Full Research in Society, for Society



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#### MESSAGE

## President's Message Thoughts for the New Year 2013

## FEATURE

## **Stem Cell Engineering Pioneering** the Future

**From Science to Industry** 

## **Research Hotline**

UPDATE FROM THE CUTTING EDGE (October-December 2012)

## **In Brief**



## President's Message Thoughts for the New Year 2013



#### Introduction

I welcomed the arrival of the New Year with a further strengthened determination to promote open innovation befitting Japan through cooperation with corporations, universities, national agencies, and local governments, so as to revive the once thriving society and economy of Japan.

Over the past several years, the voices of people in various fields have frequently been heard pointing out the decline in the competitiveness of Japanese companies, especially those in the manufacturing industry that have been the backbone of Japan's development, and recently these voices seem to have grown much louder in light of the performance of electronics companies last fiscal year and their forecasts for this fiscal year. Many have concluded that the overall problem lies in the companies' business strategies. However, it should be seen more as a result of the failure of the business model emphasizing mass production of individual single products that has been pursued by Japanese corporations faced with the very severe business environment of a strong yen, high corporate taxes, and the delayed signing of economic partnership agreements (EPAs) in this digital age. A new competitive model must be developed. Moreover, the open innovation efforts at AIST must also be promoted based on these notions.

#### Prescription for revitalizing Japan

I personally cannot readily agree with the view that says the competitiveness of Japanese industry as a whole has declined. When examined in a clear light, the automobile industry has overcome the damage caused by the earthquake as well as the severe flooding in Thailand and is beginning to move forward on the path to recovery. Other Japanese industries including chemicals, steel, machine tools, and heavy electrical machinery can also be said to be performing comparatively better than the rest of the world despite the impact of the global economic slowdown arising from the financial instability in Europe. I believe that the fundamental strength of Japanese industry is still alive and well. The capacity of corporations to cope with the strong yen has incomparably improved compared with nearly a decade ago when I was the CEO of a corporation. Hence, if, for example, the strong yen were to weaken to the level at the time of 100 yen to the U.S. dollar, corporate profits including those of the suffering electronics companies would be greatly improved. Nevertheless, I have to admit that this is merely a dreamlike hypothesis when the very difficult financial situations of some EU countries are taken into consideration.

Setting this aside, a major correction of the strong yen does not, in any event, seem likely in the near future. We must build a strategy for preserving the fundamental strength of Japanese industry assuming that the current difficult business environment will continue for the time being. I would like to express several opinions here on what I believe to be highly important points regarding this situation, including some that I have already repeatedly expressed on previous occasions.

First, we should not forget the importance of strengthening parent laboratories and parent factories in Japan. According to recent reports in the newspapers and other media, an increasing number of companies in various industrial fields are establishing production centers overseas and in more than a few cases, the total overseas production volume is exceeding the volume of production in Japan. This trend is expected to continue into the future and the establishment of overseas R&D centers will also become more common among large corporations. However, the current situation is all the more reason for promoting the further development of major domestic centers. Generally speaking, this is because for Japanese companies, intellectual property (IP) in its widest sense, which includes practical business know-how born in Japan, can become the very foundation that supports the growth and development of their overseas centers. Strategies to preserve and reinforce this flow of IP are crucial for securing sustainable competitiveness. For this purpose, it is vital that each company make more efforts to strengthen its domestic centers and for Japan as a whole to provide the environment to support these efforts.

The preservation and strengthening of small- and mediumsized companies' innovative power is also highly important. AIST is actively engaging in joint research and offering technical consultation services and other support to small- and medium-sized companies. Many executives of such companies participate in the *Full Research* Workshops held at each of AIST's regional research bases. Although we do not have the accurate statistics, it seems that the majority of these small- and medium-sized companies have already made their move overseas. The largest motive behind their decision seems to be to comply with the requests of major Japanese manufacturers who are the customers for their products. These companies do hold a competitive edge for their products at the beginning of their move overseas, but sooner or later the products will become outdated and R&D will be needed for their next generation of products. However, this new R&D is a high hurdle for such small- and medium-sized companies that have moved overseas and tend to lack resources, and they must therefore be supported by both academia and public institutions.

A change in the method of innovation from technology push to market pull is also required. The era in which the champions of the domestic market also became champions in the global market and in which all manufactured products were bought no matter how many were produced has ended. Although it is not easy, it is essential for Japanese companies to come up with ideal images of sustainable products and businesses that can withstand the highly competitive global environment surrounding Japanese companies and to formulate realistic innovation strategies from this starting point. In the linear innovation model originating in scientists' intellectual discoveries, the difficulties encountered in the "valley of death" are something that must be overcome, but when viewed from the exit point-or, in other words, from the business side-numerous technologies that lie in this valley can be seen as a group of potential gems just waiting to be polished to shine. By starting with the formulation of a market pull business strategy, it can be expected that the direction of business selection and concentration, an ideal balance in the domestic-to-overseas ratio, the generation of IT based services that Japan is considered to be weak in, and other such positive developments will naturally begin to take shape.

We must also reorganize our thinking on the <u>connection between</u> <u>standardization and competitiveness</u>. In this modern world, where not only the developed countries but also the developing countries with their large markets, workforces, and resources have a significant impact on the global economy, the importance of global standards is greatly increasing, as an intellectual infrastructure that can assure just and fair economic competition and support sustainable development. Some say that because everything is made open and accessible through standardization, our competitors, especially foreign companies in lowcost countries, can easily catch up with us. However, this is a major misunderstanding. As I mentioned earlier, since competitiveness is influenced by a wide range of factors including the strong yen and the tax system, etc., thinking that the cause lies solely in standardization only results in a misconception. Standardization does not mean that we make everything open and accessible. What is needed here is to strategically differentiate between areas that will be made open and accessible so that technologies developed by one's company can be utilized by growing numbers of people, and areas that will not be made open and accessible and will be used to characterize and distinguish one's business. It is very disappointing to see that there are many corporate executives who are still confused as to the meanings of global standards and corporate standards, which are used to improve business efficiency within a company and should be shared as much as possible.

The decision as to whether to aim at the American model or the German model, or the Japanese model seen in the 1980s, is also important when planning to strengthen our competitiveness. Excluding the inconceivable return of the Japanese model of the 1980s, it seems that the majority of commentators' views are strongly inclined toward accepting the American model as the ideal model for improving Japan's competitiveness. The media are filled with criticisms such as that we have no Google of our own, that we failed to create the iPad, and so forth. However, on the contrary, the American model is not the only model that we should use as a benchmark when discussing industrial competitiveness. Since Japan is heavily dependent on the manufacturing industry, I consider that we need to look more closely at the German model, whether in making things or services. We should distance ourselves from superficial arguments and develop a model most suited to Japan's current situation. By being conscious of the points mentioned above and with our sincere and hardworking character, I believe that a new competitive power befitting Japan, which may be close to the German model but surpasses it and even incorporates the advantages of the American model, will be born.

## AIST Workshop for Innovation Ecosystem in Thailand and the AIST Open Lab 2012

The *Full Research* Workshops that I mentioned earlier are widely attended by participants from various local governments, the industrial sector, and academia. At the end of October 2012, we held the first "international version" of such workshops in Bangkok, Thailand.

Many Japanese corporations are doing business in Thailand. People working for such companies must have an understanding of globalization based on actual work experience, but in fact a wide range of actions have been taken during the globalization process of Japan as a whole. AIST has also been working to build a strong, long-term cooperative relationship with national research institutes in Thailand. We take pride in the fact that we have played a significant role in the modernization of Thailand's industrial sector. We decided to hold this workshop in Thailand in the hope that mutual understanding of this relationship between Japan and Thailand will contribute to the deepening of economic ties between the two countries.

The workshop was composed of four sessions. The first three were a series of talks on the following topics: "Metrology Standards to Support Manufacturing in Thailand," "Standards and Conformity Assessment to Guarantee Product Reliability," and "Green Innovation through Japan-Thailand Collaboration." The final session was a panel discussion on the theme, "Future Prospective Calibration, Standards and Conformity Assessment in Thailand."

Metrology standards, and calibration and conformity assessments based on the metrology standards are indispensable for guaranteeing product quality, and their significance is increasing year by year. Even though the number of European organizations entering this field has recently been sharply increasing, it is my impression that we were able to demonstrate the solid functional improvements made by Japanese organizations and Thai national research institutes through the abovementioned talks and panel discussion. We at AIST are determined to continue our support of these efforts.

A large number of international collaborative projects are being carried out in Thailand with the support of the Japan International Cooperation Agency (JICA), the New Energy and Industrial Technology Development Organization (NEDO), and others. Among these projects, presentations were given on the research and development of biofuels and photovoltaics by both Japan and Thailand. The presentations of Thailand were made by Thai national research institutes and the presentations of Japan were made by company representatives, with each presentation offering insights on their current situations and challenges. Through these case studies that are successfully utilizing the fruits of AIST's efforts in business development and the formulation of global standards, I believe that the participants from the industrial sectors of the two countries were able to confirm the effects of collaboration among national research institutes.

The workshop was attended by a total of approximately 230 people. Of these, around 100 were corporate participants mainly from Japanese companies and their affiliates. About 100 were from Thai national research institutes and universities and some 30 were from AIST; the Ministry of Economy, Trade and Industry (METI); the Japan External Trade Organization (JETRO); and NEDO personnel in Thailand. In each of the talk sessions and the panel discussion, the participants actively asked questions and voiced their opinions.

This workshop was supported by a wide range of organizations both in Japan and Thailand including the National Science and Technology Development Agency (Thailand) (NSTDA), the Thailand Institute of Scientific and Technological Research (TISTR), the National Institute of Metrology (Thailand), the Thai Industrial Standards Institute, METI, JETRO Bangkok, NEDO, the Technology Promotion Association (Thailand-Japan), and the Japanese Chamber of Commerce, Bangkok. JETRO in particular greatly supported us from the planning stage through the preparations and to the final workshop, and I would like to express my sincere gratitude for their cooperation.

AIST also has tripartite collaborations with partner countries' national research institutes and Japanese corporations in countries other than Thailand; for example, Indonesia, Australia, and China. I believe that we need to further strengthen our emphasis on these efforts.

We also held a large event in Japan called the "AIST Open Lab 2012" on October 25 and 26. As a new endeavor, we scheduled talks by the presidents of two global companies, President Masayoshi Matsumoto of Sumitomo Electric Industries, Ltd. and President Taketsugu Fujiwara of Asahi Kasei Corporation; talks by executives of successful local companies; a talk to commemorate the receiving of the Ig Nobel Prize; and various other talks. The number of visitors at the event surpassed last year's figure thanks partly to these talks, with more than 4,700 attending. As has been the case every year, the majority of the visitors were from corporations, accounting for about 80 % of the total. Slightly more than 20 % of the corporate visitors were from small- and medium-sized companies. With each passing year, increasing numbers of high-level personnel from companies' technical departments are coming to the event. It seems that not only more people are visiting us in search of raw gemstones that can propel innovation, but that their enthusiasm is also increasing.

## First Global Summit of Research Institute Leaders

The 9th Annual Meeting of the Science and Technology in Society (STS) Forum was held in Kyoto early last October. This meeting was established as the result of a proposal and initiatives by Japan and has now achieved a presence as a venue where more than 1,000 influential people from around the world in the fields of politics, industry, and academia gather and engage in serious discussions. With the aim of creating an opportunity for exchanges of ideas among representatives of the world's public research organizations prior to this STS forum, RIKEN and AIST jointly hosted the First Global Summit of Research Institute Leaders (RIL Summit).

Sixteen institutes from 12 countries participated, with RIKEN

and AIST serving as the participants from Japan. AIST has currently concluded memorandums of understanding on research cooperation with 35 institutes around the globe. Among these, the representatives of the following institutes participated: the Commonwealth Scientific and Industrial Research Organisation of Australia; Centre national de la recherche scientifique and Commissariat à l'énergie atomique et aux énergies alternatives of France; Badan Pengkajian dan Penerapan Teknologi of Indonesia; the Agency for Science, Technology and Research of Singapore; NSTDA and TISTR of Thailand, and the Oak Ridge National Laboratory of the United States. I believe that the summit was a good opportunity to discuss various issues surrounding research institutes, with global competition and collaboration becoming increasingly common. We may also further expand our circle of collaboration. The following is my personal summary of the summit.

Research institutes have two missions: to challenge and solve global-scale problems facing humankind, and to promote R&D that benefits their own country. As regards the first mission, the urgency with which such problems must be solved may differ according to the country and region that each institute belongs to, but each institute needs to tackle these issues, not passively but actively, with a mediumto long-term vision. Collaboration among research institutes is certainly important, and especially cooperation between institutes that focus on basic research and institutes skilled in practical application can be expected to produce a great synergistic effect. As regards the second mission, each institute must comply with the policies of its country when engaging in R&D and play a leading role in solving the country's unique problems. When executing these missions, it is vital that we join hands with the industrial sector, academia, etc. to prepare world-class R&D infrastructures and nurture and attract a sufficient number of highquality human resources. Moreover, investment in innovation that leads to sustainable growth is extremely important.

When viewed from the perspective of the developing nations, the existence of a brain drain may seem to be a problem, but from the perspective of the developed nations, we believe that this will eventually bring about "brain circulation" and provide benefits to all nations concerned. Thus, each and every research institute must strive to offer a research environment and/or an innovation environment that is attractive to first-class researchers.

The representatives of the 16 participating institutes agreed that the summit was generally a good opportunity for exchanging views and decided to continue it in the future as a program in conjunction with the STS Forum.

As for the main STS Forum, the discussions on the "light and

shadows" of science and technology were impressive. The need for a calm, sincere, scientific, and technological examination that does not turn a blind eye to the impact of the Fukushima Daiichi Nuclear Power Plant disaster triggered by the Great East Japan Earthquake, was suggested from various angles. We human beings are enjoying the benefits of scientific and technological development. On the other hand, we are also facing the difficult problems of environmental pollution, climate change, resource depletion, infectious diseases, various ethical problems, and so on. From now on, therefore, science and technology innovation must not only increase the benefits (light), but also be prepared to solve difficult problems (shadows). I strongly felt that these perceptions are especially important for public research institutes such as AIST.

#### Closing

AIST, with the support of Nikkei Inc., founded the Vitalization Council for Industrial Technology of Japan in the autumn of 2011. In addition to participants such as researchers and technology specialists from AIST, corporations, and universities, corporate executives and government officials also joined this group to diligently discuss measures for the vitalization of Japanese industries. Last December, the results of these discussions were compiled and publicized as "Proposals for Revitalizing Japan," comprising a set of general proposals as well as individual proposals in the following fields: energy and resources, innovative medical care and drug development, state-of-the-art materials and manufacturing technologies, IT and service technologies, human resource development, and global standardization. The details of the proposals were introduced at a symposium held in January. It is our hope that these proposals will contribute to the development of a new competitive edge in the industrial sector of Japan.

#### Addendum

I wrote the Japanese version of this manuscript in December last year before the change in government of Japan. After that event, the currency exchange rates also changed, and the Japanese industry seems to be gradually recovering its vitality. I hope that this trend will continue, and together with the government, AIST will strive so that the Japanese industry will be further revitalized.

### Tamotsu NOMAKUCHI

President National Institute of Advanced Industrial Science and Technology (AIST)

## tem Cell Engineering Pioneering the Future - From Science to Industry -

## **Current State and Future Prospects of Stem Cell Research**

#### Introduction

Research on stem cells including those of humans or research on regenerative science have recently attracted a great deal of attention. The fact is, however, that these fields have a long history from a biological standpoint, having already been researched since the 18th century.

Regenerative phenomena are phenomena in life science that most typically represent the essence of life. They are broadly categorized into two types: physiological regeneration and damage regeneration.

Physiological regeneration is a process that takes place in the human body on a daily basis. Take blood cells, for example. Some 60 million of these cells are produced and destroyed every second. In the entire human body, about 120 million cells are produced and destroyed every second, but your body today does not seem any different from how it was yesterday.

Then there is the other phenomenon, damage regeneration. When we receive a cut on our hand or foot, the cut will heal on its own. If our liver is cut to one-third the original size by a surgical operation, the remaining one-third has the capability of returning to its original size. When one of the two kidneys is removed, the remaining kidney will increase in size to the equivalent of two kidneys (which is a type of damage regeneration and is technically due to metabolic hypertrophy). A newt, for example, regenerates the original form and function of limbs even



Fig. 1 Pluripotent cell mass in the embryo of a vertebrate animal

when they are severed.

As explained above, our body metabolizes all the time by its very nature, with old cells constantly being replaced by new cells. Even though we may suffer damage from some physical cause, we have the capability to restore the damaged part. Research is being carried out to clarify the actors that accomplish this capability (that is, stem cells and others) and its mechanism, so that the findings of this research can be applied to practical medical treatment.

#### Stem cells in regeneration

The body is thought to heal itself in two ways when it suffers damage. One is the process in which a cell, which has already been differentiated, becomes temporarily dedifferentiated and is then differentiated again. The theoretical mechanism of regeneration for a newt whose limb has been severed then later repaired and regenerated to its original form is that the cells of muscles or bones at the severed

part are dedifferentiated into mesenchymal stem cells and then redifferentiated into muscles or bones as required to deal with the cut surface.

On the other hand, it is known that there are undifferentiated stem cells (somatic stem cells or tissue stem cells) throughout our body from the top of our head down to the tips of our toes. Even adults are known to have large numbers of stem cells in the skin, heart, bone marrow, muscles, brain, and almost all tissues and organs of the body. It is believed that these somatic stem cells are deeply related to the repair of damaged parts.

#### What is a "pluripotent stem cell"?

Stem cells, which are being actively investigated today, are broadly classified into three categories in terms of characteristics.

In the process of embryogenesis, a cell, after fertilization, divides into a larger number of cells. When cell cleavage reaches the blastocyst stage,



a collective mass of undifferentiated or pluripotent cells is seen in part of the embryo. Examples of such a cluster include animal cap cells in the animal pole during the blastocyst stage of a frog, blastodermal cells in chicken embryos, and inner clumps of cells in mammals such as human beings or mice (Fig. 1). When an inner mass of cells of a mouse, for example, is taken out and cultivated, it becomes embryonic stem cells (ES cells). These cells are capable of differentiating into any type of cells that constitute a body; that is, they are pluripotent in differentiation (Fig. 2). However, there is an ethical issue under discussion with respect to these ES cells because they are produced from embryos, each of which may become an individual life.

In one of the fields of recent regeneration research, some researchers are working on dedifferentiation of already differentiated cells in the tissues or organs of a mature body, such as that of an adult human.

In 1953, King and Briggs of the United States took out the nuclei of cells in the blastocyst stage, which are divided somatic cells, in a frog egg, put them in an enucleated unfertilized egg, and successfully turned the differentiated cells back into undifferentiated cells. In 1963, Gurdon of the United Kingdom cloned a frog by transferring a cell nucleus in the intestines of a tadpole into an enucleated unfertilized egg, turning the differentiated nucleus back into an undifferentiated state, then fertilizing the egg to restart embryogenesis and create a tadpole. This process is called reprogramming, as it uses the power of the cellular cytoplasm







Fig. 3 Measurement of stem cells for standardization of stem cells

of the unfertilized egg to dedifferentiate differentiated cells.

A recent ongoing study focuses on a method that uses transcription genes to reprogram differentiated cells into undifferentiated cells with multipotency or pluripotency. These cells are known as induced pluripotent stem cells (iPS cells). Yamanaka *et al.* transferred four genes, Sox2, Oct3/4, c-Myc, and Klf4, into a fibroblast and created undifferentiated cells with induced pluripotency.

This means that iPS cells reprogrammed differentiated cells into totipotent cells. Unlike ES cells, iPS cells cause no ethical problems. As this technique creates iPS cells from the cells of a patient, it can promote research that reflects the condition of the patient's disease or facilitate the development of cell transplant therapy with no rejection occurring. Research on the redifferentiation of these pluripotent cells (iPS cells) into those of various organs such as the heart by the introduction of various genes from outside or chemical treatment is actively conducted.

However, iPS cells result in a wide variety of responses in differentiation, depending on the production or cultivation method. It is difficult to remove cancerous cells from a differentiated cell mass. Since it is known that cancer occurs even if only one out of 10,000 cells is cancerous, the important point is how to create iPS cells that will not become cancerous. To this end, it is necessary to standardize stem cells by investigating the nature of cells (Fig. 3).

#### Future prospects of stem cell research

From the perspective of stem cell research as described above, it is evident that there are a wide variety of stem cell types. It is therefore necessary to select the appropriate way of producing or using stem cells depending on the purpose. This will definitely require standardization of stem cells. This, in turn, means that usable and unusable stem cells must be distinguished clearly.

Future stem cell research is certain to change and evolve in significant ways because of various positive prospects such as cooperation between medicine and engineering, development of new materials such as scaffolds necessary to create tissues of two- or threedimensional structures, improvement of culture fluids and culture methods, improvement of methods to collect stem cells, development of applications for stem cells, use of bioinformatics, and development of medical equipment related to stem cells. We can look forward to a bright future for stem cell research as we will be seeing the development of a variety of new technologies including the development of new treatment methods and new drugs using hitherto untapped types of cells, and the development of new medical equipment.

Remarkable advances are undoubtedly taking place in regenerative medicine and regenerative technology, but we must never forget that the most important points are safety, certainty, and reproducibility. If we continue our efforts by always respecting these top priorities from the perspective of the natural history of all life forms including humankind, we will surely be able to attain great advances in regenerative medicine and regenerative science. We cannot be too careful in our handling of regenerative medicine, including reproductive medicine, which may affect future generations. Although it is highly commendable that we actively conduct research on pain mitigation in one generation and development of new drugs, this does not mean that we should try to achieve everything, because such new developments and treatments may affect our descendants for generations to come. We must clearly understand that scientists will be held responsible for the outcome of such research and that their ethics will be tested.

There are now high expectations for the development of new drug screening systems using various stem cells. Also important is research on how to revitalize stem cells in our body to treat ourselves or maintain our health. Cell therapy will be an effective treatment of various diseases and injuries, including diabetes, Alzheimer's, Parkinson's, spinal damage, and eyeball damage, that cannot be easily cured with drugs or other existing means. Since there are strong expectations in society for economic effects and for recovery from diseases by the future advancement of this research, further growth in this field is very much anticipated.

Director Research Center for Stem Cell Engineering Makoto ASASHIMA

## A Trailblazer in Stem Cell Engineering Research – Fabrication of bone tissues from stem cells and transplanting them to patients –

When part of our body, such as a bone, a cardiac valve, a blood vessel, a cornea, or other tissues, is severely damaged, normal tissues are sometimes transplanted to repair the damage. Many tissues for transplanting are taken postmortem, as people may know from cases of organ transplants. This means that donors are necessary, and yet there is no denying the possibility that infectious diseases originating from such donors may occur. In fact, there are reports of recipients of organ transplants having died of serious infectious diseases. In addition, the tissues of such transplants are those of other people and can cause rejection unless immune suppressors are used.

Considering these concerns, if the cells of patients themselves can be used to create the necessary tissues and the cells can be taken in a noninvasive manner, patients will not require donors and their

treatment will be more gentle and safe. We have been working on the creation of various tissues with engineering techniques using mesenchymal stem cells present in our bone marrow for many years. We have proliferated mesenchymal stem cells from bone marrow tissues and succeeded to show cellular differentiation of the stem cells into osteoblast cells and bone cells, which showed in-vitro threedimensional mineralized bone tissues (figure). For a human being, the amount of bone marrow necessary to create these bone tissues is only a few milliliters. The bone marrow can be taken with minimal invasiveness, using a syringe, requiring no incision by a surgical knife. These in-vitro created bone tissues were transplanted to arthropathic patients at Nara Medical University Hospital. This transplant treatment had never before been attempted in the world and was unprecedented as

(A) Image of confocal X–Z section obtained along the plane across one of the mineralized nodules. Confocal X–Y sectional views of mineralized matrix region (B, h=4.95 µm from the base) and outer surface region (C, h=19.8 µm from the base) of the nodule. (B) was taken as line 1 in (A), and (C) was taken as line 2. The mineralized matrix is indicated by green, the actin microfilaments by red, and the nuclei by blue. The round-shaped cells lay in the mineralized matrix (arrowheads). Courtesy T. Kihara *et al.: Biochem. Biophys. Res. Commun.* 341 (4), 1029–35 (2006), with permission. clinical research. Safety thus had to be given the highest priority. In particular, the procedure of handling cells in vitro required conditions allowing the cells to stay alive as well as a sterile environment. Fortunately, as if in synchronicity with the progress of our stem cell research, a cell processing center (CPC) was constructed at AIST Kansai (see the article by Shunsuke Yuba, leader, Tissue Engineering Research Group, on page 12-13), which enabled us to handle the cells in a sterile environment. Since the establishment of this facility, mesenchymal stem cells have been proliferated and used for treatments of patients with various diseases. These treated cases have shown no complications such as infection or tumor incidence after transplantation, and excellent postoperative clinical results have been reported.

> Invited Researcher, Health Research Institute and Director of Orthopedics, Ookuma Hospital Hajime OHGUSHI

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Establishment of bone tissues by in-vitro cell manipulation using mesenchymal stem cells (confocal laser scanning microscope photos)



## **Discovery of New iPS Cell Inducing Factor, Glis1**

#### Production of safe iPS cells

In a joint research with Prof. Shinya Yamanaka, director of the Center for iPS Cell Research and Application, Kyoto University (and joint winner of the 2012 Nobel Prize in Physiology or Medicine), we found that transfection of Glis1 factor into fibroblast together with Yamanaka's three factors (Oct3/4, Sox2, Klf4) or four factors (Oct3/4, Sox2, Klf4, c-Myc) allows us to efficiently produce far safer iPS cells.<sup>[1]</sup>

Prof. Yamanaka's group has so far successfully produced iPS cells by transfecting the three or four factors into fibroblast using retrovirus vectors. However, they encountered some problems, including the risk of cancer formation, presumably due to the influence of the transfected factor c-Myc, as well as an extremely low establishment rate of iPS cell without c-Myc. Practical use of iPS cells in regenerative medicine still requires the solution of these problems. We looked for new iPS cell inducing factors to establish a method for the efficient production of iPS cells safe enough for clinical application. We used the world's largest human cDNA library created so far, which has been built by us, in our search for appropriate factors.<sup>[2]</sup>

#### Utilization of human cDNA library

We selected 1,437 transcription factors from the human cDNA library,<sup>[3]</sup> as mentioned above, and looked for new iPS cell inducing factors. Conventional iPS cell inducing factors were found in genes that are frequently expressed in ES cells. However, we decided not to simply follow the past



We selected human proteome expression resource (HUPEX) from the human cDNA library (http:// www.HGPD.jp) to establish a retrovirus expression clone and looked for new iPS cell inducing factors.

successes and looked for new factors from a comprehensive library. As a result, we found a new iPS cell inducing factor, Glis1. Almost none of the functions of Glis1 have been clarified. It is therefore a gene with no known functions. In addition, it is rarely expressed in ES cells. Thus, no researchers have listed it even as a candidate for initialization factors. When transfected into fibroblast of a mouse or a human together with Yamanaka's three or four factors, Glis1 can efficiently induce quality iPS cells. In addition, chimera mice produced from iPS cells using Glis1 showed no occurrence of conspicuous tumors or signs of shorter lifespan as seen in the case of production with c-Myc.

#### **Future schedule**

Transfection of Glis1 has a possibility of efficiently producing highly safe iPS cells as demonstrated by our research, and is expected to make a great contribution to the establishment of a clinically applicable iPS cell production method. We intend to use the human cDNA library that we have created so as to establish production techniques for various differentiation-induced cells in the future.

Biological Systems Control Team Biomedicinal Information Research Center Naoki GOSHIMA

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## **Glycans and Stem Cells**

#### Background

Often referred to as the "signature of a cell," glycans vividly represent a cell's characteristics. However, analysis of glycans requires the skills of professionals as the structure of glycans is very complicated and they exist in diverse forms. An advanced glycan analysis technique called "glycan profiling" was recently developed, which has allowed researchers to actively conduct applied research. One such research theme attracting attention is glycan markers. Glycans are the basis of many widely known cancer markers, including AFP-L3 (for liver cell cancer) and CA19-9 (for digestive system cancer), as well as SSEA-3/4 and Tra1-60/81, which are undifferentiated markers for ES cells, iPS cells, etc.<sup>[1]</sup>

## Evaluation and diagnosis of cells using comparative glycan profiling

This technique uses a lectin array, a glass plate on which a series of glycan binding proteins (lectins) that recognize and specifically bind the glycan structure are placed. Since the glycan structure reflects not only the type of cell but also the difference in differentiation stage of cells, the glycan profile differs for each cell. Using this principle, we can find glycan



Schematic illustration of cell quality control by glycan profiling using lectin array

markers for ES cells and iPS cells that reflect the undifferentiated state. In fact, we found a new lectin, rBC2LCN, which recognizes a glycan structure that is never expressed in somatic cells but is commonly expressed in undifferentiated cells such as ES cells and iPS cells. We further found that H type 1/3 (Fuc  $\alpha$  1-2Gal  $\beta$  1-3GlcNAc/GalNAc) is the very structure bound by that lectin.<sup>[2]</sup>

## Future development: Stem cell diagnosis by lectins

The figure shows a schematic illustration of quality control of stem cells (cell diagnosis) using the lectin array. Antibodies are conventionally used in marker detection, but our newly found lectin rBC2LCN can be produced in E. coli, which allows us to conduct research at lower cost. For example, this technique may be applied to differentiation monitoring of ES cells and iPS cells during culture and even removal of undifferentiated cells likely to cause cancer in addition to detection of ES cells and iPS cells. It can also be applied to mesenchymal stem cells, whose early practical application to regenerative medicine is expected.

Prime Senior Researcher Research Center for Stem Cell Engineering Jun HIRABAYASHI

#### **Joint Researchers**

Makoto Asashima, Hiroaki Tateno, Yuzuru Ito, Yasuko Onuma, and Katsuhisa Horimoto (AIST); Akihiro Umezawa and Hidenori Akutsu (National Center for Child Health and Development); and Masashi Toyoda (Tokyo Metropolitan Geriatric Hospital and Institute of Gerontology)

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P. W. Andrews: *Nat. Biotechnol.*, 29 (9), 803–805 (2011).
H. Tateno *et al.*: *J. Biol. Chem.*, 286 (23), 20345–20353 (2011).

#### Research result

Precise evaluation of iPS cells with rapid glycan profiling technique announced on, June 22, 2011. (in Japanese)

This research and development project has been carried out with the support of the New Energy and Industrial Technology Development Organization (NEDO).



## Development of Mesenchymal Stem Cell Production Technology Supporting Regenerative Medicine

Since mesenchymal stem cells (MSCs) can be easily separated from various tissues such as bone marrow or adipose tissue and proliferated, they have been widely used in regenerative medicine applications. We have confirmed not only the effectiveness of MSCs but also their safety in our clinical research with some 100 cases of mainly adult patients. Recently, we have been promoting regenerative medicine for infants and have achieved outstanding treatment results, including the demonstration of clear treatment effects in clinical research of a genetic disease that disturbs bone formation throughout the entire body.

AIST established a full-scale cell processing center (CPC) that can maintain a high level of cleanliness to produce MSCs for regenerative medicine, and started clinical application of MSCs ahead of any other institutes in Japan. CPCs are essential, as it is difficult to remove microorganisums from produced cells due to the fact that MSCs themselves are alive. Since then, with our CPC serving as a basic model, many CPCs have been constructed around the country at university hospitals and medical ventures. The total number of CPCs of various sizes established so far is around 90, including 50 designed according to GMP\*. GMP facilities have clean rooms for strictly sterile management like those



Our future expectation is that the existing CPCs will remain as major cell production facilities, while the isolator system will be employed by ordinary medical institutions

of semiconductor plants. However, the costs of maintenance and environmental validations necessary to maintain such a high level of cleanliness are not insignificant for a large CPC. It is not easy for an individual medical facility to own and operate a large CPC unless the CPC plays the role of a major cell production facility that provides medical cells to many medical institutions. A large amount of effort is also required to clean and sterilize the facility to prevent contamination. Considering these difficulties, there have been calls for a new cell production system to replace CPCs.

In response, we commenced the development of an isolator as a new

system to replace the conventional CPC, in collaboration with a corporation, ahead of other institutes. The isolator is like a compact box, not a room-in CPC, that contains a working space in which a high level of cleanliness is maintained completely separate from the external environment. In contrast to a CPC, where there is a risk of the operators themselves contaminating the working space for cell production, the isolator eliminates such risk by completely isolating the working space from the operators. A more practical type of isolator was recently developed based on an isolator for drug formulation targeted at pharmaceutical companies. This new isolator has now been put on the market. At the same time, we are also

#### Glossary

\* GMP (good manufacturing practice): Production management and quality control standards for manufacturing facilities with the objective of maintaining the quality of pharmaceuticals. The contents of GMP in Japan are determined by the Minister of Health, Labour and Welfare according to the Pharmaceutical Affairs Law.

working together with a corporation on the development of a device that can connect the isolator to Japanese- and foreign-made cell processing units in a sterile manner so as to enhance the extensibility of the isolator.

We are actively engaged in the development of cell production technology as a supporting technology for regenerative medicine side-by-side with clinical research on regenerative medicine, with the wish that our isolator system that can save space of production systems will disseminate to ordinary medical institutions so as to eventually help reduce the costs of cell production.

Tissue Engineering Research Group Health Research Institute Shunsuke YUBA

## **Quantification of Stemness**

#### **NEDO iPS cell project**

We participated in a NEDO iPS cell project in FY2009 and 2010. We used the genetic expression data of the iPS cell established in the project to investigate the genetic control network specific to iPS cells.<sup>[1]</sup> Then, we used the genetic expression data of other iPS cells established from a different parent cell and attempted to quantify stemness.

#### Identification of pluripotent gene sets

In order to quantify stemness, we selected a gene set that serves as an indicator of cell pluripotency.

First, we selected gene sets having an expression level statistically different from that of their parent cells, using iPS cell strains established from three different parent cells. Gene sets with an expression level different from that of all of their iPS parent cells were selected from ES cells. Then, a gene set that do not depend on the origin or passage of cells was selected from the gene set common to these four gene sets. According to the same selection rule, a gene set was selected from six



Quantification of stemness based on cell pluripotency

(A) Quantification of iPS cells measured by ourselves and their parent cells, (B) Quantification of cells that are partly pluripotent or in the differentiation stage, (C) Quantification of cancer cells

data sets including parent cells among the open data of iPS cells originating from fibroblasts in addition to our own measured data. Lastly, we compiled all of these data and selected a final gene set. The gene set selected by this procedure is considered to be an index gene set for pluripotency (IGSP) with the effects of originating cells and strains eliminated as much as possible.

#### **Quantitative evaluation of stemness**

The expression level of iPS cells that have partial pluripotency or are considered

to be in the differentiation stage (B in the figure) is quantitatively presented based on the gene set contained in this IGSP (top of A in the figure) and the gene set specific to the parent cells (bottom of A). As shown in the figure, these "halfway" iPS cells are located between the parent cells and iPS

cells. Next, we investigated the stemness of cancer cells,<sup>[2]</sup> which is a topic that has been widely discussed in recent years. By using about 13,000 sets of cancer cell data, our investigation revealed that about 20 % of the cancer cells showed stemness (C), and that a positive correlation with the grade of cancer

malignancy existed.<sup>[3]</sup> Quantification based on the IGSP obtained from strict selection is expected to realize quality evaluation of iPS cells and contribute to the further advancement of cancer research.

#### References

[1] S. Saito et al.: BMC Sys. Biol., 5 (Suppl. 1), S17 (2011).

[2] I. Ben-Porath et al.: Nat. Genet., 40, 499-507 (2008).

[3] Y. Wong et al.: In preparation.

Biological Network Team Computational Biology Research Center Katsuhisa HORIMOTO

## Approach to Technical Development of Stem Cell Evaluation Infrastructure

#### A multitude of pressing issues

The term "stem cell" is now a household word that we often hear in news reports. People have come to take it for granted that these cells may serve as a savior in the field of regenerative medicine. In particular, news reports cite iPS cells, which are a type of stem cells born in Japan (Fig. 1), as being as pluripotent as embryonic stem cells (ES cells), and iPS cells are a focus of rising expectations. This understanding is quite right if we look at it from a certain angle. However, we must also face the fact that there are a multitude of pressing issues yet to be overcome.

A variety of iPS and ES cell strains are cultured at laboratories and they must be taken care of (that is, change their media) every day. Since cells in a culture dish grow and completely fill it in about five days, they need to be diluted into many other culture dishes for subculture on a timely basis. If we apply them to regenerative medicine, we will have to make hundreds of these culture dishes ready. This fact alone clearly demonstrates how much



Fig. 1: iPS cells established with Sendai viruses (arrows: iPS cells proliferating in colonies)





Fig. 2: Experiment of continuous culture using automatic iPS culture equipment a: Appearance of the equipment b: AP staining of cells in colony under continuous culture to clarify their undifferentiation rate

labor is involved in handling these cells. What is worse, iPS and ES cells have highly unstable nature. Particularly when there is a deviation in the subculture method, they instantly undergo rapid denaturation and are no longer suitable for regenerative medicine. Further, the method of culture slightly varies from one set of iPS or ES cells to another (composition of the medium, subculture method, etc.). Without solving these problems, we cannot realize mass production of standardized cell products. And without mass production, practical application in society will not be realized.

## Solving problems to establish an evaluation infrastructure technology

Under these circumstances, we developed an automatic cell culture equipment, called Autoculture<sup>®</sup>, in a NEDO project together with Kawasaki Heavy Industries, Ltd. and the National Center for Child Health and Development. This equipment successfully incorporates a proven culture technique for these cells, which may be considered to be an "art" (Fig. 2a). Autoculture has a sterile housing, in which a robot arm conducts culture operations. Inside the housing are installed an incubator and a refrigerator, allowing operation without the need for refilling for about a week. Once the type of cell is designated, the equipment conducts individual culture operations according to the protocol installed in the equipment. When alkaline phosphatase (AP) staining of a colony of cells under continuous culture was conducted (which dyes the cells red if they

are not yet differentiated), it was confirmed that an undifferentiation rate of about 98 % was maintained. Using this equipment, more than 20 passages of continuous culture were successfully conducted (Fig. 2b). This technological feat that realizes stable culture has now almost enabled us to massproduce "uniform" iPS cells. We intend to organize stem cell evaluation items based on a large amount of the produced samples and establish a system that fully allows us to stably provide standardized stem cell products to the clinical sites of regenerative medicine and pharmaceutical companies.

Organ Development Research Team Research Center for Stem Cell Engineering Yuzuru ITOU

#### Stem Cell Differentiation Research Team Research Center for Stem Cell Engineering Akira KURISAKI

## Creating Stem Cells: Generation of High-Quality Human iPS Cells by Using RNA Virus Vector

#### Human cells in clinics and in industries

Normal human tissue cells are used widely in clinical medicine: e.g., in blood transfusion, in bone marrow transplantation, and in organ transplantation. Although autologous tissue cells are ideal source for these applications, it is difficult to obtain a sufficient number of tissue cells, except for those of blood and skin. Pharmaceutical companies also need large quantities of the normal human tissue cells. They have used various animal cells for examining safety and effectiveness of new drugs, while these substitutes do not necessarily reflect physiological functions of human cells. Therefore, it is desirable to generate large quantities of valuable

human tissue cells such as liver, heart, and pancreas cells from the cells easily obtained. Although researchers found the evidences suggesting this idea more than forty years ago, it has remained a dream despite strenuous investigation.

#### Impact of discovery of iPS cells

Human embryonic stem (ES) cells capable of differentiating into various tissue cells were first generated in 1998. These pluripotent cells are potentially useful, but have a serious ethical problem that they are generated by destroying normal human embryos which may grow up to be individuals. Immunological rejection of ESderived cells by recipient patients is another fundamental problem. Human induced pluripotent stem cells (iPS cells) were first generated in 2007 by reprogramming normal skin cells through ectopic expression of a defined number of genes. Development of iPS cells brought us closer to ideal regenerating medicine using patient-derived tissue cells, because the iPS cells are pluripotent and can become various tissue cells and, at the same time, the iPS-derived tissue cells will be transplantable without immunological rejection.

#### Future of iPS cell technology

After the discovery of iPS cells, researchers also started to pursue the direct conversion of skin cells to various tissue cells such as nerve, heart, and liver cells by using the same approach as for generating iPS cells, expressing a number of transcriptional factors in the target cells. Gene delivery and expression system applicable to human cells without safety concern is a key of success of these novel approaches. At the Research Center for Stem Cell Engineering, we are investigating a novel gene delivery/ expression technology ideal for phenotype conversion of human tissue cells, using generation of iPS cells as a model.

As for the methods of reprogramming, there are several hurdles to be overcome for making the iPS cell technology practical. For example, the exogenous genes used for reprogramming should be erased thoroughly from the cells once the reprogramming is accomplished, as these genes are potentially oncogenic. It is also important to express these genes simultaneously at a fixed balance in a single cell for guaranteeing reproducible



Production of human iPS cells using SeVdp-iPS vector We are now able to produce iPS cells efficiently from blood.

results. Generation of high-quality iPS cells from peripheral blood cells is another important challenge.

The replication-defective and persistent Sendai virus (SeVdp) vector developed at the Research Center for Stem Cell Engineering is an innovative technology for expressing exogenous genes stably without chromosomal integration. The SeVdp vector is based on a special mutant RNA virus that can co-exist with host cells without any pathogenic effect. We recently proved that the SeVdp vector can clear all of the current problems mentioned above in generating high-quality human iPS cells. In order to create valuable human stem cells flexibly at will, we will continue to challenge developing new innovative technologies for the future.

Deputy Director Research Center for Stem Cell Engineering Mahito NAKANISHI

## **Application of Neural Stem Cells Originating in the Olfactory Bulb to Drug Discovery and Regenerative Medicine**

#### Diabetes and stem cell transplants: Applying neural stem cells to diabetes treatment

Stem cells have the ability to recreate themselves and differentiate into cells that constitute organs. Diabetes will be completely cured if cells that can serve as a substitute for insulin-producing cells are transplanted. Furthermore, if we can transplant stem cells (i.e., stem cell lines) capable of developing into insulinproducing cells, the supply of insulin will be continuously ensured to maintain the treatment effect almost indefinitely.

We have developed a technique for differentiating adult neural stem cells into insulin-producing cells based on the various types of stem cell research that we have been conducting. If we realize stem cell therapy for diabetes using the patient's own neural stem cells, we can eliminate various problems including the necessity for donors or immune suppressors. In practical terms, however, it is difficult to remove cells deep inside a person's brain by surgical operation and apply them to regenerative medicine. On the other hand, the neural stem cells of the olfactory bulb can be obtained by means of a relatively simple operation such as an endoscopic procedure. We used animal experiments for evaluation, transplanting neural stem cells taken and established from the nose olfactory bulb of a diabetic rat into the pancreas, and found that the blood sugar level of the diabetic rat gradually decreased and that the rat's clinical condition eventually improved.<sup>[1]</sup>

## Stem Cell Engineering Pioneering the Future — From Science to Industry —

## Road to realization: Establishment of a customized treatment system using patient's own stem cells

There are a few problems to be solved before the diabetes treatment described above can be realized. It is important to use large animals closer to humans, such as monkeys or pigs, for evaluation. We are promoting the development of a neural stem cell culture system using monkeys, in collaboration with another research institute. It is also necessary to improve the technology for activating neural stem cells taken from the olfactory bulb into high-quality stem cells with good insulinproducing capabilities before they are transplanted to the pancreas. At present we are trying to establish a screening system, with drug discovery as one of the objectives, together with a pharmaceutical company that has a chemical compound library. If we can improve the deteriorated function of stem cells by medication, we will be able to achieve a synergistic effect on diabetes treatment along with transplant therapy.

The important points, however, are a more individualized activation process and the implementation of quick evaluation. The cellular functions of the cerebral nervous system vary significantly from person to person. Even in the case of identical twins who share the same genes, recent research has clarified that considerable differences appear in their nerve functions depending on how they live. In other words, it is necessary to realize a system of customized chemical compound screening that induces insulin activity quickly and efficiently using the neural stem cells of individual patients.



Automatic culture evaluation system for customized treatment of diabetes using neural stem cells from olfactory bulb (schematic diagram) Highly individualized stem cell activation process also leads to drug development and discovery.

It should be a regenerative therapeutic process that prepares neural stem cells with highly activated insulin-producing capability that undergo chemical compound screening within a few weeks after their removal from a diabetic patient, and transplants those cells into the pancreas of the patient (figure).

It seems that an automatic culture system that incorporates measuring and evaluation equipment will be necessary to realize this process. Needless to say, further progress of basic research on, for example, genes that serve as a key factor in the creation of individual differences is important. We believe that what we need now is to establish a joint research system that rapidly incorporates candidate factors obtained from our basic research into system development. We intend to closely work with various industries (such as the measuring instruments industry) and reagent and pharmaceutical companies in Japan and conduct R&D useful in the medical institutions to establish a new stem cell therapeutic system.

Stem Cell Differentiation Research Team Research Center for Stem Cell Engineering Tomoko KUWABARA

#### Press release

[1] Development of Regenerative Medicine Technology Using Adult Neural Stem Cells, October 7, 2011.

# **Research Hotline**

# UPDATE FROM THE CUTTING EDGE Oct.-Dec. 2012

The abstracts of the recent research information appearing in Vol.12 No.10-12 of "AIST TODAY" are introduced here, classified by research areas. For inquiry about the full articles, please contact the authors via e-mail.

**Environment and Energy** 

## **Recovery of specific kinds of electronic devices from waste printed circuit boards** Advanced physical separation process to realize strategic metal recycling

Advanced physical separation processes have been developed to realize strategic metal recycling. One is a double tube pneumatic separator and another is a magnetic-shape separator. The double tube pneumatic separator, which is one kind of gravity separators, separates particles into light, medium, and heavy products. Especially, this separator can recover specific electronic devices as the medium product by automatic control. Several kinds of electronic devices such as tantalum capacitors and ceramic capacitors, which have different densities, can be recovered automatically. The magnetic-shape separator is a hybrid separator of a low intensity magnetic separator and an inclined-belt shape separator. The inclined-belt part can recover aluminum capacitors which are cylindrical shape. The magnetic part, which has a uniform and very low intensity magnetic field, can recover only iron-rich electronic devices such as quartz resonators.



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AIST TODAY Vol.12 No.11 p.14 (2012)

Mechanism of the double tube pneumatic separator

#### High yield synthesis of terpene oxide Environmentally benign oxidation using hydrogen peroxide

We have developed a high yield synthesis method of terpene oxides by oxidation of terpenes using  $H_2O_2$  with a new catalytic system. The formation of fine chemicals from biomass with an environmentally benign system is studied energetically these days. Terpene, which is generated from gum turpentine, is one of the widely used kinds of biomass. Acid-labile terpene oxides such as  $\alpha$ -pinene oxide are synthesized in high yield from the epoxidation of terpenes with aqueous  $H_2O_2$  catalyzed by Na<sub>2</sub>WO<sub>4</sub>, [Me(*n*-C<sub>8</sub>H<sub>17</sub>)<sub>3</sub>N]HSO<sub>4</sub>, and PhP(O)(OH)<sub>2</sub> in the presence of Na<sub>2</sub>SO<sub>4</sub> as an auxiliary additive. Origin of the salt effect is considered to be the addition of a saturated amount of Na<sub>2</sub>SO<sub>4</sub> to the aqueous  $H_2O_2$  strongly inhibiting the undesired hydrolysis of the acid-labile epoxide products, despite the highly acidic reaction conditions. Generated terpene oxides are expected to be used as a perfume and a building block of sealing materials.



Life Science and Biotechnology

## Technology for production of targeted functional molecules in the body Control of gene expression by light and heat energy

Development of optical control methods of cellular functions is important for various biological applications. In particular, heat shock promoter-mediated gene expression systems by laser light are attractive targets for controlling cellular functions. However, previous approaches have considerable technical limitations because they utilize ultraviolet, short-wavelength visible, and infrared laser light which have poor penetration into biological tissue. Biological tissue is relatively transparent to light inside the diagnostic window between wavelengths of 650 and 1100 nm. Here we present a new optical biotechnological method utilizing carbon nanohorn (CNH), to transform energy from diagnostic window laser light to heat in order to control expression of various genes. Using CNH, we demonstrated that laser irradiation within the diagnostic window resulted in effective heat generation and thus caused heat shock promoter-mediated in vivo gene expression. This study provides an important step forward in the development of light-controlled gene expression technologies.



## **Discovery of a functional RNA that has opposed regulatory functions** The RNA responsible for both up- and down-regulation of specific gene expression

We have discovered a functional noncoding RNA that is involved in both up- and down-regulation of gene expression. The RNA called U7 is involved in two distinct regulatory mechanisms in histone gene expression. Histones form chromosome cores by associating with genomic DNA. Since histones are highly basic proteins, the free histones unbound with DNA are harmful to the cells. Therefore the histone synthesis is strictly restricted within S phase where DNA is replicated during a cell cycle. U7 RNA is a small noncoding RNA involved in histone mRNA processing to facilitate histone synthesis in S phase. Here we discovered another role of U7 RNA out of S phase, where U7 is required for silencing of histone gene transcription to avoid extra histone synthesis. The ultrasensitive mass spectrometry identified hnRNPUL1 as a novel U7 RNA-binding protein that is specifically involved in transcriptional silencing function of U7. The dual functions of U7 RNA are expectedly applied for designing an artificial gene switch to regulate cell functions.



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**Biomedicinal Information Research Center** 

**Tetsuro HIROSE** 

Distinct cell condition-dependent regulations of histone gene expression conducted by U7 RNA

Life Science and Biotechnology

## **Discovery of symbiotic bacteria mediating insecticide resistance to pest insects** Overturns conventional understanding that insecticide resistance is determined only by the pest insect's own genome

We have discovered that bean bugs (*Riptortus pedestris*), a kind of pest insect that attacks soybean crops and is difficult to control, develop insecticide resistance by acquiring insecticide-degrading bacteria from environmental soil and allowing them to live symbiotically in their bodies. To date, insecticide resistance has been reported in approximately 500 species of pest insects worldwide, causing serious problems for agriculture and public health. It has conventionally been believed that insecticide resistance is determined only by the genes of pest insects themselves. However, this new discovery overturns this conventional understanding, presenting a new perspective on the evolution of insecticide resistance in pest insects and for planning strategies to control pest insects.

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A bean bug on a soybean leaf



Survival rates of bean bugs after fenitrothion treatment Almost none of the specimens died if they were infected with fenitrothion-degrading bacteria.

## Antifreeze protein of high activity produced from a snow mold fungus, *Typhula ishikariensis* Crystal structure of antifreeze protein from a snow mold fungus

Antifreeze proteins (AFPs) preferentially adsorb to the surface of ice crystals, inhibiting their further growth. It is expected that AFPs can be applied to various industrial uses including frozen food and cold heat transfer. AFP from a psychrophilic fungus, *Typhula ishikariensis* (TisAFP) has been identified to exhibit ice growth inhibition effectively. In the present study, we determined the crystal structure of TisAFP and found that TisAFP is mainly composed of  $\beta$ -helical structure to fold into a semipear-like shape. In contrast to the other hyperactive AFPs with  $\beta$ -helical structures, there were much less repetitive residues aligned on the molecular surface of TisAFP. Site-directed mutational analysis revealed that the ice-binding site of TisAFP is located on the flattest surface of the molecule. In troughs of the ice-binding site there were aligned water molecules which seem to act as anchors for ice-binding. Fluorescence-based ice plane affinity analysis showed that TisAFP binds to both basal and prism planes of ice crystal, different from the other hyperactive AFPs. The unique feature of TisAFP that lacks the regularity in its ice-binding site provides the novel structural insight for hyperactive AFPs.



balls.), (c) An illustration representing that TisAFP binds to the ice surface through the IBS

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Information Technology and Electronics

## Mott transistor: a novel field effect transistor based on an electronic phase transition Electrostatic controls of the metal-insulator transition of strongly correlated materials

A Mott transition is a metal-insulator transition characteristic of strongly correlated electronic materials. Mott transistors based on an electrostatic triggering of the Mott transition are believed to surpass the conventional semiconductor FETs because of the vast functionalities and the intrinsically material-independent scaling limit. In this study, we have developed a prototype device of the Mott transistor with a CaMnO<sub>3</sub> channel, a typical perovskite-type Mn oxide showing a Mott transition. In order to accumulate a large amount of carriers in the CaMnO<sub>3</sub> channel, an electric double layer between the channel and ionic liquid was used for the gate dielectric. Gate voltage as small as 2 V was enough to induce a Mott transition and the insulating CaMnO<sub>3</sub> channel changed to the metallic one drastically. Furthermore, gate-voltage dependence of the drain current showed large hysteresis, suggesting a potential application for novel nonvolatile memories.

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Schematic picture of a Mott transistor consisting of a compressive-strained CaMnO<sub>3</sub> thin film and an example of the wiring



Gate-voltage dependence of the drain current in a CaMnO<sub>3</sub>-channel Mott transistor measured at room temperature

## **Storing spin information in germanium at room temperature** Development toward a novel transistor with ultra-low power consumption

Spintronics, a new type of technology, has recently attracted much attention because of its potential to reduce the power consumption of electronic devices, which are indispensable in our present life. It has been expected that a "spin transistor" with ultra-low power consumption can be realized if the spin information of a magnetic material can be transferred to a semiconductor.

We have demonstrated the transfer of spin information from iron into p-type germanium at room temperature. Germanium is a promising candidate as the semiconductor material for next-generation MOS transistors. This achievement is an important breakthrough in the development of a spin transistor with prospective application in the so-called green information technology.



Nanotechnology, Materials and Manufacturing

### Novel preparation technique enhancing oxidation activity of Pt catalysts Formation of efficient contacts between Pt and promoter that enables CO oxidation below room temperature

The use of additives to promote catalytic reactions over platinum group metals (PGMs) is employed in various applications. To optimize interactions between PGMs and promoters, it is necessary for uniform contacts to exist between them. We have developed a preparation technique of Pt catalyst that enables CO oxidation at low temperatures. Concretely, Pt/Fe-containing alumina catalysts were prepared and treated with water under moderate conditions. From structural analyses of the catalysts, it was concluded that Pt nanoparticles and iron oxides formed efficient contacts in the catalysts probably because of the enhanced mobility of Pt species. Surprisingly, these catalysts could catalyze CO oxidation at low temperatures—even below 0  $^{\circ}$ C. The concept of interface fabrication demonstrated in this example provides an opportunity to significantly reduce the use of PGMs in catalysts.

10 nm

**HAADF-STEM image and optical photograph of the developed catalyst** Pt particles having diameters of  $\approx 1.5$  nm are highly dispersed on the support.



Temperature dependence of CO conversions over the catalysts in  $(1 \% \text{ CO} + 0.5 \% \text{ O}_2)/\text{N}_2$ 

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Materials Research Institute for Sustainable Development

AIST TODAY Vol.12 No.10 p.17 (2012)

### X-ray spectroscopy captures signals from single atoms Improved sensitivity realized single atom identification

We have developed a high sensitivity X-ray detector and demonstrated the detection of the characteristic X-ray signals from single Er atoms in energy dispersive X-ray spectroscopy. The intensities of Er L and M lines from a single Er atom were extremely weak in contrast to the *N*-edge of electron energy-loss spectroscopy, which implies the intrinsic difficulty to sense single atoms in X-ray spectroscopy. Nevertheless, this work will certainly ensure the possibilities to obtain X-ray spectra from single atoms and to identify single atoms in the sample.



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(a) The developed analytical microscope (at Kyushu University) and (b) the schematic of the experiment

Metrology and Measurement Science

## Utilization of slow positrons under atmospheric conditions based on a microbeam technique To realize in-situ evaluation of intermolecular spaces in functional thin films

We have developed a controlled-environment positron probe micro-analyzer. This system can be used with the positron annihilation lifetime technique to evaluate open spaces such as atomic- and molecular-level defects, holes, and pores of functional thin films in an ambient gas at atmospheric pressure, i.e., in conditions which are close to the actual working environment. In this system, positrons are generated in a vacuum and formed into a focused, short-pulsed beam with low, variable energy. The beam is extracted via a thin vacuum window into the atmosphere, and then injected into the film sample, so that the positrons stop near the surface. By using this system, nondestructive evaluation of the intermolecular spaces for a polymer thin film with a thickness of a few hundred nanometers in nitrogen gas with controlled, variable, relative humidity has been achieved.



Schematic of the method used to extract the slow positron beam into the atmosphere

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## In Brief

## Symposium on International Strategy for Industrialization of Biofuels

The Symposium on International Strategy for Industrialization of Biofuels was held on September 3 and 4, 2012, at Otemachi Sankei Plaza Hall, cosponsored by Japan Science and Technology Agency (JST), Japan International Cooperation Agency (JICA), Japan International Research Center for Agricultural Sciences, New Energy and Industrial Technology Development Organization, Japan Business Federation, and AIST.

This symposium focused on biofuels, which is among the Science and Technology Research Partnership for Sustainable Development (SATREPS) programs being carried out by JST and JICA, and aimed at informing stakeholders, people from companies, policy makers and researchers about the international efforts toward commercialization of biofuels.



Symposium hall

A total of 700 people, including 500 from companies and over 150 from universities and public offices, participated in this symposium. On the first day, Vice Minister Kamimoto of the Ministry of Education, Culture, Sports, Science and Technology gave the opening remarks. There was a keynote speech given by Executive Member Aizawa of the Council for Science and Technology Policy of the Cabinet Office, and invited lectures by the governor of the Thailand Institute of Scientific and Technological Research, and the chairman and deputy chairman of the Agency for the Assessment and Application of Technology of Indonesia. There were also reports on biomass utilization by related organizations. On the second day, there were reports on international case studies by researchers, reports on commercialization of biomass by individuals from companies, and a panel discussion on the international strategy for industrialization of biofuels.

Through this symposium, it is expected that the exchange of researchers and the collaboration with companies overseas will become active and that the commercialization of the science and technology of Japan concerning biofuels will advance.

### First Global Summit of Research Institute Leaders

On October 6, 2012, 16 heads of public research institutions from 12 countries met at Kyoto International Conference Center where the First Global Summit of Research Institute Leaders (RIL Summit) was held in conjunction with the 9<sup>th</sup> Annual Meeting of the Science and Technology in Society (STS) Forum. President Noyori of RIKEN and AIST President Nomakuchi, both of representative research institutions of Japan participated.

The purpose of this summit was to recognize and promote the roles of research institutions, and to raise awareness of the importance of promoting collaboration among the leaders of the institutions as they strive to solve



global-scale problems facing humankind, upon introducing to each other the roles of their research institutes, status of their leading research, and management issues. RIKEN President Noyori and Alain Fuchs, President of National Center for Scientific Research of France, served as chairmen of the meeting.

As this was the first summit of its kind, each institute gave an introductory presentation on the number of researchers, research budget, and its history of establishment. Views were exchanged on such issues as missions of public institutions, policy for innovation in science and technology, brain drain, policy of cooperation with industry, technology transfer, and research expenditures.

The content of the discussion was reported by AIST President Nomakuchi at the 9th STS Forum held on October 8 where he served as one of the panelists in the "Research Organizations Update" session. He also expressed the importance of furthering collaboration among the leaders and the need for continuous discussion of issues surrounding research institutes. The Second RIL Summit is scheduled to be held on October 5, 2013, a day before the 10<sup>th</sup> STS Forum.

#### Cover Photos

Above: Human proteome expression resource (p. 10)

Below: Blood-derived human iPS cells (Photo by Manami OHTAKA, RCSC, AIST) (p. 16)



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