enables performance evaluations during mass production

Polycrystalline silicon is a major raw material for the global solar cell industry and is a workhorse that converts sunlight into electricity. It is imperative to check solar cell performance during the manufacturing process for rapid quality control, and this is doubly important with the increasing demand for photovoltaic systems as the world begins to take the threat of climate change seriously. However, commonly used simple checks – such as current/voltage output under simulated light – do not provide sufficiently thorough metrics to correct manufacturing problems. Thus, it can be difficult to correct solar cell fabrication errors in real time, which increases costs for the consumer.

A fundamental parameter for rigorously evaluating the performance of solar cells is the minority carrier diffusion length (MCDL). This parameter is pertinent to the efficiency of a cell's energy production. However, the laboratory-grade instrumentation that works for quantita ting the MCDL isn't suitable for the production line. A research group led by Takashi Fuyuki at NAIST explored the use of a routine imaging technology – a charge-coupled device (CCD) camera – to quantitate the electroluminescence and thus estimate the MCDL of polycrystalline silicon solar cells. The benefit of having this MCDL estimation is that it enables rapid detection of deteriorated areas in the cells.

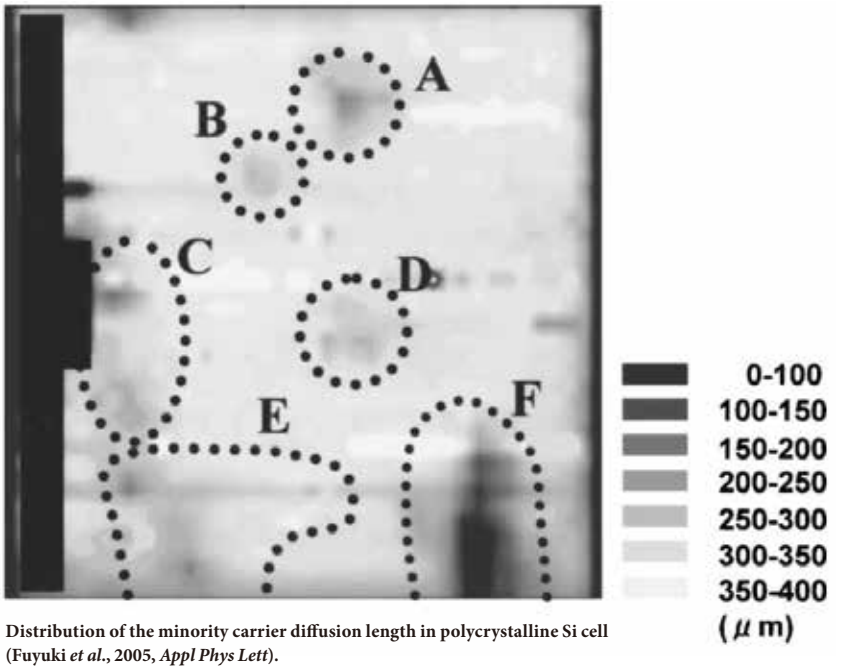
The imaging design was based on electroluminescence: applying a voltage to a solar cell and measuring the resulting emitted infrared light with a CCD camera. The intensity of the emitted light can be used to estimate the MCDL. The electroluminescence results were then compared with a standard research method – light-beam-induced current – where they used four wavelengths of light from the red to near-infrared range to exactly quantitate the MCDL. The distribution of the intensity of the electroluminescence emitted by the solar cell as measured by the CCD camera corresponded to the mapping of the MCDL. Specifically, regions in the cell that appeared dark by electroluminescence were areas of short MCDL. Furthermore, the spatial resolution of the electroluminescence method was improved by an approximate factor of 5 compared with the light-beam-induced current method, meaning that smaller defects in the cell could be identified and resolved.

To further verify these results, the researchers quantitated the relationship between the measured electroluminescence and the calibrated MCDL. The results demonstrated that a straightforward photographic survey of electroluminescence is a reliable approach for determining the MCDL in silicon solar cells, though not with absolute precision.

These results provide a means of rigorously evaluating the quality of polycrystalline solar cells by using common instrumentation. The solar cell industry can thus identify defects in the production line in real time without investing in specialized instrumentation or adding significant time to the manufacturing process. This development provided a solution to industry that will ultimately lower the cost of solar cells to global consumers and increase the economic feasibility of solar energy.

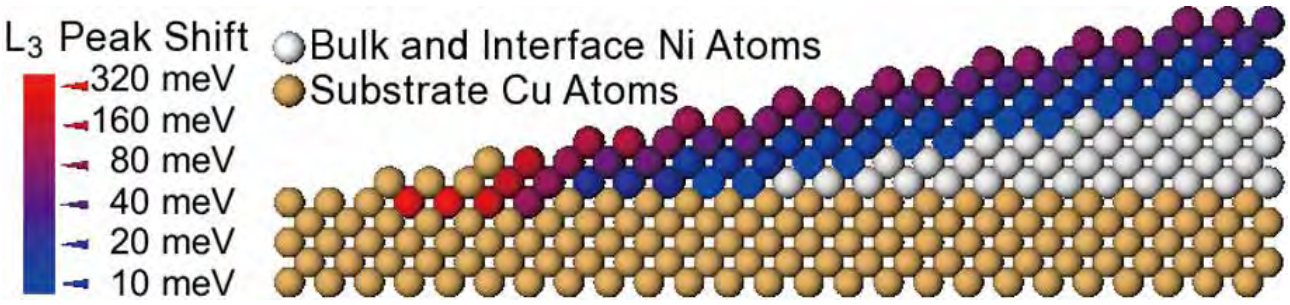
Reference

Fuyuki, T., Kondo, H., Yamazaki, T., Takahashi, Y., Uraoka, Y. 2005. Photographic surveying of minority carrier diffusion length in polycrystalline silicon solar cells by electroluminescence. *Applied Physics Letters*, 86, 262108.



Distribution of the minority carrier diffusion length in polycrystalline Si cell (Fuyuki *et al.*, 2005, *Appl Phys Lett*).

Hiroshi Daimon



Ni L_3-edge peak shift from bulk for each atomic layer in various Ni film thicknesses (Matsui *et al.*, 2008, *Phys Rev Lett*).

Atomic-layer resolution of the magnetism of nanoscale nickel

Diffraction spectroscopy is developed for quantitating the magnetism of ultrathin nickel films, yielding quantitative data that can be compared with theoretical studies of advanced data storage media

Maximizing the density of a device's data storage capacity increases the processing speed and lowers the cost of computer technology. However, there are ongoing demands for a continually increasing data storage density; diverse technical approaches are necessary to meet these demands and thus the increased memory needs of future devices. Magnetism based on nanostructured nickel would advance the state-of-the-art, but experiments have not been able to confirm the outstanding potential suggested by theoretical work.

The layer-by-layer, atomic-scale architecture of ultrathin films of nickel on copper is challenging to experimentally measure. There are means of interrogating, for example, the magnetic structure of the buried nickel–copper interface. However, direct measurements at atomic-layer resolution are required to experimentally test theoretical predictions of magnetism enabled by nanoscale architectures. A research team led by Hiroshi Daimon at NAIST aimed to address this lack of experimental testing by devising diffraction spectroscopy for direct, layer-by-layer measurements of the electronic configuration of energetic atoms.

A new spectroscopy method was first used to measure the energies and two-dimensional angular distributions of electrons emitted from ultrathin coatings of nickel on a copper (001) surface. Data that were consistent with nickel atoms in the second or third layer of atoms were obtained from one- or two-monolayer-thick coatings of nickel. This was direct evidence that some of the nickel atoms mixed with the underlying copper.

X-ray absorption near-edge structure measurements were possible up to a depth of 10 monolayers of nickel atoms. The results of these measurements differed depending on the depth of the atomic layer. One of the interpretations of these layer-by-layer studies was that from the standpoint of electronic properties, the surface of the nickel consisted of only three atomic layers.

To demonstrate unprecedented experimental interrogation of the advanced magnetic properties of nanoscale nickel, X-ray magnetic circular dichroism measurements were taken. There was in-plane magnetism for thin films consisting of eight monolayers of nickel atoms, and perpendicular magnetism when the film consisted of 15 monolayers. Thus, the spin magnetic moment of the film could be altered by adjusting the thickness of the film. This finding is consistent with theoretical predictions and is central to maximizing the efficiency of computer memory technology.

These results provided a means of experimentally testing theories of nanoscale magnetism. Ongoing questions of how such magnetism depends on atomic-level organization within ultrathin films of nickel can be addressed quantitatively through this field of research inquiry. Such knowledge could enable the high-density data storage – at a reasonable cost – that is essential for future high-speed computer technologies.

Reference

Matsui, F., Matsushita, T., Kato, Y., Hashimoto, M., Inaji, K., Guo, F.Z., Daimon, H. 2008. Atomic-layer resolved magnetic and electronic structure analysis of Ni thin film on a Cu(001) surface by diffraction spectroscopy. *Physical Review Letters*, 100, 207201.

Mikio Kataoka

First evidence for low-barrier hydrogen bonds

A combination of neutron and X-ray crystallography provides evidence for low-barrier hydrogen bonds in photoactive yellow protein, which may be important for fundamental insights into intermolecular bonding

Proteins and enzymes are important cogs in the machinery of our bodies, and investigating their molecular structures – how their atoms bond and interact – facilitates our understanding of their function and how they work. Armed with this knowledge, we can better counteract the proteins and enzymes involved in diseases, which is crucial for the design of new and improved drugs and therapies.

Low-barrier hydrogen bonds (LBHBs) were first reported to contribute to enzymatic processes and proton transfer in the 1990s, with transient LBHBs being thought to stabilize intermediates during reactions. However, in the absence of hard experimental evidence for the positions of hydrogen atoms, they proved a contentious concept until a research group led by Mikio Kataoka at NAIST demonstrated their existence in photoactive yellow protein (PYP) in 2009.

In contrast to standard hydrogen bonds where the dipoles of a donor and an acceptor atom interact, in LBHBs the hydrogen atom is shared. As a result, LBHBs have a shorter bond length than a standard hydrogen bond. Identifying the bond therefore required determining the positions of the hydrogen atom and the acceptor and donor atoms. However, because hydrogen atoms are so small, it is very difficult to detect them with crystallography techniques, which is why the subject was open to debate. To finally provide the experimental evidence that was lacking, the researchers investigated PYP using neutron and X-ray crystallography together.

PYP is a light sensor protein found in certain bacteria. It has a photoactive center that contains the small molecule *p*-coumaric acid (pCA) acid in a hydrophobic pocket. When light excites the pCA it rearranges into a different structure and the thermal reactions that follow cause reorganization of the hydrogen bonding structure.

Two short hydrogen bonds (SHBs) in PYP that are near the reactive center are known to be present even in the ground state. These SHBs were therefore selected as the focus of the investigation. Neutron and X-ray crystallography of PYP were achieved with resolutions of 1.5- and 2.5-Å, respectively.

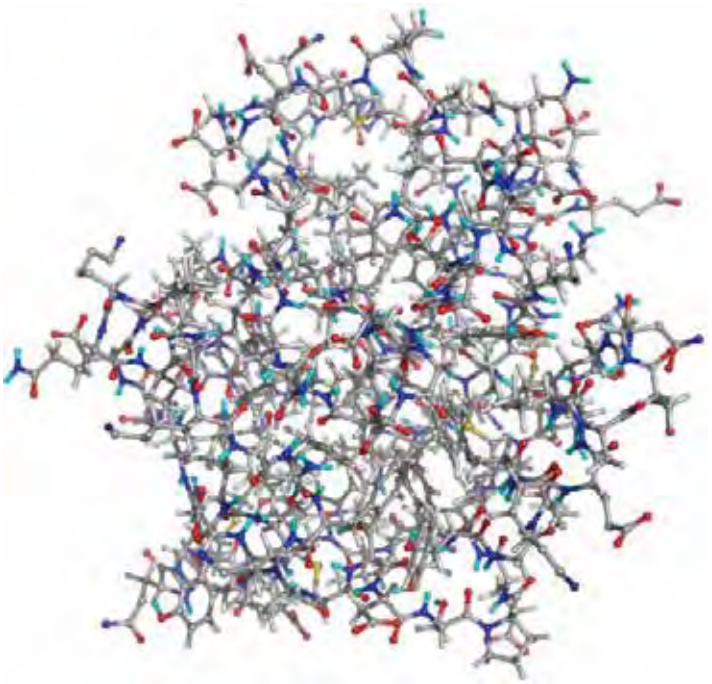
By refining both sets of data, the researchers were able to achieve nuclear density maps for 87% of the hydrogen and deuterium atoms in the protein. And based on the deuterium atoms – which have larger nuclei making them easier to detect – the researchers were able determine the locations of the hydrogen atoms participating in the bonds of interest. They found that the SHB between pCA and amino acid E46 is in fact a LBHB, while the other investigated SHB between pCA and amino acid Y42 is not and could be considered a short ionic hydrogen bond (SIHB).

The study was the first definitive example of transient LBHBs being formed and provided important insight into the additional roles played by hydrogen bonds in protein function – for example, influencing a protein's ability to stabilize an isolated charge and allow rapid proton transfer.

The implications of the findings continue to be far-reaching for the understanding of natural processes in our bodies and the world around us, which in turn provides starting points for developing vital therapeutics and treatments.

Reference

Yamaguchi, S., Kamikubo, H., Kurihara, K., Kuroki, R., Niimura, N., Shimizu, N., Yamazaki, Y., Kataoka, M. 2009. Low-barrier hydrogen bond in photoactive yellow protein. *Proceedings of the National Academy of Sciences of the United States of America*, 106, 440–444.



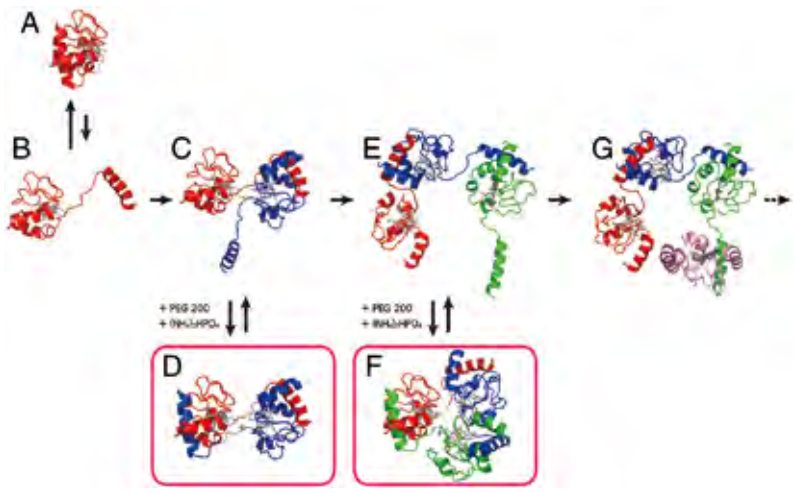
Structure of PYP including hydrogen atom positions (Yamaguchi *et al.*, 2009, *PNAS*).

Shun Hirota

Trading helices leads to protein polymerization

Structural studies reveal a protein polymerization mechanism where a helix from one monomer dissociates and plugs into another in a successive chain-linking reaction, thus providing broad insights into disease-causing protein fibril formation

Cytochrome *c* (cyt *c*) is a heme-containing protein that plays a key role in mitochondrial respiration, although it is also involved in the steps preceding apoptosis (cell death). This protein naturally exists and functions as a monomer (single protein unit) comprising three α-helices, but it is also known to form polymers or oligomers (comprising many or several protein units, respectively), albeit through an undetermined mechanism. In fact, when cyt *c* is heated, monomers aggregate to form amyloid fibrils similar to those associated with neurodegenerative disorders, such as Parkinson's disease. Therefore, understanding the structural changes and thermodynamics that govern cyt *c* oligomerization could provide critical insights into other protein polymerization processes and hence, numerous other pathologies and potential therapies.



Schematic summary of cyt *c* polymerization (Hirota *et al.*, 2010, *PNAS*).

To this end, a research group led by Shun Hirota at NAIST treated monomeric cyt *c* with ethanol and isolated its dimer, trimer and tetramer forms. The X-ray crystal structure of the dimer revealed that the C-terminal helices of the two monomers had swapped places; that is, a helix from one monomer now occupied the analogous helix's place in the other monomer's folded structure, and vice versa, thus linking the two protein units into a "closed-end mutually swapped" dimer. In the trimer's structure, the C-terminal helix displaced from the second monomer was instead inserted into a third monomer's structure whose displaced helix was fit into the first monomer's vacant site, thus forming a "cyclic" trimer structure. These results confirmed the so-called domain-swapping phenomenon.

The oligomers were all stable at low temperature but could be converted back to the monomer form by heating or addition of surfactant. Certain other additives could convert the less-stable linear configurations (where the final C-terminal helix does not occupy the vacant region of another monomer but instead dangles in an open-ended fashion) to the mutually swapped dimer or cyclic trimer structures. Together, these results suggested a certain equilibrium between the monomer and oligomer forms, and between the linear (open) and closed structures. The linear forms with "free" helix protrusions highlight the potential for higher-order polymeric structures because the ethanol solvent can stabilize the lone helix to allow for successive domain-swapping processes. Indeed, cyt *c* polymers with up to 50 protein units were identified with configurations similar to those of the smaller oligomers.

The oligomerization was also monitored according to the heme-iron environment in cyt *c*, specifically, the coordination of an axial methionine (Met) residue. This aspect was particularly crucial because Met dissociation from the iron allows for hydroxide coordination, which triggers peroxidase activity – an initial step of apoptosis. Disruption of the Met-iron coordination during cyt *c* oligomerization enabled the C-terminal helix swap, but the original heme coordination environment could be recovered upon heating to obtain the monomers. Moreover, the Met-iron de-coordination contributed 90% of the thermodynamic driving force for oligomer dissociation.

The characterization of cyt *c* oligomers confirmed a successive C-terminal domain-swapping mechanism, thus offering key insights into protein polymerization processes, including those linked to disease states. This knowledge presented a step forward in understanding the pathogenesis of such diseases, and may assist in the development of therapeutic interventions.

Reference

Hirota, S., Hattori, Y., Nagao, S., Taketa, M., Komori, H., Kamikubo, H., Wang, Z., Takahashi, I., Negi, S., Sugiura, Y., Kataoka, M., Higuchi, Y. 2010. Cytochrome *c* polymerization by successive domain swapping at the C-terminal helix. *Proceedings of the National Academy of Sciences of the United States of America*, 107, 12854–12859.

30 YEARS OF HISTORY

2011–2021

Kenji Kohno

Endoplasmic reticulum stress: How the cell prevents misfolded proteins

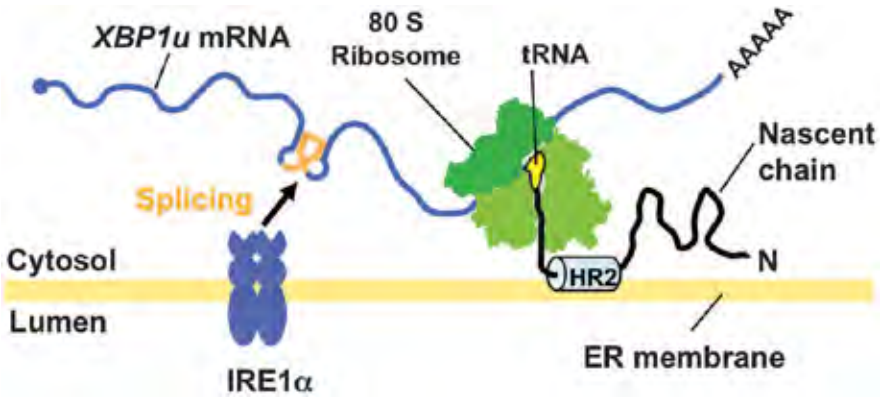
mRNA encodes the transcription factor that controls the ER-stress regulation pathway, facilitating its instant reaction to the build-up of ER stress

The endoplasmic reticulum (ER) serves as a site for protein synthesis and folding in cells and thus involves a major interplay of many important factors. For certain proteins, translation begins at ribosomes throughout the cytosol, but association with the ER and completion of translation require the signal recognition particle (SRP). This critical function means that the ER is susceptible to effects of cellular stress conditions, such as a viral infection. ER stress occurs when there is an imbalance between new proteins entering the ER and its folding capacity, and the ER cannot keep up with its expected workload. Understanding the mechanisms controlling this is important, because ER stress can lead to misfolded proteins and is associated with the pathogenesis of some degenerative disorders like Alzheimer's disease.

The inositol requiring enzyme 1α (IRE1α)-X-box-binding protein 1 (XBP1) pathway is the main eukaryotic mechanism for addressing ER stress. IRE1α is an ER membrane protein that is activated upon ER stress, which allows its RNase domain to cleave two sites of *XBP1* precursor mRNA (*XBP1u*). This spliced mRNA (*XBP1s*) encodes the XBP1s transcription factor, which promotes the transcription of ER stress-alleviating genes.

Because of the importance of ER stress in human disease, this pathway became a primary focus of a research group led by Kenji Kohno at NAIST. The group previously showed that the *XBP1u*-encoded protein, known as the mRNA-ribosome-nascent chain (R-RNC), can recruit the *XBP1u* mRNA-ribosome complex to the ER membrane through its hydrophobic region 2 (HR2) as it is being translated, serving to increase splicing efficiency during ER stress. In a 2011 paper published in *Science*, the group used *in vitro* translation experiments to demonstrate that *XBP1u* mRNA contains a signal that causes translational pausing. Serial truncation of a luciferase-XBP1u fusion protein indicated that the translational pausing is mediated by a C-terminal 26 amino acid region of XBP1u. Interestingly, this region is evolutionarily conserved, indicating that its function is important and not limited to certain species.

The *in vitro* observations were also seen in mammalian cell lines, where it was determined that L246A and W256A mutations could prevent the translational pausing, while the S255A mutation could increase it. Additional *in vitro* translation experiments using pausing-defective L246A and



A model for nascent chain-mediated membrane-targeting of the *XBP1u* mRNA (Yanagitani *et al.*, 2011, *Science*).

W256A mutants resulted in XBP1u peptide accumulation in the soluble fraction rather than the membrane fraction, implying that translational pausing is necessary for the XBP1u protein to be targeted to the ER membrane. Similar findings were seen *in vivo* in HEK293T cells. Furthermore, the L246A and W256A mutants had significantly reduced splicing efficiency of *XBP1u* mRNA, suggesting that translational pausing is also important for splicing.

The exact details and mechanism governing the recruitment of XBP1u-RNC to the ER were not fully clarified in the paper published in 2011. The team continued investigating this pathway and published a follow-up article in *PNAS* in 2016. Using immunofluorescence, they observed XBP1u protein localized in the ER and absent from the mitochondria and Golgi, suggesting it is specific to the ER membrane. A protease-treated microsome showed significantly lower affinity to XBP1u, implying that an ER membrane-based protein component is needed for this protein to be targeted there.

The research group investigated this protein component further through coimmunoprecipitation and mass spectrometry approaches. Proteins associated with SRP and the Sec61 translocon were identified and confirmed to interact with XBP1u. Because the Sec61 translocon is a membrane protein complex involved with transport to the ER, the researchers hypothesized that SRP recognizes the XBP1u-RNC and recruits it to the Sec61 translocon. However, a mechanism controlling this required further study. The association with SRP was confirmed to be in an HR2-dependent manner. Following membrane-flotation assays, it became evident that the presence of SRP is indispensable for XBP1u-RNC to be targeted to the ER. This was also seen in cells, where knockdown of SRP54 protein resulted in inhibited ER targeting of *XBP1u* mRNA, as well as a modest reduction of *XBP1u* splicing efficiency under ER stress.

Furthermore, observed interactions of XBP1u-RNC with SRP and the translocon were weakened with the W256A mutant and strengthened with the S255A mutant, suggesting that the translational pausing is critical for these associations. It was also shown that XBP1u does not enter into the ER lumen but stays on the membrane, implying that a noncanonical mechanism is at play.

These groundbreaking findings by Kohno and colleagues identify a novel mechanism where HR2 in XBP1u-RNC is recognized by SRP but does not result in translocon pore opening and entrance to the ER lumen. Furthermore, their data highlight the critical need for translational pausing for this to proceed properly and the cell to efficiently respond to ER stress. This work may lead to the identification of other mRNAs that are targeted and spliced in this manner. Importantly, a better understanding of how the cell responds to ER stress can aid in the investigation behind the mechanisms of conditions such as Alzheimer's disease which are associated with misfolded proteins. Gaining clarity over the innate cellular mechanisms responsible for preventing protein misfolding may strengthen efforts to develop therapies for treating diseases associated with ER stress.

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Yoshiko Takahashi

Identifying the key control center for sympathetic nervous system development in early embryos

The diversification of the sympathetic nervous system is coordinated by signaling from the dorsal aorta, enhancing our fundamental understanding of a key step in embryonic development

The autonomic nervous system (ANS) plays a key role in various involuntary physiological functions including stress defenses (fight-or-flight response) and homeostasis. ANS dysfunction can occur by itself or in diseases such as diabetes and autoimmune disorders, and usually results in an inability to properly regulate biological processes such as blood pressure or heart rate. Proper functioning of the ANS requires multiple cooperating cell types; however, how the different cell lineages of the ANS develop in the early embryo remained largely unknown.

The ANS comprises a variety of cell types that are responsible for specific processes. These include the adrenal medulla that secrete hormones such as adrenaline in response to stress, the sympathetic neurons that control the body's rapid response to stressful stimuli, and the parasympathetic neurons that control involuntary functions of the body at rest, such as salivation and digestion. The sympathetic (Sg) and medullary (Am) lineages both develop from common progenitor cells, known as SA progenitors, that are formed from neural crest cells during embryogenesis.

Previous research had shown that the SA progenitors first migrate towards the dorsal aorta, the first blood vessel that forms in a developing embryo. Then, in the region around the aorta, they begin to differentiate into the separate Sg and Am lineages. This indicated that signaling from the dorsal aorta may play a role in this differentiation process. However, the exact mechanism of action had proved difficult to uncover, because it was very challenging to manipulate both the dorsal aorta and the cells of the SA lineage separately yet at the same time in a single embryo.

In 2012, a research team led by Yoshiko Takahashi at NAIST identified the dorsal aorta as a key relay point that coordinated the diversification process using a combination of mouse genetics and avian gene manipulation to show that the dorsal aorta acts as a critical developmental relay point, known as a morphogenetic center, for SA progenitor cell migration and the subsequent separation of Sg and Am cells. Bone morphogenetic proteins (BMPs), a class of signaling molecules, were also

shown to play a key role in this process.

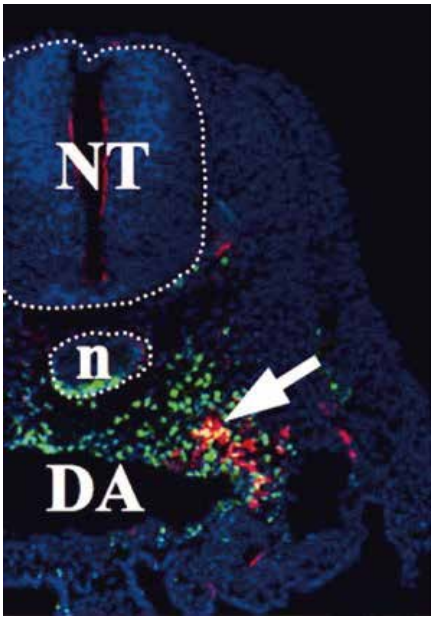
Using cell lines in which the expression of certain genes had been reduced or removed, alongside antibody staining to determine the precise locations of cells that were expressing key proteins, the team showed that BMPs from the aorta induced the expression of two other cytokines in the region around the aorta. These cytokines are known as SDF1 (stromal cell-derived factor 1) and NRG1 (neuregulin 1). The expression of SDF1 and NRG1 went on to directly regulate the differentiation of the SA progenitor cells.

In vivo tissue transplantation experiments and *in vitro* time-lapse imaging were then used to show that SDF1 and NRG1 were responsible for the migration of the SA progenitor cells towards the dorsal aorta by acting as chemical "attractants." They also showed that inhibition of the receptors of SDF1 and NRG1 interfered with the accumulation of SA progenitor cells around the dorsal aorta. Transplanting a piece of dorsal aorta or an aggregate of cells that were producing SDF1 and NRG1 elsewhere in the embryo caused the SA progenitor cells to migrate towards the implanted tissue and form an ectopic accumulation.

Upon further investigation into the segregation of the SA progenitor cells into the Sg and Am lineages, BMP signaling was found to be essential. The dorsal aorta is the most likely source of the BMP signaling molecules BMP4 and BMP7. These molecules caused segregation of the Am cells from the Sg cells, and also set up a localized pattern of the cytokine NRG1. NRG1 then attracted the Am cells, causing a physical displacement of the different cell types.

This study outlined the mechanisms, both molecular and cellular, that lead to the establishment of both the sympathetic nervous system and the adrenal medulla. The dorsal aorta is a critical control point for all three processes: the migration of the SA progenitor cells, the segregation of the Sg and Am cells, and the subsequent movement of the Am cells. This process involves multiple factors that are regulated not only temporally throughout the process but also spatially within the embryo. The cells alter how responsive they are to the various signaling factors that they encounter. This allows the various events to occur in the correct sequence to result in the different cell types being established in the appropriate regions of the embryo.

This work provided significant insight into a complex cascade of signaling which controls one of the earliest interactions between neurons and blood vessels guiding the development of embryos. The outcomes of this study support the identification of new therapeutic targets for the treatment of developmental disorders of the autonomic nervous system.



SDF1 and NRG1 act directly on migrating SA progenitors (NT, neural tube; n, notochord; DA, dorsal aorta) (Saito *et al.*, 2012, *Science*).

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Taku Demura

Adapt and thrive: How a group of proteins may have helped plants to colonize land

NAC transcription factors, which regulate the differentiation of water-conducting and support cells in vascular plants, may have helped them to adapt to living on land

The water-to-land transition was a key step in plant evolution, facilitated by the emergence of specialized cells that conduct water and provide mechanical support. Xylem vessel elements perform these functions in vascular plants, enabling them to colonize the terrestrial environment. In this plant group, NAC transcription factors are the proteins that control the differentiation of these cells, as well as regulating the development of fiber cells, such as those found in the supporting sclerenchyma tissue of stems and bark. This suggests that there is an evolutionary link between fiber cells and vessels, and that the system that regulates transcription has been evolutionarily conserved, providing potential clues to the evolutionary origins of how land plants developed the vascular systems that enabled them to invade land. However, exactly how plants with xylem vessel elements evolved from nonvascular plants was not previously well-understood.

To investigate the novel features needed by plants to adapt to land, a research team led by Taku Demura at NAIST studied genes that encode NAC proteins in the moss *Physcomitrella patens*. In *P. patens*, these genes regulate the development of stereids and hydroids, which are specialized water-conducting and structural support cells. Prior to this study, it was unclear whether there was an evolutionary relationship between hydroids and stereids and xylem vessels and fiber cells, respectively. There are eight loci in the *P. patens* genome similar to the VND/NST/SND transcription factors seen in vascular plants, and the team sequenced complementary DNA from these genes, naming the gene family *PpVNS* [*VND-*, *NST/SND-*, *SMB* (*SOMBRERO*)*-related protein*]. The team searched publicly available transcriptomic and genomic sequence data for phylogenetic analysis; the results suggested that VNS genes underwent an early expansion in prevascular plants.

The tissue-specific expression patterns of *PpVNS* in tissues from protonemata and gametophytes were then examined. In *P. patens*, after haploid spore germination in the gametophytic generation, filamentous cells called protonemata are formed, and leafy gametophores develop from these. The results suggested that *PpVNS* genes are involved in midrib development, as well as that of other tissues. Consequently, the researchers investigated three of the *PpVNS* genes that were prominently expressed in the midrib. Water transport in leaves from three loss-of-function mutant lines was examined and, compared with the wild type, was revealed to be deficient, which may have resulted from malformation of

hydroid cells. The mutants also exhibited abnormalities in their stereids and sporophyte cells, indicating that *PpVNS* genes play crucial roles in the development of these specialized cell types in *P. patens*.

The research group then conducted overexpression of the *PpVNS* genes in *P. patens* and *A. thaliana*, and the results suggested that the PpVNS family is involved in programmed cell death in *P. patens*. Further analysis indicated evolutionary conservation of VNS downstream genes. Building on existing research, the team concluded that the expression of genes that support the formation of water-conducting cells is promoted by the overexpression of *PpVNS* genes, and in *P. patens* and *A. thaliana*, those developmental mechanisms lead to ectopic thickening of cell walls and programmed cell death.

The results of this study demonstrated that hydroid cell differentiation is regulated by PpVNS proteins via induced cell death, and that these proteins regulate the differentiation of stereid cells by enabling the thickening of cell walls and the clearance of cellular contents. In mosses and vascular plants, the same gene families are regulated by PpVNS, indicating an evolutionary conservation of the genetic basis for the formation of both supporting and water-conducting tissues among these groups. In mosses, stereids and hydroids are developed during the gametophytic generation; in vascular plants, the vascular system is established in the sporophytic generation. Therefore, it appears that in the gametophyte and sporophyte generations, the VNS-based gene regulatory networks are homologous, and during vascular plant evolution were expanded in the sporophyte generation. Additionally, water is conducted much more readily via dead cells than by the living cells of the parenchyma. Therefore, water conduction may have been facilitated by the evolution of the cell death program. Similarly, the production of large, complex plant bodies requires supporting elements. During the transition of plants to land, the NAC transcription factors may have played a role in the evolution of both supporting and water-conducting cells. This research enhances our fundamental understanding of the genetic changes in the evolution of land plants and may contribute to future developments in agricultural biotechnology.

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Masaaki Umeda

Forming giant cells in response to DNA damage

In response to double-strand DNA breaks induced by radiation, plant cells replicate the nuclear genome to help survive the effects of genotoxic stress and avoid cell death

DNA can be damaged by many toxic stimuli, such as radiation and chemicals. If this damage goes undetected, it can lead to serious consequences, such as cancer. Thus, identifying and repairing areas of DNA damage is a crucial cellular process that is built into the cell cycle in the form of checkpoints, which give the cell a chance to detect damage and decided what to do with it before replicating and potentially passing on a damaged genome to its daughter cells. In metazoans, double-strand breaks halt cell cycle progression at the DNA damage checkpoint. This checkpoint is vital in ensuring genome integrity by giving the cell time to either repair the break or induce apoptosis. While there is some conservation of DNA damage checkpoint and double-strand break repair system components across species, plants seem to lack many of the factors known to participate in these processes in animals. Therefore, a research group led by Masaaki Umeda at NAIST sought to investigate the factors involved in the cellular response to double-strand breaks in *Arabidopsis*, a common plant model, to better understand how cells manage what could otherwise be a catastrophic cellular event.

To do this, the researchers treated *Arabidopsis* roots with zeocin, an agent that mimics radiation damage, and found that the plants responded by forming giant cells, indicating the occurrence of endoreduplication, which is replication of the entire genome without cell division. Gamma irradiation, another agent that causes double-strand breaks, had a similar effect on *Arabidopsis* cells, suggesting that this type of DNA damage specifically induces endoreduplication.

Further investigation of the signaling pathways involved in this process showed that ATM and ATR, which are orthologs of metazoan proteins that are known to play key roles in the DNA damage response, as well as SOG1, a plant-specific transcription factor, all helped mediate endoreduplication. In addition, cells that demonstrated endoreduplication after treatment with zeocin exhibited significant changes in the expression of cell cycle–related genes and induced degradation of CDKB2, an important cyclin-dependent kinase.

Taken together, the findings from this study demonstrated that double-strand DNA breaks affect the expression of cell cycle regulators to induce a programmed response of endoreduplication in *Arabidopsis*. This strategy represents a third option for responding to the damage induced by ionizing radiation, in addition to the classical options of delaying cell division or inducing cell death. Although SOG1 was involved in this signaling process, its specific role appeared to vary depending on whether it was active in the ATM- or ATR-dependent pathway, opening up new avenues for research into activity of this transcription factor in *Arabidopsis*. In addition to revealing an important aspect of double-strand DNA break repair in plants, the findings from this study provide crucial insight into a universal process that is key to maintaining genome integrity in all living organisms.

Reference

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Cell enlargement (right) in root tips (Adachi *et al.*, 2011, *PNAS*).

Naoyuki Inagaki

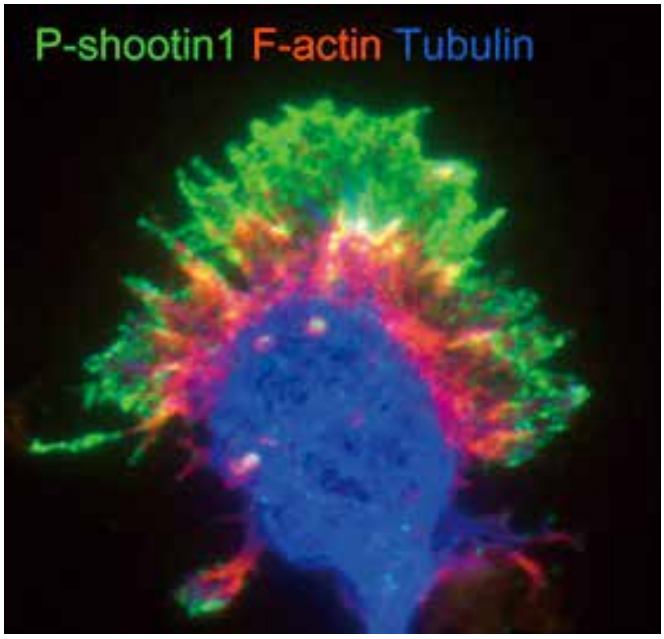
Shootin1 phosphorylation converts a chemical signal into mechanical force to promote axon growth

Phosphorylation of shootin1 by Pak1 enhances its interactions with actin filaments, enabling them to couple more strongly with extracellular substrates and create traction force to promote axon outgrowth

Cell movement and growth can be directed by soluble guidance cues that modulate adhesion and cytoskeletal dynamics. The combination of actin filament (F-actin) polymerization at the leading edge of motile cells and myosin II activity causes F-actins, which couple with extracellular substrates, to flow backwards within cells, resulting in movement or growth. One example of this phenomenon is the growth of the long neuronal cell extensions called axons, which occurs at the tip of axons from a region known as the growth cone. Retrograde flow of F-actins, coupled with a variety of extracellular molecules known collectively as substrates, is thought to generate traction force that enables outgrowth of the axon. However, it was unclear how neurons regulate this process, and specifically how cell signaling affected the efficiency of coupling between F-actins and these substrates to create traction and facilitate growth.

To address this, a research group led by Naoyuki Inagaki at NAIST tested the effects of netrin-1, which was known to promote axonal outgrowth, on axonal growth cones. They utilized traction force microscopy to detect filopodium extension and fluorescent speckle microscopy to visualize F-actin flow. They also investigated the expression and phosphorylation of downstream effector molecules using standard techniques, including *in vitro* kinase assays, immunoblotting, and Immunocytochemical analysis, to gain a more detailed understanding of the cellular signaling systems regulating these processes.

The results showed that netrin-1 promoted traction forces at growth cones. This process involved phosphorylation of shootin1, a linker molecule known to promote axon outgrowth by coupling F-actin flow with extracellular substrates, by Pak1, which had also been shown to play a role in axon outgrowth. Shootin1 was phosphorylated at two sites, Ser101 and Ser249, and this modification increased when Pak1 was expressed constitutively and when the cells were stimulated with netrin-1. Furthermore, phosphorylated shootin1 was expressed at high levels in areas of the axon growth cones where retrograde F-actin flow and traction forces were also observed, suggested an enhanced association between shootin1 and F-actin flow at growth cones. This resulted in greater coupling with substrates, thereby generating traction force that "pulled" the growing axon forward.



Fluorescence image of an axonal growth cone (Toriyama *et al.*, 2013, *Curr Biol*).

Taken together, these findings suggested that the dynamic interactions between shootin1 and F-actin at axon growth cones are regulated by Pak1-mediated phosphorylation of shootin1. Importantly, this discovery provides clear evidence for the conversion of a chemical signal into traction force, which in this case functions to promote axon outgrowth. This molecular level of insight into cells regulating the signaling process to control growth and movement could potentially have important clinical implications – for example, in the fields of regenerative medicine and neurology, where directing nerve regrowth after injury would have a crucial impact on recovery from catastrophic spinal damage.

Reference

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Hiroshi Takagi

Mpr1 is a yeast antioxidant with a unique chemical structure

The crystal structure of the antioxidant Mpr1 reveals key differences from other members of the same enzyme superfamily that account for its unique substrate specificity

Oxidative stress occurs when there is an imbalance in free radicals and antioxidants within an organism. Free radicals, also known as reactive oxygen species, can damage DNA, fats and proteins, and in humans this can contribute to the development of serious conditions such as diabetes, hypertension, and neurodegenerative disease. Antioxidants, whether generated by the cell or acquired from the environment, play an important role in neutralizing reactive oxygen species and thereby reducing the amount of damage they can cause.

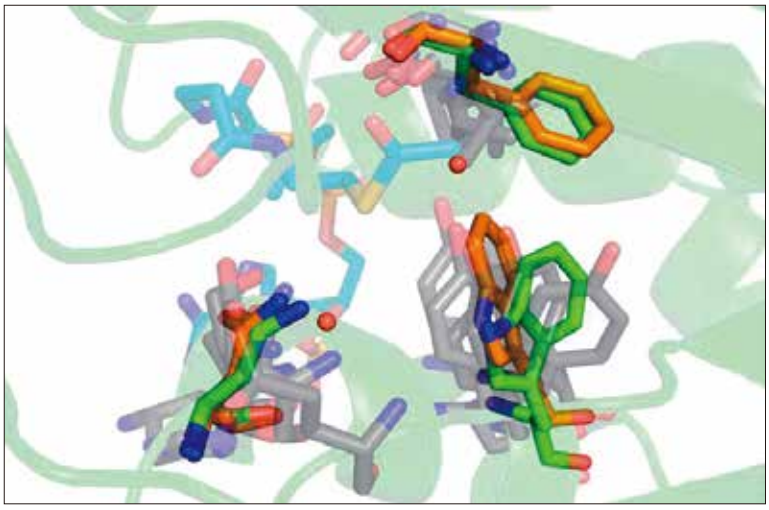
Mpr1 is a protein and antioxidant enzyme found in yeast that provides protection against oxidative stresses, such as those that occur under extreme temperature. In contrast to other members of the Gcn5-related N-acetyltransferase (GNAT) superfamily of enzymes, which act on primary amines, the two known substrates of Mpr1 are cyclic secondary amines. Furthermore, Mpr1 has little homology with other GNAT enzymes, suggesting that it may have a unique mechanism by which it recognizes substrates and catalyzes reactions. However, the structural features conferring these unique properties were previously poorly understood. A research group led by Hiroshi Takagi at NAIST showed the X-ray crystal structure of Mpr1, providing important insights into the structure-function properties of this specific enzyme and wider evolutional relationships among the biologically important *N*-acetyltransferases.

To determine the structure of Mpr1, the researchers engineered a stable form of the protein and subjected it to single-wavelength anomalous dispersion. They also analyzed the structure of Mpr1 bound to one of its known substrates to clarify the interactions within this complex. Analysis of X-ray diffraction data resulted in high-resolution three-dimensional structures of both unbound and bound Mpr1.

The results showed that, even though Mpr1 exhibits poor sequence homology with other GNAT enzymes, it has a similar structure, with eight-stranded mixed β-sheets and six α-helices. In addition, the substrate was bound at the expected site. Interestingly, however, Mpr1 lacks a β-bulge structure near the active site that is common to several other GNAT proteins.

Mutational analysis of Mpr1 based on the crystal structure showed that residues Asn135 and Asn178 are important for substrate recognition and catalysis, respectively. Furthermore, mutation of either of these two residues reduced the ability of Mpr1 in yeast to acetylate P5C, which normally inhibits mitochondrial respiration and leads to the generation of reactive oxygen species, thus demonstrating an important physiological role for Mpr1 in protecting yeast from oxidative damage.

Taken together, the findings from this study show that Mpr1 has a similar structure to other GNAT superfamily member proteins, but that key differences account for its distinctive substrate specificity, highlighting the diversity of this enzyme superfamily. Given that expression of Mpr1 increases the resistance of industrial yeast strains to oxidative stress such as ethanol and freezing, engineering a form of Mpr1 based on the structural findings from this study could have applications in improved industrial fermentation for the production of biofuel or as new chemical catalysts for the synthesis of natural products. Mpr1 is only found in yeasts and fungi; therefore, understanding the mechanism of Mpr1 may also have important clinical application regarding the development of antifungal drugs and combating antifungal drug resistance.



The structure of Mpr1, superimposed with those of other GNAT proteins shown in green, orange and gray (Nasuno *et al.*, 2013, *PNAS*).

Reference

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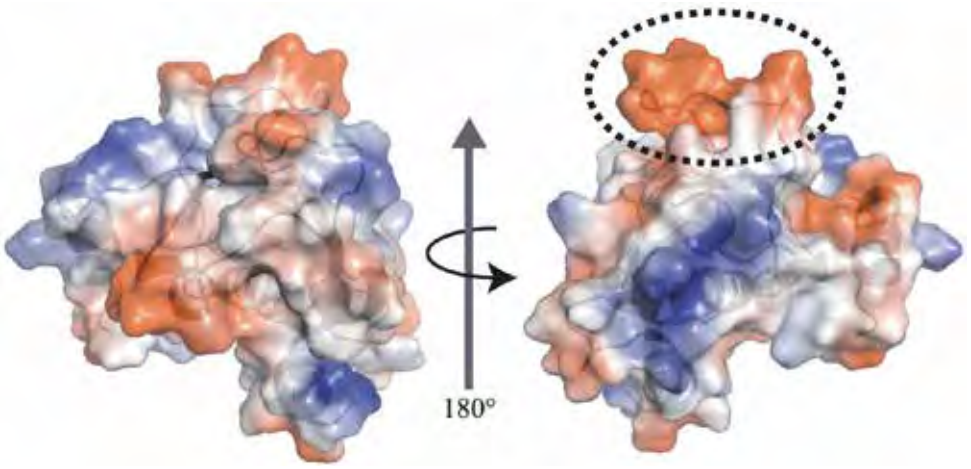
Kazuhiro Shiozaki

Original Sin1: Sin1 recruits substrates to TORC2 for phosphorylation

A central ubiquitin-fold domain in yeast Sin1 is required for recruitment of substrates to the TORC2 complex, where they are phosphorylated and activated



The molecular surface of SpSin1CRIM colored according to the electrostatic potential ranging from positive (blue) to negative (red) charge (Tatebe *et al.*, 2017, *eLife*).

Several key molecules and signaling pathways are implicated in more than one cancer, and as such make attractive targets for designing new treatments that could be effective against a variety of tumors. One of these central players in cancer development is mammalian target of rapamycin complex 2 (mTORC2), a multi-subunit complex that mediates phosphorylation of several important cellular targets, notably AKT. However, even though the function of mTORC2 has been relatively well characterized, how it recognizes and targets specific substrates for phosphorylation remained unknown.

To address this, a research team led by Kazuhiro Shiozaki used the yeast equivalent of mTORC2, known as TORC2, and a yeast target protein with a high degree of similarity to human AKT, known as Gad8, to determine the mechanism by which this complex targets specific proteins for modification. Because previous studies had shown that a subunit of the TORC2 complex called Sin1 plays a critical role in this process, the researchers chose to focus on exploring the function of Sin1 in this context.

The results showed that Sin1 is not required for assembly of the TORC2 complex, as the complex formed normally even when the gene encoding Sin1 was deleted. Because it was not important for TORC2 structure, the researchers next sought to determine whether it was required for TORC2 function by deleting domains that had been previously associated with Sin1 function. Unexpectedly, removal of both known functional domain in Sin1 had no effect on Gad8 phosphorylation. Instead, the conserved region in the middle (CRIM) was required for Sin1 function, although deletion of this domain had no effect on complex formation. Importantly, deletion of the CRIM in human Sin1 made it unable to phosphorylate its target proteins. The researchers then performed a structural analysis of the Sin1CRIM and found that it forms a ubiquitin fold, which is common to proteins that interact with a select group of target proteins. In addition, the Sin1CRIM exhibited a protruding loop structure that was essential for recruitment and phosphorylation of its target proteins. Finally, Gad8 that was artificially brought into proximity with the TORC2 complex lacking Sin1 was phosphorylated, indicating that the primary role of the Sin1CRIM is to recruit target proteins to the complex.

The findings from this study suggest that Sin1 is the substrate-recruiting element of the TORC2 complex, and that its CRIM domain, and specifically the protruding loop part of the ubiquitin fold, is responsible for this activity. Importantly, these findings in yeast appeared to hold true in humans as well, as phosphorylation of AKT by the human SIN1 protein also required this region.

Given that over-activation of the mTORC2 complex is a central event in the development of a variety of cancers, inhibition of this complex could be an effective means of controlling tumor growth. The functional and structural insights provided by this study could therefore be valuable in designing small-molecule inhibitors that disrupt the interaction between mTORC2 and its target proteins.

Reference

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Toshiro Ito

Fine-tuning flowers: Plant genes work in balance to get flower formation right

The plant gene CRABS CLAW regulates auxin homeostasis, which controls the transition from floral meristem termination to gynoecium development

In plants, all development after the embryonic stage is underpinned by stem cells. Because of this, the balance between the differentiation and proliferation of plant organs must be carefully established. For flowering plants to reproduce successfully, the control of floral stem cell termination must be coordinated with the start of female reproductive organ development, both in time and space. The source of these stem cells, floral meristem, is where flowers are formed. Floral meristem is of particular significance to humans, because this gives rise to flowers and fruits, from which most agricultural and horticultural products are derived. A detailed understanding of mechanisms behind developmental processes in flowers is beneficial, for example in applications for crop improvement.

In the model plant *Arabidopsis thaliana*, the transcription regulation pathway for floral meristem termination had been documented. However, prior to this study conducted by a research team led by Toshiro Ito at NAIST, the mechanisms underlying the coordination of these stages were unclear. In *Arabidopsis*, the gene *AGAMOUS* (*AG*) is pivotal to the process of floral meristem termination and establishment of the identity of reproductive organs. Floral meristem termination is dependent on activation of the genes *CRABS CLAW* (*CRC*) and *KNUCKLES* (*KNU*), which are direct targets of *AG*. Previous studies had revealed that auxin homeostasis plays a role in generating auxin maxima that are key to development. Although it was known that auxin plays a crucial role in gynoecium development, its potential function during floral meristem termination was unknown.

Previously, a *crc knu* double mutant had been demonstrated to exhibit strong indeterminacy in its floral meristem, and therefore, the *crc* mutant phenotype could be partly rescued with an auxin transport inhibitor. Because of this, the NAIST researchers used *A. thaliana* mutants to investigate the role of auxin at the termination of floral meristem. They found that *CRC* and *KNU* coordinate to regulate the repression of the *WUSCHEL* (*WUS*) gene, which is essential for meristem maintenance. Additionally, *CRC* was demonstrated to repress expression of the *TORNADO2* (*TRN2*) gene and control the determinacy of floral meristem. It was further shown that *TRN2* regulates auxin homeostasis, demonstrating a relationship between gynoecium development and floral meristem termination via the fine-tuning by *CRC* of auxin homeostasis.

The results of the study revealed the molecular framework for *AG*-mediated floral meristem termination via auxin, which is needed for the subsequent development of the gynoecium. The researchers found that in the developing gynoecium, *CRC* controls auxin homeostasis via negative regulation of *TRN2*, therefore avoiding floral meristem overgrowth when *KNU* is mutated. *CRC* and *KNU* work together to regulate the termination of floral meristem, and mutation of these genes, which act downstream of AG, disrupts determinacy of the floral meristem. The verification of the remarkable, synergistic mutant phenotype seen in *crc knu* highlights the key roles played by these genes.

The mechanisms revealed in this study provided an important addition to the knowledge of flowering plant reproduction. This improved understanding of floral development has implications for the study of plant biology overall, and especially to agriculture and horticulture.

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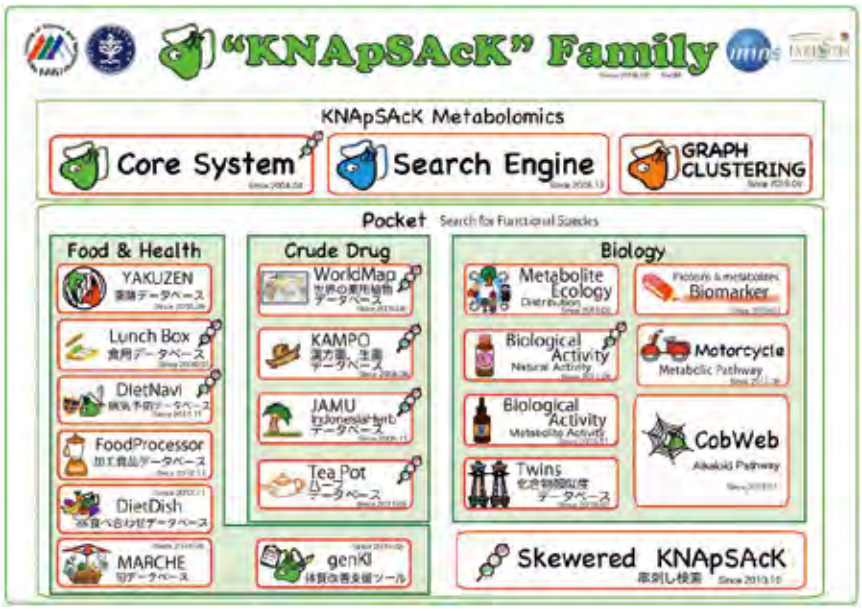
Arabidopsis thaliana

Shigehiko Kanaya



Main window of the KNApSAcK family.

Integrated species–metabolite databases for plants distributed around the world

A family of databases to facilitate research on plant metabolites, with a focus on medicinal plants

Plants produce a broad variety of metabolites, which include an array of medicinal compounds. Studies on the effects of these plant metabolites in humans provide invaluable insights, particularly in the field of healthcare and medicinal plants. Owing to their great importance, many scientists have investigated and characterized plant metabolites. Such studies have generally employed mass spectrometry, which can accurately measure the masses of thousands of compounds within a single sample. From various studies, metabolite data have been collected for numerous organisms. However, systematic evaluations of the relationships between metabolites and their originating plants have been hindered by the vast number of plant metabolites in existence. In a major step towards connecting widely dispersed data and plant use knowledge, a research team led by Shigehiko Kanaya at NAIST developed an integrated family of species–metabolite databases for plants, called the KNApSAcK Family Databases.

By collecting information available in the scientific literature, the research team developed a family of plant metabolite databases that can facilitate systematic research on metabolites. Several databases were developed as part of this initiative. As a primary database within this family, the KNApSAcK Core Database contains information for more than 100,000 species–metabolite relationships based on over 20,000 species and over 50,000 metabolites. For this database, the investigators developed a search engine, enabling researchers to search for metabolites based on various characteristics, such as metabolite mass, name, spectra, or molecular formula.

Other databases include a world map function that provides information regarding medicinal and edible plants for different geographic zones. A biological activity database provides information on the biological activity of medicinal and edible plants. Two additional databases provide the formulas of medicinal plants based on Kampo (Japanese medicine) and Jamu (Indonesian medicine). These databases are highly extensive: the 15,240 medicinal plants in the database correspond to 72% of all medicinal plants listed by the World Health Organization.

By statistically analyzing the metabolites in the database, the research team predicted that approximately one million plant metabolites exist on Earth. This large number highlights the need for computational tools, such as the KNApSAcK Database, to allow for timely and comprehensive studies of plant metabolomics.

The researchers have made these databases, along with an instruction manual, freely available online. By providing free access to this resource, scientists worldwide are able to systematically study plant metabolites, particularly metabolites that may be of medicinal value. In addition to accelerating discoveries in the use of medicinal plants, this family of databases will contribute to our holistic understanding of plant function and healthcare, bolstering efforts to create sustainable societies.

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Keiichi Yasumoto

Gamification of crowdsourced monitoring tasks

Adding game-like elements encourages participation in a mobile phone app based on feelings of accomplishment and increased social status

Online monitoring services, such as those that engage in the real-time recording of store crowd sizes, sometimes enlist people in the area to detect and relay accurate information to a central server via an app. Participation in this "crowdsourcing" of sensing tasks can be substantially enhanced if the monitoring service offers incentives, financial or otherwise, to users. To minimize the cost of paying these benefits, status-based rewards, such as badges or ranking lists, can also be included. While many companies now implement non-financial inducements to encourage increased user engagement, systematic studies of apps that can promote real-world sensing tasks have been lacking.

To help address this need, a research team led by Keiichi Yasumoto at NAIST has proposed an algorithm that combines both a financial incentive, such as airline miles, with status rewards. This idea was based on the psychological concept of "gamification," in which users are much more likely to complete difficult or onerous tasks when presented in the form of a game with points to be scored. The hypothesis is that the satisfaction users receive from the sense of accomplishment will compensate for reduced financial incentives.

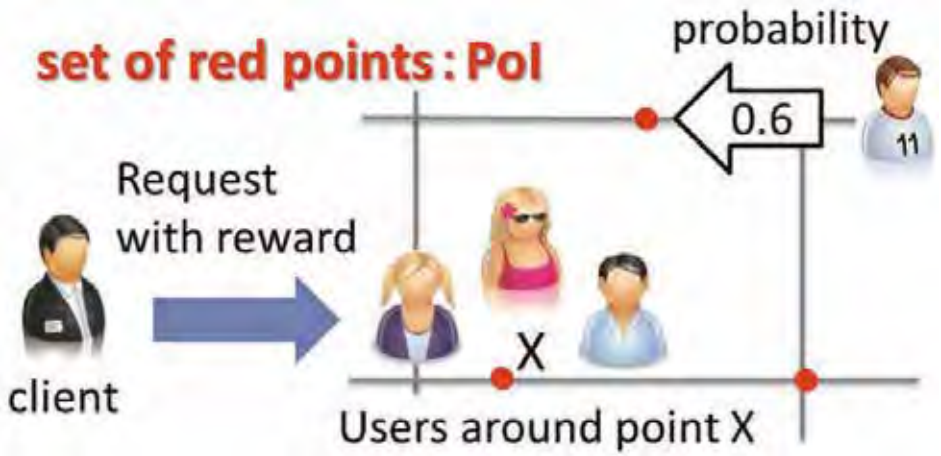
A heuristic algorithm was created to determine which set of users should receive each request, and the appropriate number of reward points to offer as an incentive. In addition, a status scheme was added that classifies users into ranks based on points accrued. Users with higher status were offered increased reward points for additional sensing tasks. Rankings were made public to all users with leader boards. Finally, digital badges could be earned, along with bonus points, for accomplishing conditions set by the system.

The application was then built on the *Foursquare* platform, which is a popular smartphone app in which users "check in" at various locations. During the experiment, the new algorithm decided to whom requests should be sent by sorting users based on their expected performance per cost, which was defined as the ratio of probability of participation divided by the reward points offered for the task. These requests came in the form of an email that contains the content of the sensing task related to the location where the user checked in, the reward points, and the time limit. For this test, the tasks all involved uploading a photograph within 15 minutes. For example, the user might be asked to submit an image of a restaurant's menu or cars in a parking garage to gauge usage rate. To find the impact of gamification, users were randomly offered either a monetary incentive only, or a monetary incentive coupled with a gamification-based reward.

When they trialed this approach with 18 users over 30 days, the researchers found that their gamification algorithm increased participation probability to 73%, up from 53% without gamification. Possible applications of this research are very broad, including the real-time monitoring of weather condition or highway congestion at various locations. Optimizing the gamification aspects of crowdsourcing apps could greatly increase participation rates while not incurring excessive payment costs.

Reference

Ueyama, Y., Tamai, M., Arakawa, Y., Yasumoto, K. 2014. Gamification-based incentive mechanism for participatory sensing. *Pervasive Computing and Communications Workshops*, 98–103.

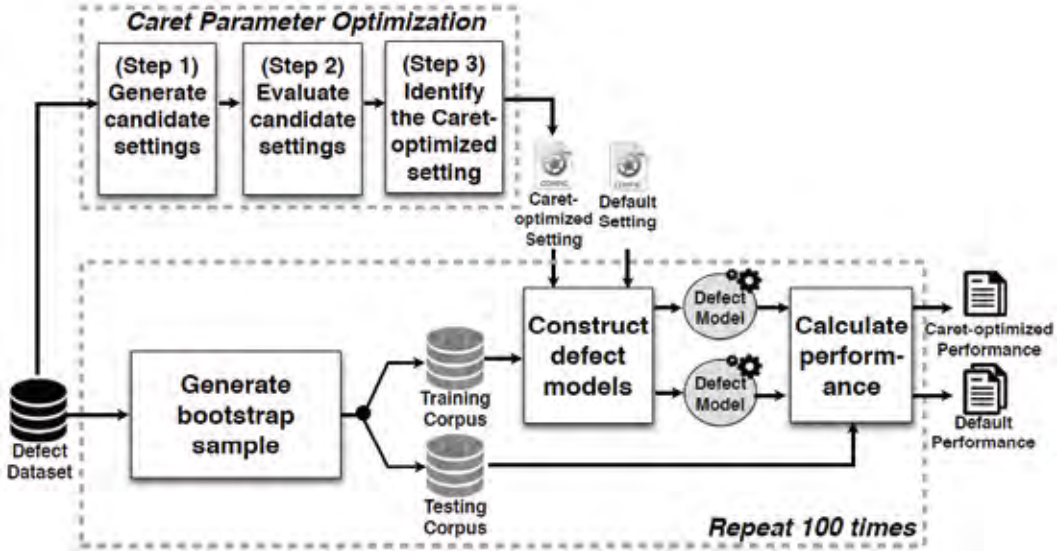


The overview of the participatory sensing system (Ueyama *et al.*, 2014, *PERCOM*).

Kenichi Matsumoto

Improving software defect detection with parameter optimization

Optimizing the model parameters of machine-learning algorithms used for identifying software bugs may lead to more accurate artificial intelligence



An overview of the approach employed (Tantithamthavorn *et al.*, 2016, *ICSE*).

The complexity of modern software has rapidly outstripped the comprehension of any single coder. The length of the source code that lies behind a major computer program or website can easily run to tens of millions of lines. It has become standard practice for organizations to test each module for bugs that could potentially lead to failure or suboptimal performance under certain future conditions. As part of this effort, the software quality assurance process usually employs specialized automated machine-learning prediction models trained to identify software that may be vulnerable to defects. These tools are based on statistical or machine-learning classification approaches that use predefined search parameters, for example, the number of decision trees to use in a random forest classifier. However, the optimal settings for these parameters are not known ahead of time, and there is reason to believe that defect prediction models may suffer from reduced accuracy if they are trained under suboptimal parameter settings. Because the number of possible parameter permutations is far too large to try exhaustively, a more systematic approach is needed.

To study this situation, a research team led by Kenichi Matsumoto at NAIST used the automated parameter optimization tool called Caret using examples from 18 datasets, including both proprietary and open-source software. They found that changing parameter settings for the defect-detection models can lead to significant improvement in performance. Of the 26 machine-learning methods considered, Caret could be used for 16 of them, including commonly used approaches like random Forest, support vector machines, and artificial neural networks.

Caret works by evaluating potential combinations of the candidate parameter settings, up to a preset limit. The researchers used 100 repetitions to estimate the performance of classifiers that are trained using each of the candidate parameter settings. They found that using Caret improved the area under the curve (the primary measure of model accuracy) of defect prediction models by as much as 40 percentage points. This approach also increased the chance that a top-performing classifier would be identified by up to 83%. To ensure that the conclusions regarding the models were robust, out-of-sample bootstrap validation was applied.

The results of this work can be applied throughout the field of machine learning. In particular, the accuracy gains due to parameter optimization can be beneficial for almost any kind of classification task. This project can help guide developers determine how many resources to invest in this process based on the prospective increase in performance.

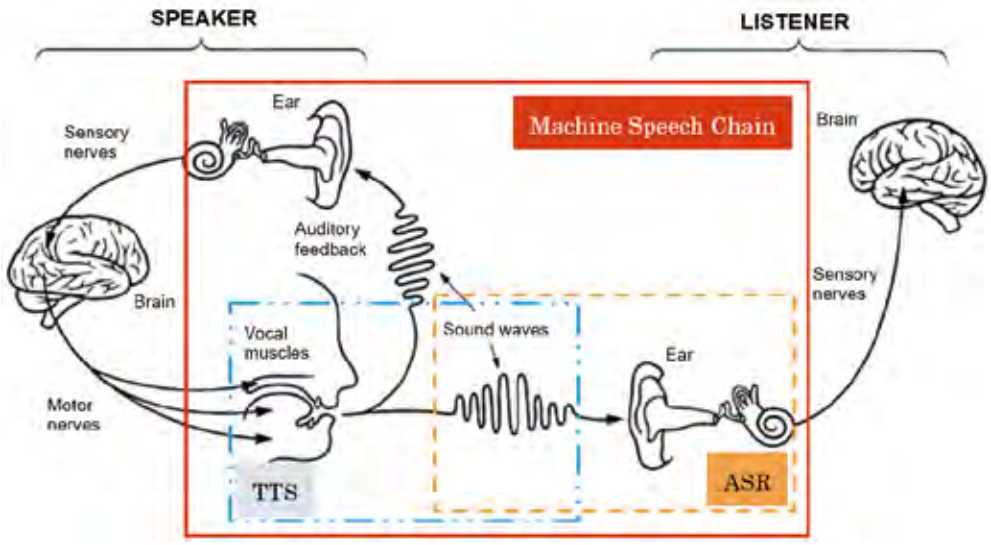
Reference

Tantithamthavorn, C., McIntosh, S., Hassan, A.E., Matsumoto, K. 2016. Automated parameter optimization of classification techniques for defect prediction models. *International Conference on Software Engineering*, 321–332.

Satoshi Nakamura

Text and speech waveform closed-loop deep learning

A machine learning system for speech that combines waveform-to-text and text-to-waveform algorithms in a closed-loop architecture, may assist in future human/computer interfaces



Speech chain and related spoken language technologies (Tjandra *et al.*, 2017, *ASRU*).

The neural processes used by the human brain to decode incoming audible language signals and those that encode outgoing speech are closely related. For example, it has been observed that children with hearing difficulties often have trouble speaking as well, due to, at least in part, the fact that they do not receive the proper audible feedback to modulate their own speech. However, the current machine learning algorithms for converting a sound waveform into text (automatic speech recognition) have developed almost entirely independently from those that do the reverse and produce a waveform from text (text-to-speech synthesis). The ability to combine these two tasks into a single workflow is desired for more sophisticated and seamless computer interfaces, as with digital assistants like Siri or Alexa.

To address this need, a research team led by Satoshi Nakamura at NAIST created a "closed loop" artificial intelligence system based on a sequence-to-sequence model. This allowed the computer to operate using a combination of both labeled and unlabeled data. By using this deep neural network (DNN) approach, the computer was able to organically learn the relationships between speech waveforms and snippets of text, instead of relying on a complex model created by hand. Based on this increased flexibility, the algorithm created more robust and accurate models.

The researchers found that the key insight was the ability to jointly train the sequence-to-sequence automatic speech recognition model with the sequence-to-sequence text-to-speech synthesis model. Although the models can be trained separately if the data have labels (supervised learning), they must be trained together if the data are unlabeled (unsupervised learning). Although many labeled training sets are available, the vast majority of written and spoken language is not prepared in this way. Thus, the option to operate on both kinds of inputs is a distinct advantage.

When implementing this combined approach, the team used a large corpus of text read by a single human speaker. The speech waveforms were converted into spectrograms with a 2048-point fast Fourier Transform into 50-ms frame lengths. This permitted the machine learning features, which could be correlated by the algorithm with the corresponding text, to be efficiently extracted from the raw voice data.

The research in this study could enable the development of an artificial intelligence program that more closely mimics the way the brain creates and processes sound. This is the first deep learning model that attempts to integrate human speech production with speech perception, and so paves the way for revolutionary improvements in the ability of humans and computers to interface in a more natural fashion.

Reference

Tjandra, A., Sakti, S., Nakamura, S. 2017. Listening while speaking: Speech chain by deep learning. *IEEE Automatic Speech Recognition and Understanding Workshop*, 301–308.

Tsuyoshi Kawai

Shining a light on the structural basis for highly sensitive photo-induced cyclization in organic compounds

A molecular design strategy highlights the intramolecular interactions that govern photo-induced reactivity in a synthetic organic compound with higher light sensitivity than the biological system responsible for night-vision

Light-induced molecular isomerization (photochromism) in nature contributes to animals' night-vision capabilities. Being able to control this photochromic property in synthetic molecules, inspired by natural systems, could broaden the scope of their potential applications in photoswitches, eye-protection materials and other optical devices.

To optimize the quantum yield and sensitivity of the photochromic reaction, it is crucial to understand the molecular structural features that support the desired photoreactivity. In 2011, a research group led by Tsuyoshi Kawai at NAIST published a study detailing the synthesis, characterization and photocyclization behavior of terarylene-based compounds. They revealed that a C_2 ground-state symmetry with rationally designed intramolecular H-bonding and steric interactions could promote the highly efficient photo-induced ring-closing reaction.

Considering the weak, water-repellent, noncovalent interactions in the rhodopsin binding pocket, which hosts the photo-active molecule in the natural system, it was reasonable to conclude that such an environment would be conducive to the photoisomerization process of a synthetic molecule under mild conditions. Photochromic terarylene molecules contain three ring moieties that can undergo reversible ring-closing/opening reactions upon UV/vis irradiation, respectively, and their reported photocyclization quantum yields (>60%) are comparable to the natural system's photoisomerization quantum yield (65%).

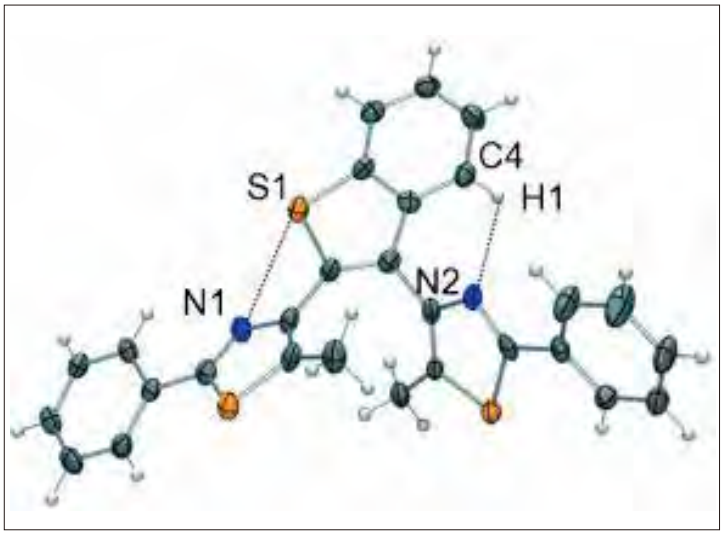
Thus, a dithiazolylarylene molecule was synthesized and characterized in the solid state (by single-crystal X-ray diffraction) and in solution. The designed molecule adopted the desired geometrical conformation and exhibited weak intramolecular CH–N hydrogen bonding, as well as S–N and CH_3–π interactions. These results were confirmed by temperature-dependent NMR studies and density functional theory calculations, which also supported the compound's potential photoreactivity.

The solid crystal dithiazolylarylene turned blue under UV light, so its photochromic performance under 313-nm irradiation was also evaluated in aprotic (hexane) and protic (methanol) solvents using UV-vis spectroscopy. Although the quantum yield for the ring-open to ring-closed photoreaction was only 54% in methanol, it reached 98% in hexane. This was one of the largest photocyclization quantum yields reported at the time and confirmed that photon absorption quantitatively triggered photoisomerization in this system. Meanwhile, the observed solvent dependence supported the proposed mechanism of stabilization via noncovalent intramolecular interactions, such as H-bonding.

The designed photochromic molecule exhibits significantly greater sensitivity than the natural biological opsin-retinal complex in animal night-vision systems because the researchers were able to precisely control the ground-state conformation to optimize its functional properties. The structure-function insights described in this study will undoubtedly continue to inspire the design of synthetic systems that can be implemented in next-generation optical devices.

Reference

Fukumoto, S., Nakashima, T., Kawai, T. 2011. Photon-quantitative reaction of a dithiazolylarylene in solution. *Angewandte Chemie International Edition*, 50, 1565–1568.



ORTEP drawing of the open-ring isomer (Fukumoto *et al.*, 2011, *Angew Chem Int Ed*).

Yoichiroh Hosokawa

Femtosecond lasers: A tool for understanding cell–cell adhesion pathologies

Intercellular adhesion has been quantified in a nondisruptive manner, yielding accurate estimates of physiological and pathological adhesion strengths

Cell–cell adhesion is foundational to neuroimmunity, inflammatory diseases, and many other life processes. However, some measurement methods for estimating the strength of this adhesion disrupt native physiology; thus, it can be difficult to apply corresponding measurements made in the lab to general medical practice. A research team led by Yoichiroh Hosokawa at NAIST used transient femtosecond laser pulses to quantitate intercellular breaking forces in a noncontact manner, providing insight into otherwise intractable biological signal transduction pathways.

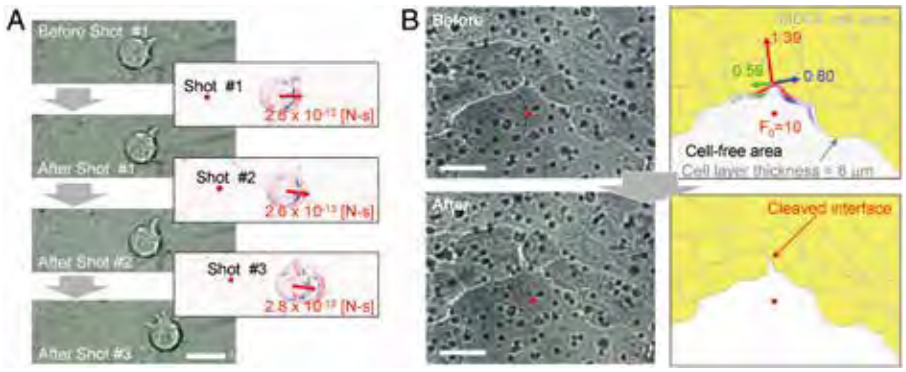
Multicellular organisms routinely modulate cell–cell adhesion as a means to regulate physiological and pathological tissue functions. Quantitative estimates of adhesion strengths are available from *in vitro* aggregation or pulling-force studies. However, the *in vivo* applicability of such studies is limited because they disrupt concurrent physiology. Hosokawa and his team aimed to address the uncertainty in quantitative estimates of *in vivo* cell–cell adhesion strengths through their research using a combination of atomic force microscopy and femtosecond laser pulses.

To solve this problem, the researchers first used an 800-nm, 120-femtosecond laser to apply a transient impulsive force onto the water near the top of an atomic force microscopy cantilever. By measuring the nanometer-scale oscillations of the cantilever, they were able to quantitate the corresponding impulsive force. By using these data in experiments that measured the minimum impulsive force required to dissociate cell–cell adhesion, the researchers were able to quantitate this adhesion in an otherwise nondisruptive manner.

The researchers first checked their experimental approach against a conventional cell aggregation assay and a microsphere model system. The cell aggregation assay was based on a typical connective tissue cell line NIH3T3 that expressed the adhesion molecule CADM1. The laser didn't appear to damage the cells, but did dissociate cell–cell interactions in a predictable manner. The microsphere model system provided a useful measure of the uncertainty of the impulsive force estimates (and thus the calculated adhesion strengths). On the basis of several hundred measurements of streptavidin-coated microspheres on a biotin-coated substrate, the measurement accuracy was ~7%.

To demonstrate clear applicability to biologically pertinent cell cultures, the researchers next quantitated the adhesion in two scenarios: either between leukocytes and endothelial cell monolayers, or between kidney cell monolayers. In the former, the impulse required to induce slipping along the monolayer was on the order of 10^{-13} N·s. In the latter, the impulse required to disrupt adhesion was on the order of 10^{-12} N·s. These measurements are comparable to the best estimates derived from *in vitro* work that nevertheless requires disruptive mechanical contact.

These results provide a means of reliably measuring cell–cell adhesion strengths without otherwise disrupting concurrent physiology. Ongoing questions of how cell–cell adhesion modulates the complex crosstalk that characterizes neuro-immune signaling can be addressed quantitatively through this research. Such knowledge could assist medical practitioners to treat serious immune disorders and characterize other cell-adhesion pathologies, such as those which cause cancer.



Femtosecond laser-induced intercellular dissociation of HL-60 leukocytes attached to HUVECs (A) and the MDCK cell–cell interface (B) (Hosokawa *et al.*, 2011, *PNAS*).

Reference

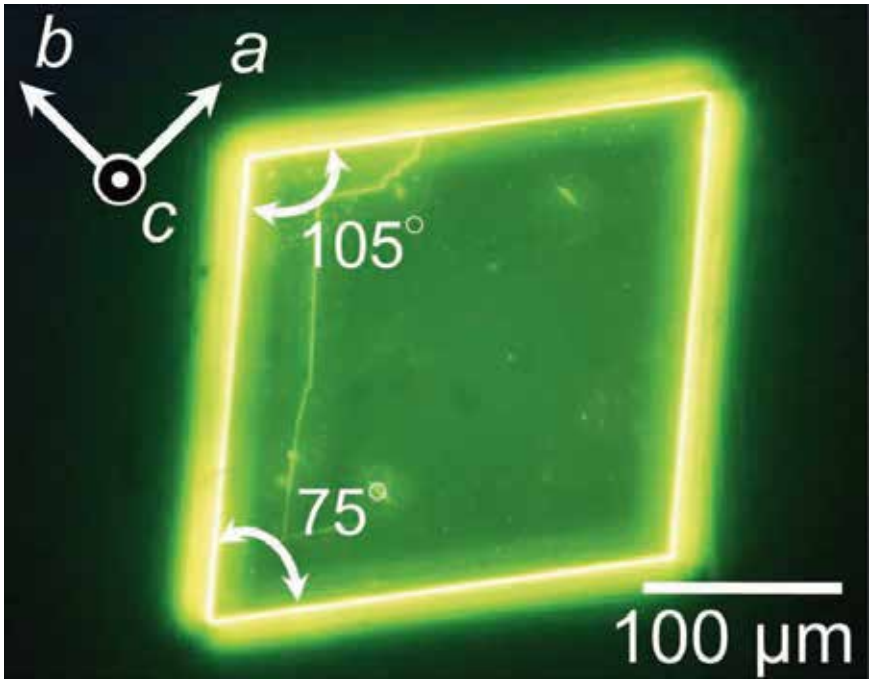
Hosokawa, Y., Hagiyama, M., Iino, T., Murakami, Y., Ito, A. 2011. Noncontact estimation of intercellular breaking force using a femtosecond laser impulse quantified by atomic force microscopy. *Proceedings of the National Academy of Sciences of the United States of America*, 108, 1777–1782.

Hisao Yanagi

Chemically modified gain medium developed for organic lasers

X-Ray crystallography and photoluminescence spectroscopy are used to study chemically modified thiophene/phenylene co-oligomer crystals, which are found to be suitable as the gain material for organic lasers

Lasers based on organic molecules have many advantages over conventional devices. For example, the intrinsic properties, including emission wavelength and gain, are much easier to tune to desired values using chemical modification. In addition, organic-based lasers can be fabricated directly on flexible substrates without the need for extreme conditions, such as high temperatures or toxic reagents. However, the integration of organic lasers into photonic devices, including those used in telecommunication fiber optics that support broadband internet service, has been slow. New, optimized molecules are needed before organic lasers are able to be widely adopted.



Fluorescence micrograph of single-crystal BP2T-OMe excited at 365 nm (Mizuno *et al.*, 2012, *Adv Mater*).

One solution to this problem was investigated by a research team led by Hisao Yanagi at NAIST using thiophene/phenylene co-oligomer (TPCO) crystals. This class of polymers had already been recognized for their ability to act as an efficient laser gain medium. The gain medium is the most crucial part of a laser because it is the location where energy is added so light amplification can occur. For best performance, its light emission wavelength bands should be narrow, and the energy losses should be low. It was theorized that small modifications to the chemical formula could change the resulting crystal structure, which in turn would impact its emission and absorption properties. Single crystals of 5,5'-bis(4'-methoxybiphenyl-4-yl)-2,2'-bithiophene were created, also called BP2T-OMe, and its structure analyzed using X-ray diffraction. They found that BP2T-OMe molecules crystallized into an orthorhombic form, as compared with the zigzag monoclinic structure of unsubstituted TPCO.

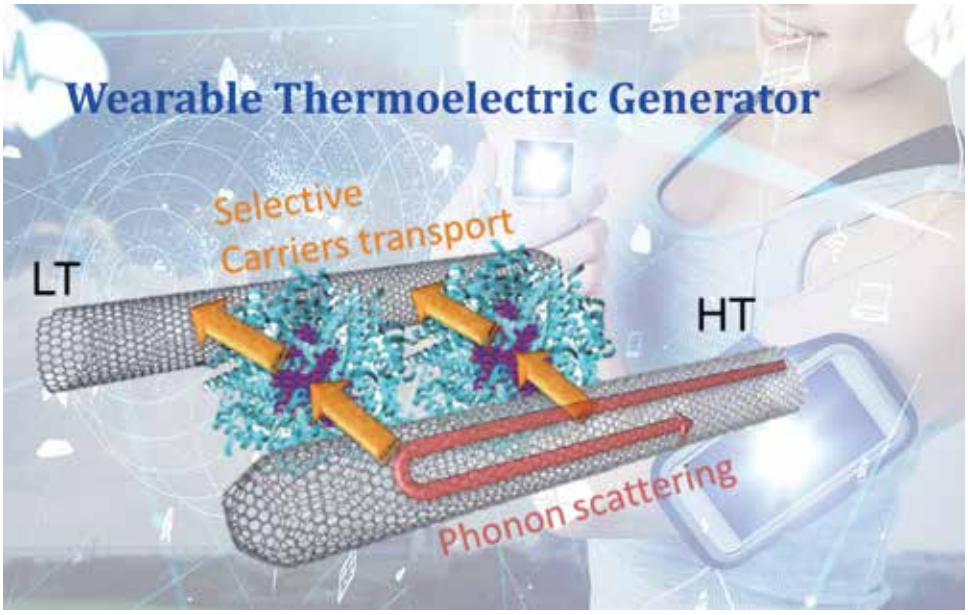
The optical absorption and photoluminescence spectra were then measured at room temperature. The bright green fluorescent light emitted from the edges of the crystal indicated that the photonic energy remained confined inside the crystal, as seen previously for other TPCO crystals. Absorption bands occurred at 455 and 415 nm, while photoluminescent emission bands were observed at 525, 565 and 605 nm. These results suggested that the unusual luminescence bands were the result of the altered molecular orientation and crystal structure caused by the chemical substitutions.

It was also found that the regular orientation of the molecules in these organic crystals leads to significant anisotropy – meaning the luminescence bands differed in strength depending on the direction of measurement. This was reflected in the optical properties in the form of polarized light emission. On the basis of this approach, crystals like BP2T-OMe seem closer to being incorporated as the gain medium of next-generation organic lasers. This could lead to substantial reductions in cost for internet infrastructure, which could help close the "digital divide" by bringing high-speed telecommunication technology to remote areas.

Reference

Mizuno, H., Haku, U., Marutani, Y., Ishizumi, A., Yanagi, H., Sasaki, F., Hotta, S. 2012. Single crystals of 5,5'-bis(4'-methoxybiphenyl-4-yl)-2,2'-bithiophene for organic laser media. *Advanced Materials*, 24, 5744–5749.

Masakazu Nakamura



Control of charge and heat transports by molecular junctions for wearable thermoelectric generators.

Weaving carbon nanotubes into fabric for harvesting human body heat

A fabrication process for carbon-nanotube composite threads that can form flexible thermoelectric generator textiles is developed to recover waste body heat

The process of recovering electricity from waste heat, such as that produced based on the temperature difference between a person's body and the ambient environment, is a promising avenue for energy harvesting. Thermoelectric generators (TEGs) produce electricity directly from waste heat and offer the best approach for harnessing this process, and it is hoped that they can be applied for use in self-powered, durable and low-cost wearable technologies.

However, development of TEGs for energy harvesting is burdened by several technical challenges. For instance, one promising approach relies on solid-state TEG materials, which convert heat directly into voltage. Unfortunately, these materials require a rare combination of properties. In particular, a TEG needs to have high electrical conductivity while simultaneously possessing low thermal conductivity. Moreover, the thickness required for optimal efficiency often prevents the resulting devices from being flexible enough to be woven into clothing. Overcoming these limitations would allow small wearable electronics to be powered by the user's own body heat alone, which would reduce the need for batteries and stops for recharging.

To address these limitations, a research team led by Masakazu Nakamura at NAIST used carbon-nanotube threads to produce a flexible "thermoelectric fabric". Using carbon nanotube composite threads allowed the team to control the thickness of the final material. The resulting thermal conductivity could be made to be less than 0.1 W/K·m, while the thickness was large enough to produce a sufficiently large temperature difference between the inner and outer surfaces. The researchers found that this permitted the system to operate even with the relatively small temperature difference between the human body (about 37°C) and the surrounding air (usually around 22°C).

To produce the fabric, carbon nanotubes were spun into a thread along with binding polymers. The composite thread was then sewn into a felt fabric for structural stability. To create an electrical device, alternating "stripes" of p-type and n-type doped regions were made by exposing the fabric to chemicals that removed or added electrons, respectively. The sample remained intact even after 160 test cycles of bending and stretching. The touch of a finger was enough to generate a voltage in a prototype thermoelectric fabric. Within 4 seconds, an electrical potential of 2.3 millivolts registered.

The proof of concept demonstrated by this research could be extended to other flexible thermoelectric materials besides carbon nanotubes, including conducting polymers, or organic/metal composites. This has great potential for a wide variety of products – from biomedical devices to countertops – that would benefit from its own source of power.

Reference

Ito, M., Koizumi, T., Kojima, H., Sato, T., Nakamura, M. 2017. From materials to device design of a thermoelectric fabric for wearable energy harvesters. *Journal of Materials Chemistry A*, 5, 12068.

Jun Ohta

World's smallest IR wireless energy receiver developed for implantable biodevices and smart sensors

A semiconductor photovoltaic platform that can absorb infrared light energy is developed, which may help power implanted medical devices and smart objects that will contribute to the "Internet of Things"

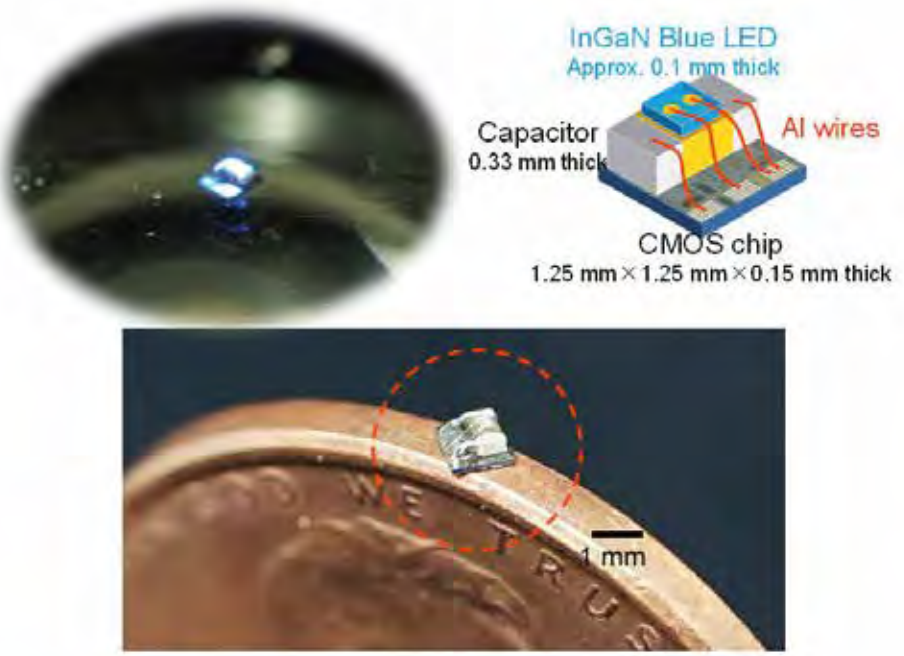
With the advent of small medical implants, there is an increasing need for wireless power systems that can allow these devices to function without the need for batteries or wires that cross the skin. Current solutions often rely on electromagnetic induction with radio-frequency waves to transmit energy from a source outside the body to the device. However, these systems require large induction coils – often bigger than the rest of the device – and suffer from low efficiency. While this is sufficient for certain applications, such as wireless charging of smartphones or powering RFID tags, alternatives to electromagnetic induction for contactless energy transmission are needed. This is particularly true as we enter an era of the "Internet of Things (IoT)" in which many more connected devices will need to be powered remotely.

To address this need, a research team led by Jun Ohta at NAIST has created a complementary metal-oxide semiconductor (CMOS) photovoltaic receiver chip that works by receiving infrared light. Like any other solar panel, the light energy can be converted directly into an electrical voltage. For biomedical applications, the wavelength of light must be carefully chosen based on efficiency and safety considerations. Red or infrared light sources placed on the body are preferred, because they can pass more easily through tissue while also not causing cell damage. For IoT devices, smart connected nodes could be configured to run on indoor ambient light.

The researchers built a 1.25-mm × 1.25-mm prototype and demonstrated the proof-of-concept by powering a blue LED light. The integrated circuit eliminated the need for a voltage booster by monitoring the voltage of the internal capacitor and switching between the power-receiving photovoltaics and the load circuit as appropriate. An optogenetic neural stimulator that had a volume of just one cubic millimeter was then fabricated. This type of device is used in certain animal experiments, in which gene expression is turned on or off inside the body by activating light-sensitive proteins. The team found that the stimulator could be powered solely by a commercially available IR flashlight.

This approach to making implantable medical devices and IoT nodes more cost efficient and reliable holds great potential for widespread adoption. For example, a temperature sensor placed on the exterior of food packaging to ensure freshness could operate without batteries based on the indoor lighting. More sophisticated medical devices for internal use, like a pacemaker, could be continually charged by an IR LED the patient wears over their heart.

By eliminating the need for on-device energy storage, nodes may be substantially streamlined, and used in many more applications than they are today.



Size and design of the 1 mm³ optogenetic stimulator.

Reference

Tokuda, T., Ishizu, T., Nattakarn, W., Haruta, M., Noda, T., Sasagawa, K., Sawan, M., Ohta, J. 2018. 1 mm³-sized optical neural stimulator based on CMOS integrated photovoltaic power receiver. *AIP Advances*, 108, 045018.

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