

OCARINA通信

The OCU Advanced Research Institute for Natural Science and Technology

—Special Project—

Present State of the Tenure-track Project and its Prospects

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OCU Advanced Research Institute for Natural Science and Technology

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The 2013 OCARINA Annual International Meeting

OCARINA Seminars

The first Osaka City University Tenure Track Teachers Research Presentation Event

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VOL.4

■ Special Project: Present State of the Tenure-track Project and its Prospects

“We hope to grow as researchers and educators, making use of a research environment that integrates humanities and sciences.”

Discussion with researchers invited through the Tenure-track Promotion Project



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Osaka City University started the Tenure-track Promotion Project in 2014 to establish a research environment in which young researchers would be able to study independently. This is part of a project conducted by the Ministry of Education, Culture, Sports, Science and Technology, and an unprecedented undertaking for our university.

We are presenting a discussion by the three tenure-track teachers belonging to the OCU Advanced Research Institute for Natural Science and Technology, Prof. Amao, their mentor, and Vice President Miyano.

A Wide Variety of Fields of Study – The Advantage of a Comprehensive University Offering Both Humanities and Science Programs

Dr. Miyano / Only a few municipal universities have been selected for the tenure-track promotion project of the Ministry, and we are proud to be one of them. I am truly pleased to have such brilliant teachers as you with us.

It is my understanding that one of the reasons our university was selected for the project was that we offer well-balanced fields of study in both the humanities and sciences, and have a high potential for their integration, even though our university is not as big as the major national universities.

Also, both the Advanced Research Institute for Natural

Science and Technology and the Urban Research Plaza, to which the tenure-track teachers belong, are conducting state-of-the-art research, and they are also engaged in programs with foreign institutes involving the acceptance of researchers from abroad.

Almost one year has passed since you three joined the Advanced Research Institute. Could you tell us your impressions of our university, your research topic and your hopes?

Dr. Amao / Our tenure-track project was launched in August of 2013, and we soon began recruiting. I suppose it might have been difficult to prepare in a short time. Could you tell us why you decided to apply to our university?

Dr. Nakadai / It was just when I was starting my 10th year

after obtaining my degree. The time limit to apply for the tenure-track project is within 10 years of obtaining a degree. I was thinking about becoming an independent researcher in the future. Then, Dr. Nishikawa of this university posted the information about the open recruitment of researchers of nematodes on the mailing list. The field of recruitment was slightly different from my main field of study, but Dr. Nishikawa also deals with nematodes as his model animals; therefore, I expected that my research topic might be accepted, and applied for the recruitment.

Dr. Yamaguchi / I had been conducting research in the U.S.A., and it was time for me to decide whether to stay there and acquire permanent residency or go back to Japan. There are many independent researchers in the States, and I also hoped to establish my own project and conduct research independently. I looked for the recruitment information of many institutions and found the recruitment of OCU. There was an English website, so I expected that applications from countries other than Japan would also be accepted.

Dr. Amao / I was present at your interviews, and I was impressed by Dr. Yamaguchi's presentation given in English. Dr. Asoh, you applied for the field of chemistry, which was highly competitive. Why did you decide to apply?

Dr. Asoh / Like the other two, I was seeking an environment that would give me independence. I had been working as an assistant professor at Tokyo University of Science and looking for an opportunity to become independent, so I was paying attention to information about the recruitment of tenure-track teachers. I thought tenure-track status with abundant funds would be ideal for my purposes. This university offered chances for a wide range of fields of chemistry, so I expected that bio-material, my field of study, would be included.

Dr. Miyano / All of you hoped to be independent, as I assumed. The Tenure-track Project aims to provide researchers with an opportunity to study independently; therefore, it goes along with your purpose. Didn't you have any hesitation in applying to a municipal university rather than a national university?

Dr. Asoh / This university is a comprehensive university offering both humanities and science courses, and it is not inferior to national universities. I did not worry with regard to that point.

Dr. Yamaguchi / I was teaching at a state university in the U.S.A., so the type of organization was not important to me; what was important was to be able to conduct research as I wanted to.

Dr. Nakadai / I did not mind the fact that this university is municipal, but I had little information about OCU since I'm from Kyushu. I asked my husband, who is from the Kansai District, and he told me that this university is a good, comprehensive university, so I decided to apply.

Expansion of the Fields of Research through the Tenure-track Project

Dr. Amao / I appreciate your comments. Now, could you please give a brief outline of your field of research.

Dr. Nakadai / I am studying the mechanism of byophylaxis, using the model animal *C.elegans*, which is a 1mm-long nonparasite nematode. For all creatures including humans, it is a kind of biophylaxis to perceive danger by means of the neural system and senses. I am analyzing the actions of sensing and avoiding danger, using nematodes. I am also studying the mechanism of biological response to infection at the levels of individual cells and molecules.

Dr. Yamaguchi / The types of microorganisms that I am studying are bacteria. In general, bacteria do not have a positive image, but they have been recently found to produce a toxin that could kill them. Almost all of the bacteria that have been found so far have a similar toxin, but we have not discovered the reason yet. The targets of each toxin are different, and the study of the targets is also interesting. I think if we can make use of such toxins, they can be applied to the treatment of HIV or cancer.

Dr. Asoh / I am conducting research on polymer bio material. Basically, I am studying the chemical design of materials that are used in or on the human body. My ultimate, major goal is the study of artificial muscle.

Dr. Amao / The three of you now belong to a department of the Advanced Research Institute or another affiliated section. I hope you will establish a new field of study there in the future.

Dr. Miyano / The Advanced Research Institute now focuses on the research of artificial photosynthesis, but it was originally expected to cover a wide range of science.

■ Special Project



Director/Vice President, OCU

Michio Miyano

Completed the doctoral program of the Graduate School of Engineering, Tokyo Metropolitan University in March 1980 without obtaining a doctorate. Obtained a degree of Doctor of Engineering. Was a full-time lecturer, professor, Dean of the Graduate School of Human Life Science and the Faculty of Human Life Science of OCU, and became Vice President in 2010. His books include "Great East Japan Earthquake - Volunteering Activities and Temporary Housing" (Kenpaku-sha, 2014), "Long-period Ground Motion and Seismic Resistance of Buildings" (Architectural Institute of Japan, 2007), "Disaster Prevention Encyclopedia" (Tsukiji-shokan, 2002) and "Great Hanshin Earthquake - Short yet Long Five Years" (Gakugei Publishing, 2000).

Professor, OCARINA

Yutaka Amao

Professor, OCARINA
Obtained a Doctorate in Engineering at the Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology in March 1997. Was a researcher at the Kanagawa Academy of Science and Technology Foundation and the National Aerospace Laboratory of Japan (present-day JAXA). In addition, he was a lecturer, an assistant professor, and then an associate professor at the Faculty of Engineering, Oita University. He became a professor of OCARINA in April 2013. He has also been a pioneering researcher at the Japan Science and Technology Agency since April 2010.



The expansion of the fields due to your research is a welcome development.

The most important objective is for you to gain the capabilities to become independent, and to accomplish many projects during your tenure-track term. Please let us know about any requests or proposals for improvement of our system that you may have thought of through conducting your research.

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Dr. Nakadai / This is the first period of the project and that might be the reason, but I sometimes feel that the term "tenure-track" and the system itself have not been widely recognized.

Dr. Miyano / The system has definitely not been sufficiently recognized, particularly among teachers of liberal arts. We began the Tenure-track Project in the fields of science first, although we hope to spread it into the fields of humanities in future. The project is sometimes discussed at the meetings of professors of the three graduate schools of science, engineering and human life science, so the teachers of these schools should know about the system. However, we should publicize the system to disseminate the information more widely.

Dr. Yamaguchi / I am really grateful that not only the teachers of the Graduate School of Science but also other people care about me, since I started my research here. Many of them often ask me if I need something or talk to me every time they see me. My only trouble is this: there are many instruments I would like to use in my experiments, and I guess they might be used by someone at another graduate school, but I do not know how to contact them. Of course, I have only been here one year, so I am

still not very familiar with this university.

Dr. Miyano / Other teachers might have the same problem with regard to sharing the experimental instruments. This also needs to be publicized. We will provide information about the tenure-track teachers regarding which sections they belong to and what they are studying so that you can collaborate with other teachers.

Dr. Yamaguchi / Thank you very much. In that sense, it was good that many teachers attended the research report meeting the other day. I think that they have been gradually gaining an understanding of our research.

Dr. Asoh / I do not have any problems at the moment. Three teachers have constantly been following my research. I give them reports on the progress of my research, and they conduct a strict check once every two months. First, I had some difficulty in understanding my position; however, partly because of the characteristics of this region, Osaka, I feel very close to them and it is easy to communicate, since they honestly give me both good comments and harsh comments.

Dr. Amao / You are the advisor of two chemistry students, Dr. Asoh. In the future, you will be required to have the skills of an educator. I think you should start gaining experience in endeavoring to train good students.

Dr. Miyano / I agree. It is definitely an important goal for you to be an independent researcher, but I hope you will also improve your ability to teach students. Please commit yourself to education during this period to the extent that it will not be detrimental to your research. As part of this



Specially appointed tenure-track lecturer,
Graduate School of Science (Chemistry), OCARINA

Taka-aki Asoh

Research theme: "Development of Stimulation-responsive Polymer Materials that Act in an Aquatic Environment" and "Construction of Next-generation Materials by the Bonding of Hydrogel" Obtained a Doctorate in Engineering at the Graduate School of Engineering, Osaka University in March 2008. Was a fellowship researcher of the Japan Society for the Promotion of Science (DC2, later PD) and an assistant professor of the Faculty of Industrial Science and Technology, Tokyo University of Science, and joined OCARINA in March 2014.

Specially appointed tenure-track associate professor,
Graduate School of Science (Biology), OCARINA

Yoshihiro Yamaguchi

Research theme: "A Study on the Physiological Role of Apoptosis-like Cell Death in Microorganisms" Obtained a Doctorate in agricultural science in the post-doctorate course at the Graduate School of Agricultural Science, Tohoku University in 2005. Was a fellowship researcher of the Japan Society for the Promotion of Science (DC2), a post-doctoral researcher and a Research Teaching Specialist III of Rutgers University. He joined OCARINA in 2014.



Tenure-track Promotion Project, it is also necessary to train young researchers who will follow in your footsteps. I hope that you will have many experiences of collaboration with graduate students and young researchers that you will be able to apply to education.

opportunity to explain my research. Also, I think I should have been aware of the audience and explained in such a way that people of different fields could understand easily, since I started with very technical topics.

A New Challenge Identified at the Research Report Meeting

Dr. Amao / I hear that there was a large audience at the research report meeting Dr. Yamaguchi mentioned earlier. Did you find anything difficult in reporting your research results?

Dr. Asoh / Not really. I emphasized the fact that I had started something new.

As for the commentator, the one in the field close to mine could not attend the meeting, but a renowned researcher in the field of polymers, who is my mentor's superior, attended the meeting and gave a comment from a perspective different from ours. That was very meaningful. After the presentation, the director of OCARINA gave me a lot of encouragement, and I renewed my determination to work hard.

Dr. Yamaguchi / At the meeting, I learned about what other tenure-track teachers were working on. I did not know how I should explain my topic to the teachers of fields of study that are totally different from mine, and I could not manage my reporting time well. However, I was very inspired by the questions, which were from a wide range of perspectives.

Dr. Nakadai / Yes. I also think that it was a good

Dr. Amao / You are still young, so I recommend that you use this kind of report meeting as a means of accumulating experience in preparation for applying for JST's PRESTO (Sakigake) program and other subsidized research programs that involve hearings as part of the approval process. My research was adopted for PRESTO, and when I was applying for that, I felt the same way as you did; since the judges were not from the same field as me, I should have given a more detailed introduction and talked about basic things. If you are in the same situation, I will give you advice, so please let me know. Securing research funds is an important process for advancing your career.

Aiming to Be a Researcher Who Can Give Students Dreams

Dr. Amao / Finally, do you have an image in your mind of the kind of teacher you would like to become?

Dr. Asoh / I hope to be a strong researcher. I would like to continue with the research that I want to do, be a recognized researcher and an educator who can convey what I have learned to students. In addition, I think I will be rigorously evaluated over the next five years, so I will train myself mentally and physically to steadily acquire capabilities. Ultimately, I aim to become a full-fledged researcher and educator.

■ Special Project



Specially appointed tenure-track associate professor, Graduate School of Human Life Science (Food and Nutrition), OCARINA

Eriko Nakadai

Research theme: A Study of the Molecular Base in Organisms for detection of and Protection from Environmental Stress

Obtained a Doctorate in Pharmaceutical Science at the Graduate School of Pharmaceutical Sciences, University of Tokyo in 2004. Was a researcher for a pharmaceutical company, an assistant professor and lecturer of the School of Medicine, Tokyo Women's Medical University, and joined OCARINA in February 2014. She also belongs to the Graduate School of Human Life Science.

Dr. Yamaguchi / I have long aimed to become a researcher who continues to take on challenges and enjoy science; however, I need to not only conduct research but also educate students for these five years. I think the best thing will be for me to spend time with students in a meaningful way, and let them know that science is enjoyable.

Dr. Nakadai / What I want for my students is for them to become strong. I hope to support them in becoming strong researchers and educators. Also, there are many female students in the graduate school, and I will be happy if seeing me working inspires them to pursue a career as a researcher.

Dr. Miyano / I think all of us decided to become researchers because we had a good mentor, a model researcher whom we aimed to resemble. As Dr. Nakadai said, I hope you will be a researcher who will be able to give dreams to young people. This will be an important perspective when you begin to teach students as an independent researcher.

Soon, there will be new, young graduate students. I would like to have opportunities to communicate with them through you.

Dr. Amao / It may be a good idea to use this institute for that purpose, and to start some kind of an association for young members. Providing an occasion for students of different fields to get together may lead to innovative ideas.

Dr. Miyano / There are many researchers in this institute who study in fields such as artificial photosynthesis that do not have much to do with my own field, and I have a number of opportunities to obtain inspiration from them. I hope you will also make use of this kind of environment. Speaking of the university as a whole, there are many fields that have integrated humanities and sciences. Please take advantage of this feature as well.



■ Research Introduction

Toward X-ray crystallography on the reaction intermediate of Photosystem II

Photosystem II (PSII) is a membrane protein complex which undergoes a primary reaction of photosynthesis. PSII binds oxygen-evolving complex (OEC) that catalyzes the water-splitting and oxygen-evolving reaction. The structural model of OEC at a resolution of 1.9 Å has been reported on 2011 (Umena, Kawakami, Shen, and Kamiya, *Nature*, 2011, 473, 55-60). In order to elucidate further the principle of oxygen-evolving photosynthesis, we are studying about light-induced structural-change of PSII.

PSII exists in thylakoid membrane of plants and algae, performing the water splitting and intra-protein electron-transfer reactions. PSII contains many cofactors such as OEC, chlorophylls, carotenoids and plastoquinones, among which electron transfers take place. The functional role of OEC is the oxidation of water molecule and the donation of electron to other cofactors. It has been known that OEC has five intermediates in the PSII reaction (Figure). These five states have different oxidation numbers and alter sequentially by individual photon absorptions. These intermediates are denoted as S1, S2, S3, S4, and S0. S1 is the most stable state under dark condition. Molecular models in the S2 state have been proposed by spectroscopic techniques, such as EPR and XAS. On the other hand, by protein crystallography or X-ray diffraction techniques (XRD), there is no knowledge about the S2 state structure at high resolutions, at which water molecules can be distinguished obviously. It is important to clarify the structure of water molecules in PSII, because PSII utilizes water molecules as the substrate. We are focusing on the structure analysis of the S2 state by XRD.

Spectroscopic researches previously reported have elucidated that the S2 state can be trapped at a cryotemperature range of 200 – 240 K. We also confirmed,

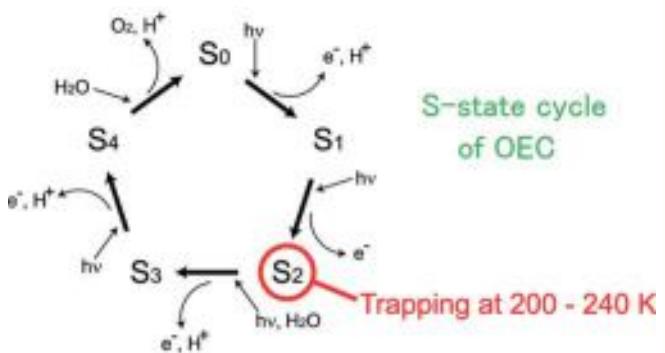
OCU Advanced Research Institute for Natural Science and Technology Specially Appointed Assistant Professor

Yoshimasa Fukushima

B.S. in Physics, Nagoya University School of Science. M.S. and Ph. D., Nagoya Univ. Graduate School of Sciences. After serving as a researcher at the Nagoya Univ. Graduate School of Sciences and the same university's Center for Gene Research, began serving in his current position in November, 2012



by EPR spectroscopy on our PSII solution, that the S2 state was induced and trapped by illumination at the cryogenic temperature. The similar protocol may be available to achieve the S2 state in crystals, however, we have to overcome a problem in the case of XRD. The matter is the ice formation in protein crystals. If the protein crystal contains ice, it will perturb the diffraction pattern from the protein crystal, and the dataset quality may become quite low. In general, the ice formation can be prevented by adding of cryoprotectants, such as polyethylene glycol, into a crystal-enfolding solution. We examined for experimental conditions that suppress the ice formation, changing the crystal-enfolding solution to oils and modifying crystal-cooling protocol. Recently, we found out a new condition that suppresses the ice formation at the temperature around 240 K, which will enable the structure analysis of PSII at the S2 state.

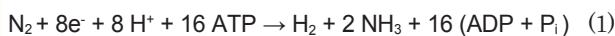


■ Research Introduction

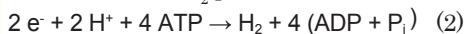
Improvement of photobiological hydrogen production by heterocystous N₂-fixing cyanobacteria

Photobiological production of H₂ by cyanobacteria is an attractive source of renewable energy because these microorganisms have simple needs: readily available sunlight for energy and water as the electron donor. H₂ is an efficient substrate for fuel cells that cleanly generate electrical and thermal power with water as the final product.

In cyanobacteria, H₂ gas is generated by either hydrogenase or nitrogenase. Both enzymes are sensitive to inactivation by O₂. In contrast to hydrogenase, nitrogenase catalyzes the following reaction under optimal conditions for N₂ fixation:



Whereas, in the absence of N₂ (e.g., under Ar), all electrons are allocated to H₂ production:



The above reactions are essentially irreversible and thus nitrogenase does not consume H₂ as a substrate. For the heterocystous N₂-fixing cyanobacterium used here, *Anabaena* sp. strain PCC 7120, nitrogen deficiency leads to the development of specialized cells called heterocysts. Heterocysts lacking photosystem II activity, have increased respiration and are surrounded by a thick cell envelope that impedes the entry of O₂, providing a microaerobic environment to protect the nitrogenase from inactivation by O₂ (Fig. 1). Because of their ability to generate energy by oxygenic photosynthesis while forming a separate space for anaerobic reactions, heterocystous cyanobacteria are good candidates for optimization of photobiological nitrogenase-based H₂ production through gene engineering [1,2].

One of the major obstacles to efficient solar energy conversion to H₂ is the presence of the uptake hydrogenase (Hup) that reabsorbs the H₂ produced by nitrogenase. When the Hup activity is eliminated by genetic engineering, the cyanobacterial mutants showed 3-7 fold

Special-appointment Associate Professor,
OCARINA

Hajime Masukawa

Received a Ph.D. (Doctor of Science) from Waseda University in March 2003. He previously worked as a postdoctoral fellow at Advanced Research Institute for Science and Engineering, Waseda University, an assistant professor at Department of Biology, School of Education, Waseda University, JSPS Research Fellow (PD) at Kanagawa University, Research Associate at DOE Great Lakes Bioenergy Research Center and Department of Microbiology and Molecular Genetics, Michigan State University, and JST PRESTO researcher at Kanagawa University. He has been in his current position since October 2014.



increased H₂ production activity under Ar gas compared with wild-type cells and accumulated a high concentration of H₂ (about 7-30%) even in the presence of O₂ [1,2].

According to equations (1) and (2), one expects to be able to increase the H₂ production activity of nitrogenase by decreasing the electron allocation to N₂ fixation. Mutagenesis of nitrogenase offers an alternative mechanism to redirect electron flow and overcome N₂ competition which causes a decrease in H₂ production activity in the presence of N₂. In an attempt to direct electron flow selectively toward proton reduction in the presence of N₂, 49 variants with amino acid substitutions presumed to be located in the vicinity of the catalytic 1Mo-7Fe-9S-1C-homocitrate] FeMo-cofactor of nitrogenase were constructed [3]. Some of the variants photobiologically produced H₂ under N₂ at rates comparable to those under Ar over the course of several days (Fig. 2). Of the variants, the two mutants, Q193S and R284H, rapidly produced H₂ under N₂-rich gas at higher sustained rates than under Ar-rich gas over 3 weeks [4]. These site-directed variants have a potential cost advantage in sustaining H₂ production at high rates by periodic gas renewal using the less expensive N₂ gas without changing the culture medium.

References

- [1] Masukawa H. et al., *Ambio* 41, 169-173 (2012).
- [2] Sakurai H., Masukawa H. et al., *J. Photochem. Photobio. C* 17, 1-25 (2013).
- [3] Masukawa H. et al., *Appl. Environ. Microbiol.* 76, 6741-6750 (2010).
- [4] Masukawa H. et al., *Int. J. Hydrogen Energ.* 39, 19444-19451 (2014)

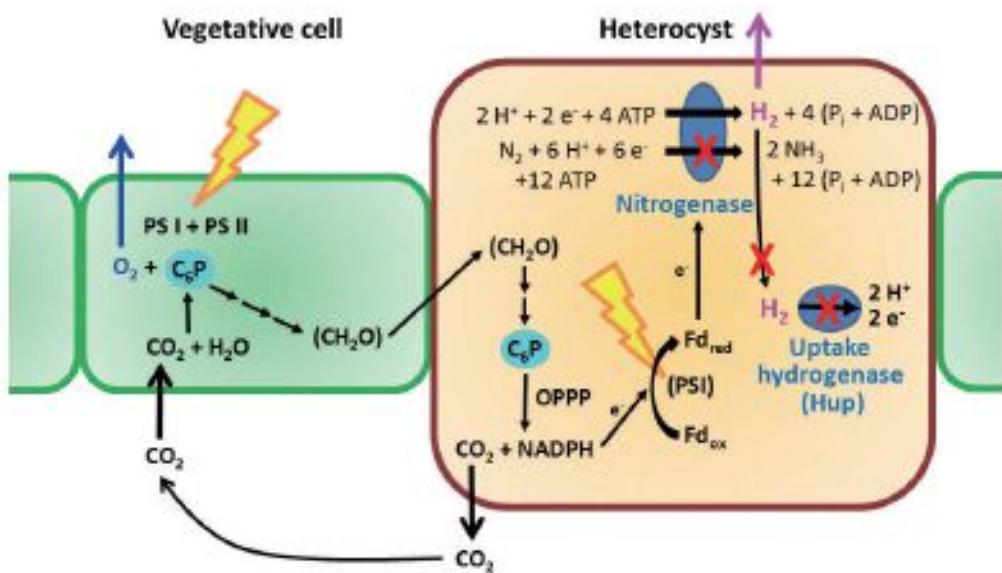


Fig. 1 Photobiological H₂ production by heterocyst-forming cyanobacteria (adapted from [ref. 1])

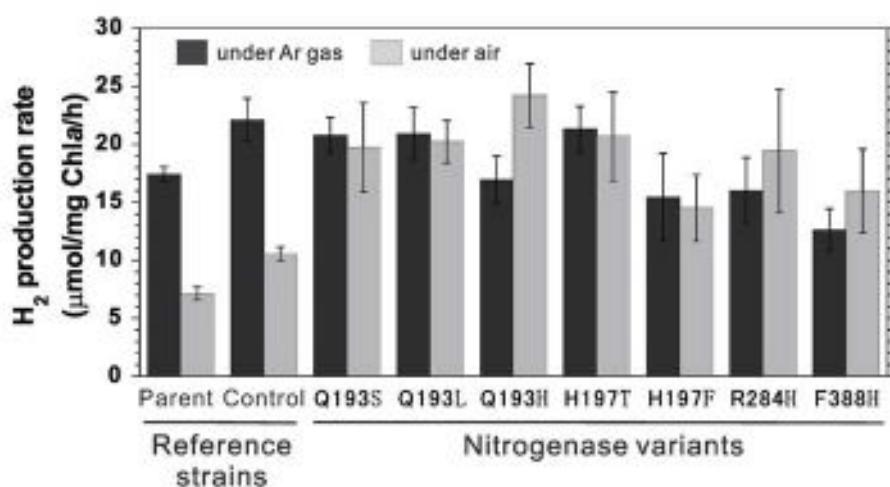


Fig. 2 *In vivo* H₂ production activity of the several nitrogenase active site variants under Ar or air

■ Research Introduction

Tenure-track Associate Professor

Yoshihiro Yamaguchi

2005 Graduate School of Agricultural Science,
Tohoku University
2006 Postdoctoral fellow, Rutgers University.
2010 Research and teaching specialist III, Rutgers University.



Why bacteria contain many TA systems?

Regulation of cell death is essential for living organisms. In the eukaryotes, apoptotic cell death is crucial for developmental process, for maintenance of normal cell homeostasis as well as for elimination of cells damaged by stress or pathogen infection. In bacteria, regulation of cell growth and cell death is also important under various stress conditions. Almost all bacteria contain genes encoding a small toxin protein consisting around 100 amino acid residues, which targets various cellular processes including DNA stability, DNA replication, mRNA stability, protein synthesis, cell-wall biosynthesis and ATP synthesis to inhibit cell growth (Figure 1)^{1,2)}. In normally growing cells, however, these toxins are co-expressed from an operon with their cognate antitoxins forming a stable toxin-antitoxin (TA) complex so that the toxicity of the toxins cannot be exerted. This growth-regulatory system is called a toxin-antitoxin (TA) system.

The TA systems are not essential for normal cell growth, nevertheless they are widely prevailing in bacteria and archaea. This fact suggests that they may have been playing subtle, advantageous roles for cells to survive in their natural habitats, for example, in cellular adaptation to ever changing natural habitat by either reducing the growth rate or completely inhibiting cell growth or leading some cells to die. A TA system was first identified on a plasmid, which plays an essential role in plasmid maintenance. When a plasmid encoding the TA system is lost from a cell, the toxin is released from the existing TA complex, since the antitoxin is more unstable than toxin. This results in cell growth inhibition and eventual cell death. Since this discovery, a number of different TA systems have been identified to be encoded from bacterial genomes. The toxins from these TA systems are proposed to be associated with various cellular processes in regulating gene expression, bacterial population

control and programmed cell death.

Until now, there are about 50 different types of TA systems have been found in bacteria. It is interesting to note that *Mycobacterium tuberculosis* contains more than putative 80 TA systems, while its nonpathogenic counterpart, *M. smegmatis* has only two putative TA systems. It seems that the number of TA systems may be related to the pathogenicity of *M. tuberculosis*, and the TA systems in this pathogen may play important roles in the maintenance of the extremely long dormancy of this bacterium inside macrophage. On the basis of this consideration, it is perhaps surprising that *E. coli* also contains comparatively a large number of TA systems.

Since these TA systems have likely been maintained through the evolution, one may speculate that each one of them might have played a positive role in survival of the bacterium under harsh natural environments over those cells which had fewer or no TA systems. Characterization of these TA systems, including the identification of their cellular targets, the mechanisms of their toxic actions and the regulatory mechanisms of their expression, therefore, is essential for our understanding of the roles of the TA systems in bacterial physiology, pathogenicity and evolution. Among the cellular targets for TA systems, mRNA is the most frequently used target for toxins.

MazF from *E. coli* cleaves mRNA at ACA sequences to effectively inhibit protein synthesis. Since then, a large number of MazF homologues have been identified from bacteria to archaea, and some bacteria have more than one MazF homologues. MazF homologues so far identified specifically cleave mRNAs at either three-, five- or seven-base recognition sequences. It is not clear at present why these cleavage sites consist of only odd base numbers, but it is interesting to see how the RNA cleavage site expands

by two bases at a time from three-base sequences to five- and to seven-base sequences. The RNA recognition and cleavage specificity appears to have been extended mostly at the 3' -end site, which is particularly evident when the *E. coli* MazF cleavage site (A^{CA} or ^ACA) is compared with the cleavage sequence of the MazF homologue (MazF-hw) from a superhalophilic archaea, *Haloquadratum walsbyi* (UU^AACUCA)³⁾. It seems that more space is available on the MazF-dimer interface for RNA substrate binding, which allows it to extend the recognition specificity at the 3' -end⁴⁾. As the recognition sequences become longer, the target mRNAs become more limited so that MazF homologues cleave more and more specific mRNA species in the cells. MazF-hw, a 7-base cutter from a coastal brine pool on the Sinai Peninsula in Egypt seems to specifically cleaves the rhodopsin transcriptional activator as its mRNA contains unusually as many as three of the 7-base sequences³⁾. It should be noted that in comparison with DNA restriction enzymes, which require a palindromic sequence for cleavage, the number of mRNA interferases, which may be called mRNA restriction enzymes, have potentially so much more, since they do not require any palindromic sequences for sequence recognition. For example, there are only maximum 64 DNA restriction enzymes for six-base sequences, while there are as many as 4096 (= 64 x 64) possible cleavage sites for single-stranded six-base RNA. Although we have found a 7-base specific mRNA interferase cleaving only one out of a total of 16,384 different 7-base sequences, it remains to be elucidated how many of 7-base specific mRNA interferases actually exists in

nature. There could be only a few such enzymes. The cellular function of MazF homologues appears to be highly diverse from those which eliminate almost all cellular mRNAs such as ACA-specific *E. coli* MazF to those which are highly sequence-specific such as, MazF-hw that eliminate only a specific group of mRNAs in the cells³⁾. Recently, the X-ray structure of a UACAU-specific MazF homologue from *Bacillus subtilis* forming a complex with a 9-base long un-cleavable substrate RNA⁴⁾. The X-ray structure of the RNA-MazF complex provides insights into how a specific RNA sequence can be recognized by MazF. On the basis of the X-ray structure and other information, it is now possible to explore to design MazF homologues cleaving other RNA sequences or sequence-specific RNA binding proteins.

As mention above, the study of TA systems will open new avenue of bacterial physiology and biotechnology.

- 1) Yamaguchi, Y., J. H. Park & M. Inouye, (2011) Toxin-antitoxin systems in bacteria and archaea. *Annu Rev Genet* 45: 61-79.
- 2) Yamaguchi, Y. & M. Inouye, (2011) Regulation of growth and death in *Escherichia coli* by toxin-antitoxin systems. *Nat Rev Microbiol* 9: 779-790.
- 3) Yamaguchi, Y., H. Nariya, J. H. Park & M. Inouye, (2012) Inhibition of specific gene expressions by protein-mediated mRNA interference. *Nat Commun* 3: 607.
- 4) Simanshu, D. K., Y. Yamaguchi, J. H. Park, M. Inouye & D. J. Patel, (2013) Structural basis of mRNA recognition and cleavage by toxin MazF and its regulation by antitoxin MazE in *Bacillus subtilis*. *Mol Cell* 52: 447-458.

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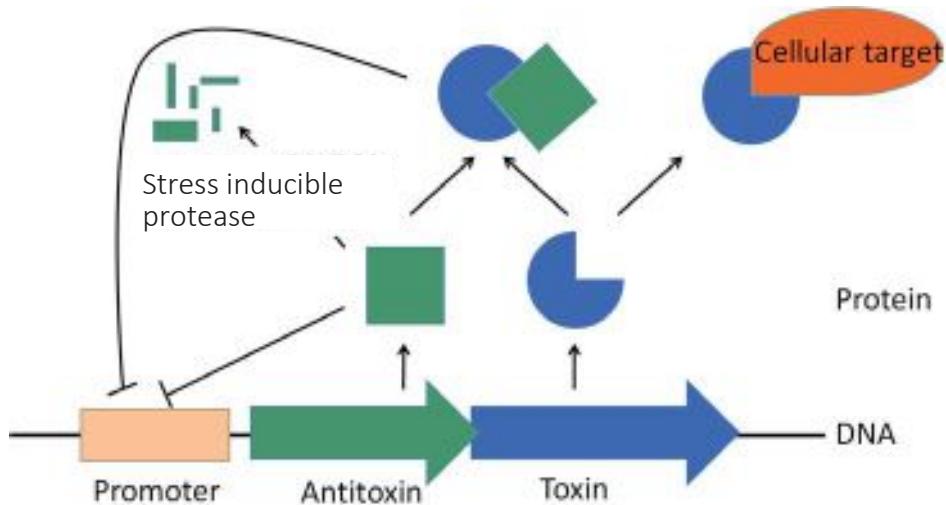


Figure 1. TA systems

■ Research Introduction

The OCU Advanced Research Institute for Natural Science and Technology (OCARINA)
Special-appointment lecturer

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July/2011 Division of Material Science (Physics), Graduate School of Science, Nagoya University Doctoral course
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"Glass leaf" which enables artificial photosynthesis under aerobic condition

Leaves convert carbon dioxide and water to organic substance and oxygen, at room temperature, atmospheric pressure, under an oxygen atmosphere with high efficiency. A problem that is inhibited by oxygen should be overcame to achieve the material conversion reaction (artificial photosynthesis) under aerobic condition. A light-induced electron transfer system combined with a photosensitizer, an electron mediator and catalyst is required to synthesize hydrogen or as formic acid carrier of hydrogen energy by sunlight energy (Fig. 1(a)). This panel shows a standard light-induced hydrogen production system. Photosensitizer, electron mediator and hydrogen evolution catalyst are Ru(bpy)₃²⁺, MV²⁺ and hydrogenase, respectively. Under anaerobic condition, effectual light-induced hydrogen was detected (□ in Fig. 1 (b)). On the other hand, under aerobic condition, the efficiency was decrease to parts per thousand (■ in Fig. 1 (b))). This result indicates that electrons necessary for hydrogen evolution were deprived by oxygen as shown red arrow in Figure 1(a). However, we have found that an effective hydrogen evolution reaction occurs even in aerobic condition by introducing the light-induced hydrogen evolution system in the pore of 50 nm in the porous glass plate. Light-induced hydrogen production efficiency of porous glass system was increased to 3300 times of solution system (● in Fig 1 (b)). This result indicates that photoreaction is faster than inflow of oxygen to inside pore (Fig. 1 (c)).

In OCARINA, I want to clarify the reaction mechanism that is specific reactions achieved inside porous glass. Furthermore, I want to create a new artificial photosynthesis system that has an electron source water "glass leaf (Fig. 1 (e))" driving under an oxygen atmosphere by immobilizing light-harvesting system, photosystem I, II of photosynthetic organisms and various

catalysts.

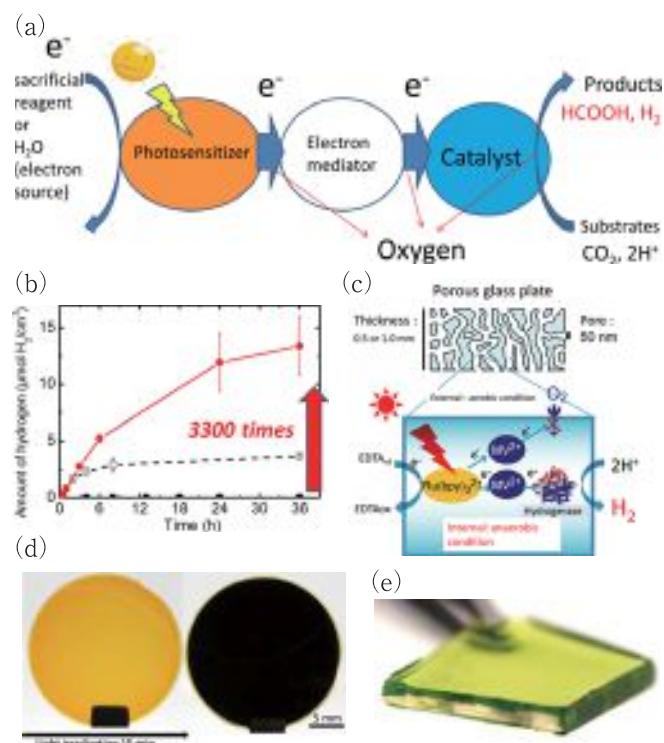


Fig.1(a) Overview of solar material conversion reaction and the inhibition by oxygen. (b) Light-induced hydrogen production by solar simulator. Porous glass plate system under aerobic condition (●). The solution system under anaerobic (□) and aerobic conditions (■). (c) Overview of Photoreaction inside porous glass plate (pore diameter of 50 nm, thickness of 0.5 mm). The flow rate of oxygen is reduced at the interface of the external solution and glass nanospace. The center in the porous glass becomes low oxygen concentration condition by photoreaction. Them, the hydrogen generation reaction is achieved. (d) Porous glass plate immobilized Ru(bpy)₃²⁺ and MV²⁺. Color o glass was changed to black by accumulation of MV²⁺. (e) Porous glass plate of green immobilized photosystem I.

■ Facility Introduction

Research Center for Artificial Photosynthesis Analysis Equipment

<Facility Overview>

Research Center for Artificial Photosynthesis(ReCAP) at OCU, established in June 2013, is a collaborative research institute equipped with analysis equipment. The sophisticated equipment within this facility was installed for the purpose of making a significant contribution to the vitalization of the industry through the provision of equipment and expertise, as well as advancing research within the university. The facility is equipped with a nuclear magnetic resonance apparatus, an ion cyclotron resonance mass spectrometer, and an X-ray diffractometer, etc. With precise and rapid analysis performed by technical staff using advanced equipment, ReCAP can respond flexibly to the needs of users. As a shared facility, the center also provides services such as entrusted measurement, training of machine users and consultation related to special measurement.

Nuclear magnetic resonance apparatus

AVANCE III NMR (for liquids) with 600MHz cryoprobe
(Bruker Biospin Inc.)



General Performance:

The machine determines the structure of organic compounds by detecting the structure and state of materials by means of nondestructive nuclear spinning. It can hasten spatial structure analysis performed in water solutions of protein molecules.

Features:

Since the machine is equipped with a cryoprobe, it has a higher sensitivity compared with other conventional 600MHz machines. Spatial structure analysis of low molecular weight organic compound solutions (molecular weight 1,000 or less) can be performed.(However, only proteins in which substitution of carbon cores and nitrogen cores has been conducted by means of a biochemical method, and with a molecular weight of 20,000 or less can be analyzed.)

Ion cyclotron resonance mass spectrometer

(FT-ICR-MS, MALDI, Solari X-OCU with an ESI source,
Bruker Daltonics Inc.)



General Performance: This machine accurately measures the mass of ionized material to determine the atomic composition, and has the highest resolution among other mass analyzing machines.

Features:

With extremely high mass resolution (error of 1ppm or less), this machine only requires accurate mass number information to identify molecular formula, making it very powerful in determining the structures of unknown compounds. This machine includes not only an ESI source but also the ground-breaking MALDI source, which increases the ionization rate of protein peptides. During protein analysis it is also possible to perform primary structure analysis (amino acid sequence analysis) by means of the fragmentation technique, using the FT-MS. This machine can conduct not only typical amino acid sequence analysis (where proteins are identified using a database of known sequences) but also de novo sequence analysis, which determines unknown amino acid sequences using the mass number, obtained through the extremely high-precision measurement of mass.

Single Crystal X-ray Diffractometer

(XRD, R-AXIS VII, Rigaku Corporation)



General Performance:

X-rays are projected from a table-top X-ray generator into the crystal structure of the target material, and the diffracted patterns are analyzed by the X-ray detector. Amino acid residues of the target protein is identified and its structure is clarified at the atomic level.

Features:

Protein crystal structures are measured and analyzed, using X-ray diffraction. Since this machine can irradiate high flux X-ray beams, the measurement of smaller crystal structures, which was difficult using conventional machines equipped in ordinary laboratories, is now possible. In addition, the detector reading time has been improved, increasing measurement efficiency.

Inquiries:

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■ Activity Report

The 2013 OCARINA Annual International Meeting

Organizer

The OCU Advanced Research Institute for Natural Science and Technology (OCARINA)

Sponsor

The OCU Strategic Key Research Project
COI-T Next Generation Hydrogen Energy Society Realization Project

Special Speaker

Harue Masuda (Professor, Advanced Research Institute for Natural Science and Technology / Department of Geosciences, Faculty of Science, OCU)

Guest Speakers

Tim Storr (CA/ Associate Professor, Simon Fraser University)

Rudi Berera (NL/ PD, Vrije Universiteit Amsterdam)

Yasushi Nishihara

(Professor, Department of Chemistry, Faculty of Science, Okayama University)

Masaki Ihara

(Assistant Professor, Project Research Promotion Center, Faculty of Agriculture, Shinshu University)

Ryutaro Tokutsu

(Assistant Professor, Division of Environmental Photobiology, National Institute for Basic Biology)

Yasuhiro Funabashi (Professor, Graduate School of Science, Osaka University)

In-house speakers

Taka-aki Asoh (Specially Appointed Lecturer, Tenure Track Project of the Advanced Research Institute for Natural Science and Technology)

Yasuyuki Tsuboi (Professor, Advanced Research Institute for Natural Science and Technology/ Department of Chemistry, Faculty of Science)

Masato Miyata (Professor, Advanced Research Institute for Natural Science and Technology/ Department of Biology, Faculty of Science)

Yoshikazu Nishikawa (Professor, Advanced Research Institute for Natural Science and Technology/ Graduate School of Human Life Science)



The 2013 OCARINA Annual International Meeting was held in the Osaka City University Media Center on the 4th and 5th of March, 2014.

The Advanced Research Institute for Natural Science and Technology has held annual meetings regularly since 2008, and this was the fifth international symposium. Harue Masuda, a professor from OCU, presented her research report on the mechanism of arsenic pollution as a special lecture, and six other guest speakers (two from overseas) and four in-house speakers gave presentations on their research reports from various fields.

At the student poster presentation, 30 posters were presented during the tight schedule of the symposium. The meeting finished a success.

The 1st Osaka City University Tenure Track Teachers Research Presentation Event



Date: Wednesday, December 3, 2014 Venue: Osaka City University Media Center

Presenters: The collaborative graduate school is shown in <>

Satoshi Endo (Specially Appointed Associate Professor, Tenure Track Project of the Urban Research Plaza)
<Graduate School of Engineering>

Taka-aki Asoh (Specially Appointed Lecturer, Tenure Track Project of the Advanced Research Institute for Natural Science and Technology)
<Graduate School of Science>

Cai Kai (Specially Appointed Associate Professor, Tenure Track Project of the Urban Research Plaza)
<Graduate School of Engineering>

Yoshihiro Yamaguchi (Specially Appointed Associate Professor, Tenure Track Project of the Advanced Research Institute for Natural Science and Technology)
<Graduate School of Science>

Eriko Nagadai (Specially Appointed Associate Professor, Tenure Track Project of the Advanced Research Institute for Natural Science and Technology)
<Graduate School of Human Life Science>

OCARINA Seminars

The Advanced Research Institute invites leading scientists from Japan and abroad to its occasional OCARINA seminars. In addition to the regular seminars, guests are also invited to a series of major seminars known as “DISCO Parties” (see note*). Also convened as OCARINA seminars, with students forming the main constituent, DISCO Parties provide a common place for people with various occupations, including teaching staff, to discuss students’ research

17th	Date	February 3rd, 2014	Venue	Faculty of Science Conference Room
	Guest	Jitendra K. Bera (Professor, Indian Institute of Technology Kanpur)		
	Theme	Bifunctionality in Organometallic Catalysis		
18th	DISCO Party major seminar No. 7	Student: Keisuke Kawamoto (OCU Kinoshita Laboratory D2)		
	Date	June 18, 2014	Venue	Building 2, 220A
	Guest	Yuzuru Yoneda (Professor, Radiocarbon Dating Laboratory, The University Museum of the University of Tokyo)		
	Theme	Human evolution from the viewpoint of the ecology of eating		
19th	DISCO Party major seminar No. 8	Student: Shusaku Ikeyama (OCU Amao Laboratory D1)		
	Date	September 19, 2014	Venue	Building 2, 220B
	Guest	Kentaro Ifuku (Assistant Professor, Laboratory of Molecular and Cellular Biology of Totipotency, Graduate School of Biosciences, Kyoto University)		
20th	Theme	Development of foundation technology for the production of diatom in biofactories		
	Date	September 22, 2014	Venue	Building 2, 220A
	Guest	Makiko Kosugi (Specially Appointed Staff of the National Institute of Polar Research, Research Assistant in the Department of Biological Sciences, Faculty of Science and Engineering, Chuo University)		
21st	Theme	Ecological study of photosynthetic organisms living on the bedrock outcrop in the vicinity of Showa Station in Antarctic		
	Date	November 19, 2014	Venue	Building 2, 220B
	Guest	Nicholas Cox (Ph.D, Group leader, Max Planck Institute for Chemical Energy Conversion, Germany)		
21st	Theme	The reaction mechanism of water decomposition and oxygen evolution based on recent analysis using the photosystem II electron spin resonance method		

See the website for details.

* Doctor Course students' Incorporated Scientific Communication (DISCO) Party:

A group of doctoral students affiliated with OCARINA organizes a weekly closed mini seminar just for students, to serve as an opportunity to deepen their understanding of each other's work and promote mutual development. In addition to this, a major seminar is held several times a year.

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The first Osaka City University (OCU) Tenure-track Staff Research Presentation Event was held in the OCU Media Center on 3rd of December, 2014, in which five tenure-track staff who were selected through the public appeal made presentations on their research and received advice from invited domestic specialists in each field. This event was organized to provide an opportunity for young staff to develop their research by receiving comments from specialists. Three tenure-track staff members from OCARINA made presentations.

Michio Miyano, Vice president of OCU, made an opening address and introduced activities for the promotion and development of the tenure-track project. Presenters were encouraged through warm support and constructive criticism, and lively specialist discussions were conducted.

Isamu Kinoshita, Director of OCARINA, made a closing address giving his encouragement and expectations for future activities, and a social gathering was held.

The Media Center was almost filled to capacity, with 73 attendants from in-house and other facilities attending.



History of the Osaka City University Advanced Research Institute

2008	March	Founding Anniversary International Symposium held
	April	The OCU strategic key research project (2008-2011) started
	December	International Workshop held on the efficient use of sunlight energy
2010	March	1st International symposium held
	March	Enforcement of official regulations (start of activities as an official bureau)
	October	Building 2 renovated for research floors of OCARINA
	November	Opening symposium for building 2 of OCARINA held
	December	2nd International symposium held
2011	March	3rd International symposium, "Kakuno memorial," held
2012	March	Annual meeting and the OCU strategic key research project (2008-2011) debriefing held
	April	The OCU strategic key research project (2012-2014) started School of
	July	Science Building C completed, partial occupation
2013	March	4th International symposium held
	April	2 new full-time staff members appointed
	June	Research Center of Artificial Photosynthesis opened
2014	February	Partial occupation for the new School of Science Building
	February	One new full-time tenure track staff member appointed
	March	One new full-time tenure track staff member appointed
	March	5th International symposium held
	April	The first Osaka City University Tenure Track Teachers Research Presentation Event



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