

BUILDING AN INTERNATIONAL NETWORK EXCHANGE PROGRAM OF EDUCATION AND RESEARCH FOR GRADUATE COURSE STUDENTS IN LIFE SCIENCE AND BIOTECHNOLOGY

Tadao OIKAWA*, Shinichi UESATO**, Hiroshi TAMURA***, Hidehisa KAWAHARA**, Yasuo NAGAOKA**, Koji SHIMOKE**, Tetsuaki TSUCHIDO**, Lothar EGGELING****, Volker F. WENDISCH†, Jochen BÜCHS††, Ratana RUJIRAVANIT†††, Nazalan NAJIMUDIN††††, and Chan Lai KENG††††

(Received October 2, 2007)

Abstract

We proposed an “International Network Exchange Program of Education and Research for Graduate Course Students in Life Science and Biotechnology” to the Kansai University Special Research Fund 2007 in order to promote international student exchange in the Graduate School of Engineering. In this program, we have successfully sent 8 graduate students to study for about a month in Malaysia, Thailand, Indonesia, and Germany. We hope the result of our efforts will promote an acceleration of international student exchange in the Graduate School of Engineering.

1. Purpose of this project and the activities of the German-Japanese bilateral student exchange program

Kansai University has two big problems with the school regulations promoting an international student exchange program in the graduate school of engineering. Firstly, students must study abroad for one semester, which is too long for students, since it means that they must remain in the same class for another year. Secondly, students must enroll for an extra year in Kansai University in order to study abroad. Therefore, only second-year master course students can participate. Generally, they are very busy looking for a job doing the second year of the master course, so they have almost no chance to study abroad under the present school regulations. Therefore, during the past 10 years, only one student of the

* Correspondence to: Tadao Oikawa. E-mail: oikawa@ipcku.kansai-u.ac.jp

** Department of Life Science and Biotechnology, Faculty of Chemistry, Materials and Bioengineering, Kansai University, Suita, Osaka 564-8680, Japan

*** Department of Chemistry and Materials Engineering, Faculty of Chemistry, Materials and Bioengineering, Kansai University, Suita, Osaka 564-8680, Japan

**** Institute for Biotechnology 1, Forschungszentrum Jülich, D-52428 Jülich, Germany

† Institute of Molecular Microbiology and Biotechnology, Westfalian Wilhelms University Münster, Corrensstr. 3, D-48149 Münster, Germany

†† Biochemical Engineering, RWTH Aachen University, Sammelbau Biologie, Worringerweg 1, D-52074 Aachen, Germany

††† Petroleum and Petrochemical College, Chulalongkorn University, Soi Chulalongkorn 12, Phayathai Road, Pathumwan, Bangkok 10330, Thailand

†††† School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia

Graduate School of Engineering has studied abroad. To promote an international student exchange program in this Graduate School of Engineering, we proposed an “International Network Exchange Program of Education and Research for Graduate Course Students in Life Science and Biotechnology” receiving a grant from the Kansai University Special Research Fund 2007. In addition to 7 researchers in Kansai University, 6 foreign researchers joined and admitted the students of Kansai University to study in their laboratories. Under this program, the members of this program in Kansai University successfully sent 8 graduate students to study for about a month in Malaysia, Thailand, Indonesia, and Germany. We hope the result of our efforts will serve to further accelerate the international student exchange program in the Graduate School of Engineering.

In this program, Associate professor Tadao Oikawa (the Representative of this program) organized the researchers and was mainly responsible for the German-Japanese bilateral student exchange program. Dr. Oikawa sent 3 graduate students (Kenta Shirai, Osamu Kato, Ikuo Muraoka) from his laboratory to Forschungszentrum Jülich, RWTH Aachen University, or University of Münster, Germany. Dr. Oikawa has collaborated with Dr. Lothar Eggeling, Forschungszentrum Jülich since 2001. When Dr. Oikawa was in Jülich as a visiting scientist, he belonged to Dr. Eggeling's laboratory and was treated very well. Recently, Dr. Eggeling came to Kansai University to give a research lecture. Even after Dr. Oikawa returned to Japan, they maintained close relations and published several papers together about the acetyl-CoA decarboxylase, *Corynebacterium glutamicum*^{1, 2)}. *C. glutamicum* is an important microorganism that produces various amino acids, especially L-glutamate, a raw material for the well-known “Ajinomoto” flavoring. Mr. Kenta Shirai stayed in Dr. Eggeling's laboratory from August 1 to August 31, 2007 to study the construction of the deletion mutant of *C. glutamicum* (Fig.1). This was his first time to visit a foreign country, but he adapted easily to the German research environment and obtained a fruitful result. Mr. Osamu Kato stayed in the laboratory of Prof. Volker F. Wendisch, University of Münster, from August 1 to August 31, 2007. Prof. Volker F. Wendisch is another Dr. Oikawa's German friends: the two first met in Jülich in 2001 (Fig. 2). Prof. Wendisch has been studied microarray experiments on the *C. glutamicum* gene expression. Microarray analysis is one of the most advanced technologies in the field of Biotechnology. Mr. Kato was privileged to undertake high level experiments there that he cannot do at Kansai University. In addition to the research, Kato made many German friends and broadened his experience. Prof. Jochen Büchs of RWTH Aachen University has been one of Dr. Oikawa's German collaborators since 2002. They are studying the development of two-phase (gas/liquid) reaction systems with Dr. Oikawa's thermostable alcohol dehydrogenase from *Flavobacterium frigidimarum* KUC-1 to produce a chiral alcohol. Prof. Büchs came to Kansai University to give a research lecture in 2003. Dr. Oikawa accepted his diploma student, Mr. David Ullisch, as an exchange-research student of Kansai University in 2005. Dr. Oikawa subsequently sent one of his graduate students, Ikuo Muraoka, to study at RWTH Aachen University under this international network exchange program. Based on such individual efforts, our Graduate School of Engineering now is planning to establish an efficient student and researcher exchange program with RWTH Aachen University.

(Tadao Oikawa, Lothar Eggeling, Volker F. Wendisch, and Jochen Büchs)



Fig. 1 Dr. Lothar Eggeling, Dr. Tadao Oikawa, Kenta Shirai, and members of the Institute for Biotechnology 1, Forschungszentrum Jülich, Germany

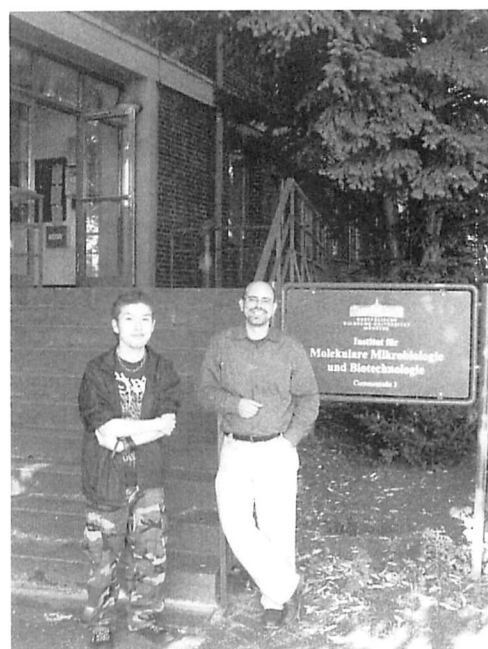


Fig. 2 Prof. Volker F. Wendisch and Osamu Kato in front of Institute of Molecular Microbiology and Biotechnology, Westfalian Wilhelms University Münster, Germany

2. Collaborative research between research groups from Kansai University and Universiti Sains Malaysia on exploration of anti-virus agents from natural resources

Some viral infectious diseases are spreading worldwide and threaten our daily life. For example, human cytomegalovirus infection (HCMV) causes serious diseases, especially for immuno-compromised patients. Additionally, new avian influenza viruses have appeared in many countries and are becoming a serious problem worldwide. A new mutation of the avian influenza virus could bring on new influenza subtypes, which might spread from human to human, leading to an influenza pandemic outbreak. Now, we are already at a high level of risk. Moreover, human immunodeficiency virus (HIV) threatens to cause death amongst AIDS patients, and this infection is rapidly increasing worldwide.

Human cytomegalovirus (HCMV) does not significantly influence the health of persons in normal health. However, its infection sometimes causes serious diseases, including retinopathy and acute hepatitis for immuno-compromised patients, such as those with AIDS, and organ-transplanted patients. Although DNA polymerase inhibitors such as ganciclovir (GCV) and foscarnet are used for treatment of HCMV, their increased and prolonged use has led to the emergence of a resistant virus, which is dangerous for immuno-compromised patients. Thus, we need to develop new antiviral drugs possessing a different mechanism of action. Professor S. Uesato group paid attention to a benzylisoquinoline alkaloid, berberine chloride, and its related compounds, since papaverine hydrochloride of the same group was reported to exhibit anti-HCMV activity. Berberine chloride showed an anti-HCMV activity comparable to that of GCV by a plaque assay. Furthermore, berberine chloride most likely has an antiviral action mode

different from that of GCV.

Influenza is a disease with a high mortality rate. Despite efforts to develop effective vaccines and therapeutic agents against influenza virus infection, it is still virtually uncontrolled. Current vaccines against influenza virus infection are still limited, owing to the frequent conversion of viral antigens. Some neuraminidase inhibitors are effective in prophylaxis and therapy. Oseltamivir (Tamiflu) is an orally-active neuraminidase inhibitor which is used as an anti-influenza virus agent all over the world. However, it is said that oseltamivir might cause CNS and GI side effects in patients. Moreover, an oseltamivir-resistant type of influenza A virus (H5N1) has reportedly appeared in Vietnam. Therefore, new agents showing a synergistic antiviral activity with oseltamivir should be exploited to reduce its dose for the purpose of controlling such a resistant influenza A virus. Prof. Uesato research group assessed the effectiveness against influenza A virus of various natural drugs which were traditionally known to ease flu, and they were able to find some promising anti-influenza virus A agents in lignan series compounds. Notably, these compounds, unlike Tamiflu, did not produce a resistant virus. Additionally, a combination of lignan-G and Tamiflu at various concentration ratios produced a synergistic effect not only *in vitro*, but also *in vivo*. They are expected to be promising candidates as influenza therapeutic agents.

As has been demonstrated so far, the natural compounds still remain a treasury for creating of new medicines. Prof. Uesato therefore visited the laboratory of Prof. Chan Lai Keng, Universiti Sains Malaysia, on 11th June, 2007 to facilitate the research project of anti-HIV agents from natural resources, and discussed the possibilities with her of collaborative research (Fig. 3). She has developed a cell culture system which can produce medically useful secondary metabolites from medicinal plant species. Her group has already succeeded in producing the desired secondary metabolites from intact plants. As examples, *Coleus aronmaticus* cell cultures produce the material which forms abnormal Aedes larvae or pupae, and Senduduk (*Melastoma malabathricum*) cell cultures produce a beautiful pigment. Prof. Chan Lai Keng is also carrying out cell culture of *Eurycoma longifolia* and *Artemesia annua*, both producing anti-microbial activities. Prof. Uesato group has a plan to start fusion assay of CD4 expressing HeLa cells and gp160 expressing HeLa cells to screen anti-HIV compounds in these cultured cells.

(Shinichi Uesato and Chan Lai Keng)



Fig. 3 The members of Kansai University who attended the inaugural lecture by Professor Chan Lai Keng. The picture was taken in the lobby of the main Hall at Universiti Sains Malaysia

3. The graduate students in Chulalongkorn University

Two graduate students of Dr./Prof. Tamura's laboratory visited the Petroleum and Petrochemical College, Chulalongkorn University from August 5th to September 6th 2007 under the guidance of Associate Professor Ratana Rujiravanit (Fig. 4). The two have collaborated with Dr. Ratana for eight years in the polysaccharide field and are on visiting terms with each other. Dr. Ratana got her PhD degree at Hokkaido University, so that she can speak Japanese very well^{3, 4)}. Therefore, there was no problem in sending her two Japanese students even though Tamura could not accompany them. In addition, they all attended the 2nd International Conference on Advances in Petrochemicals and Polymers in Bangkok in June, 2007. The students could set up their apartment on this occasion and experienced the atmosphere of the country.

However, the students experienced a lot of things, being in Thailand for about a month. They were confused by the different style of the laboratory management, as compared with Japan. They were happy to get their individual experimental table, however, they had to rent every all other equipment, because PhD students in Thailand have their own stuff.

One of the graduate student introduced unique spinning technique. He struggled to set up the spinning system and to gather the required stuff. Most items were rather different from those available in Japan. But he felt that explaining the fundamental principal was important, anyway. He was delighted to act as intermediary between the two laboratories. Dr. Ratana's group will now continue extend this tresarch using their own resources. Another graduate student tried to prepare a new ointment using chitin hydro-gel and plant extract from Thailand. We decided to continue this research as collaborate research, because there are several varieties of plant extract to test. These two graduate students contributed very much toward the future collaboration research.

Two graduate students had a chance to visit industrial firms in Thailand, Ma-Ma factory and Thai Negoro. They are the Thailand branches of Japanese companies, the former, a noodle factory, and the latter, a textile company. It was a good opportunity to earn something in the Industry. In addition, they visited Chao Phya Abhaibhubejhr Hospital, an advanced medical

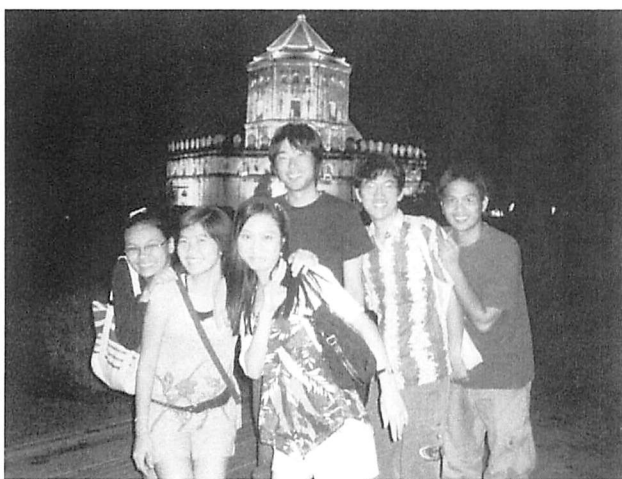


Fig. 4 Students visited the Santi Chai Prakan Pavilion and Public Park in Bangkok

center that is able to provide holistic care and apply to use traditional methods and medicine to health care. This hospital also undertakes researches into the traditional herbal productions. Visits to these places provided graduate students with much useful information. These two graduate students forged a good relationship with Thailand students. In the laboratory, the Thailand students were very kind and looked after visitors well. They traveled together to several places at every weekend.

At the begging of December, Prof. Tamura will accept two PhD students from Dr. Ratana's laboratory, based on the exchange program established between the two universities. The Japanese graduate students should make fine arrangements for them. The Kansai University Special Research Fund 2007 has giving an opportunity to the graduate students to visit Chulalongkorn University and enable us to get to know each other.

(Hiroshi Tamura and Ratana Rujiravanit)

4. Collaborations of the Laboratory of Microbial Technology at Kansai University and the School of Biological Sciences at USM

The research exchange between the Department of Biotechnology, Faculty of Engineering at Kansai University and the School of Biological Sciences at USM started in 2004. In 2005, the Laboratory of Microbial Technology at Kansai University undertook two joint international research projects into the antifreeze protein of the yeast, which was isolated in a sample from the Antarctica, together with Prof. Nazalan's groups, and into the research of tropical lichens in Malaysia, with Prof. Chang's groups. Dr. Kawahara visited both professor's laboratories in March, 2006 for a full-dress collaboration meeting and the observation of tropical lichens. As a result, Kawahara's laboratory began a collaboration with both laboratories. In this program, Sakura Higa, who is a master course student in Kawahara's laboratory, was exchanged for a student belonging to Prof. Nazalan's laboratory. Also, observation and collection of lichens in Penang was done by a group with Prof. Chang and Prof. Yamamoto, who is a president of the Japan Society for Lichenology and a professor at Akita Prefectural University.

AFP is a group of ice-binding proteins that inhibit the growth of ice. They have antifreeze properties show a thermal hysteresis value (TH), which indicates depression of the freezing-point, and ice recrystallization-inhibiting (RI) activity in the frozen state over -10°C. Since this AFP have found in the blood plasma of an Antarctic notocenoid, AFPs have been found in fishes, plants, fungi, lichens and bacteria that can grow at low temperature. However, there are no reports of AFP producing yeast.

Dr. Nazalan's group in USM has found in Antarctic psychrophilic yeast some genes having high homology with the AFP gene. As they do not have an assay system for AFP activity, they could not confirm such AFP activity in the recombinant AFP of this homologous gene. So, the measurement of this AFP activity using RI assay with 96-well microplate and the purification of this recombinant AFP are two of the important objectvies of our collaboration

Sakura Higa and Dr. Kawahara had a discussion of each other's studies and the assay system for the AFP activity using RI assay. During her two weeks stay at USM, Higa had demonstrated this assay system using a positive sample of the recombinant AFP of the AFP gene from the Antarctic bacterium, *Flavobacterium xanthum*, and checked the AFP activity of recombinant AFP of other gene from the Antarctic yeast (Fig. 5). Regrettably, this



Fig. 5 Prof. Nazalan, his students and Sakura Higa

recombinant AFP of the gene from Antarctic yeast showed no RI activity using the RI assay system. There are several possible reasons for this negative result: 1) that recombinant AFP is a protein having no AFP activity; 2) that as the recombinant AFP was not in a conformation state that can express a high level of AFP activity, the RI assay system with the 96-well microplate could not confirm such a low level of AFP activity. Now both Prof. Nazalan's group and Kawahara's group are researching in consort about the AFP gene expressions of Antarctic microorganisms.

During Kawahara's stay at USM on this program, he had some chance to observe and collect tropical lichen in Malaysia. Kawahara is one of co-supervisor of Mr. Lim Choon Leng, a master course student belonging to the laboratory of Prof. Chang Lai Keng at USM. Mr. Leng has joined the International Symposium on Science and Technology at Kansai University in 2007 and gave a presentation in the student section of this symposium.

Lichen is a symbiotic organism, between green algae and fungi, that is classified into Ascomycota or Basidiomycota. Green algae provide food for the fungi in lichen: glucose becomes the carbon source through photosynthesis. On the other hand, the fungi in lichen provide the green algae's habitation. Lichen can grow in some extreme environments such as the Antarctic, or deserts etc. Our groups took a field trip to Seberang Perai & Bukit Mertajam as the forest parks to collect tropical lichens (Fig. 6). Although Prof. Yamamoto couldn't identify some of these tropical lichens by eye alone judgment, we could confirm that almost all of the lichens isolated from these forest parks near Butterworth are the typical genus that belonging to foliose or crustose lichen. The group went around Penang island to make this

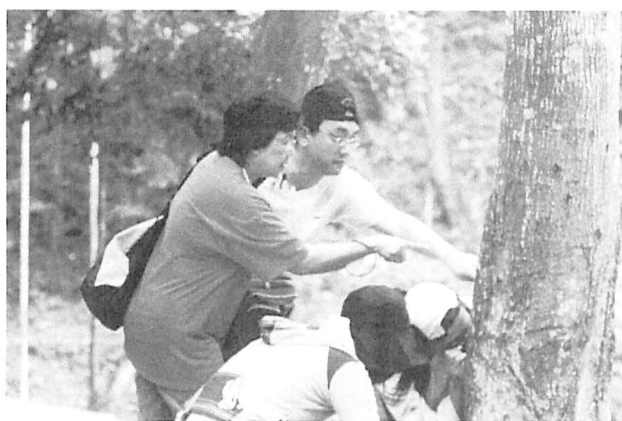


Fig. 6 Observation of tropical lichen

collection (44 samples). Among them collection, on the west coast of Penang we found fruticose lichen, which is a normal genus in the tropical region. Now Prof. Yamamoto is identifying the genera of tropical lichen in this collection using analysis of their chemical compounds. In future, Mr. Lim Choon Leng will try to separate the fungi and green algae from the lichen and then culture only the fungi in various media.

This international exchange between the two laboratories could prove very benefit for our study. Their hospitality during our stay was much approved for our exchanges in the future.

(Hidehisa Kawahara, Nazalan Najimudin, and Chan Lai Keng)

5. KU-USM collaboration based on the International Exchange Program —Explorative study of hair growth stimulants in plants from Malaysia—

Our collaboration work with Prof. Chan Lai Keng from the Universiti Sains Malaysia (USM) began on her first visit to Kansai University (KU) in 2004, when the first joint seminar was held between our department and the School of Biological Sciences, USM. The USM is located on Penang Island on the west coast of the Malay Peninsula. The USM is the largest science university in Malaysia with 23 schools, including Biological, Health and Medicinal Sciences. Since 1982 Prof. Chan has been actively involved in plant tissue and cell culture research there. Her research team comprises two research officers, eight Ph.D. and 12 M.Sc. students. They are divided into two main research groups. The first group mainly carries out research on the micropropagation of fruit trees, timber trees, ornamental and medicinal plants using various in vitro culture techniques. The main research activities of the second group are the production and optimization of useful secondary metabolites from the cell suspension cultures of various medicinal plants.

Based on her knowledge of the botanical field and our knowledge of medicinal chemistry, we joined in collaboration for an explorative study of bioactive compounds in plants from Southeast Asian countries, including Malaysia. Our first target was hair growth stimulants actively investigated in our Kansai laboratory. Prof. Chan had selected several kinds of local plants traditionally used as components of hairdressings. To try to discover natural products which induce hair growth, we examined EtOH extracts of such plants as *Eurycoma longifolia*, *Orthosiphon stamineus*, *Costus speciosus*, *Hibiscus rosasenensis*, *Gynura procumbens* and *Carissa carandas*. We applied the extracts in tests of *in vivo* anagen-phase-induction using C3H mice, and confirmed that extracts of the leaves of *Hibiscus rosasenensis* exhibits the most potent activity. We then applied further multi-solvent extraction to the leaves, successively using hexane, ethyl acetate and methanol. The extracts were examined in their growth-promoting activity on human epithelial keratinocytes, which have a close relation to hair follicle cells. The hexane extract showed the highest proliferative activity of the cells, up to 175% of control. These results indicate that the extracts of *Hibiscus rosasenensis* have a potent effect on hair growth. A further investigation to identify the active components in the extracts is in progress.

Encouraged by these auspicious results, our collaboration has expanded further to test the cultured cells rather than their mother plants. As part of this project, Aya Demizu, a graduate student of the masters course at KU, stayed two weeks from August 20, 2007 in Chan's laboratory to learn cell culture techniques (Fig. 7). This visit was supported by the Kansai

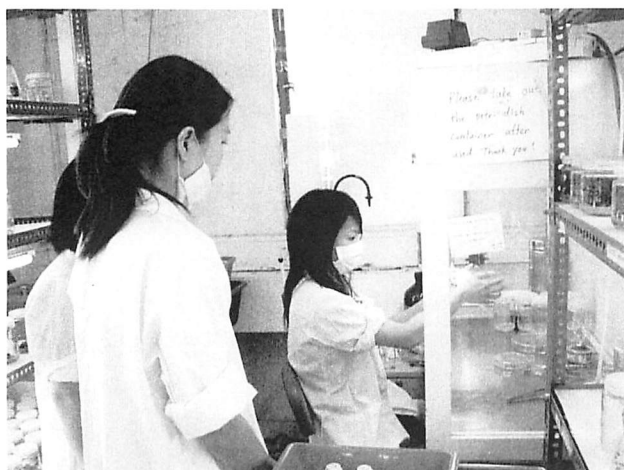


Fig. 7 Practicing of cell culture techniques
Instructor: Mrs. Tan Chee Leng, a
Ph.D. student at USM

University Special Research Fund. We are expecting further development of the collaboration between KU and USM in connection with the international exchange program for graduate students.

(Yasuo Nagaoka and Chan Lai Keng)

6. Education and research in Indonesia

Dr. Koji Shimoke visited both Indonesia University and Airlangga University together with a graduate student, Harue Sasaya, in June 2007. Ms. Sasaya had been interested in studying medicine in Indonesia, but we did not have any collaborators in Indonesia. Fortunately, Ms. Sasaya's mother (a native Indonesian) introduced some Indonesian medical doctors to me. Dr. Shimoke was thinking that he could cooperate with Indonesian scientists over a basic research in the field of neurodegenerative diseases. Dr. Shimoke has been studying basic neuropathophysiology, including creation of a new strategy for neurodegenerative disorders. Thus, during our stay, we began to discuss on the phone the idea of study with Indonesian medical doctors in both Universities. After further discussion, we agreed to study endoplasmic reticulum (ER) stress-induced apoptosis in the patients' brain of tropical diseases, because the molecular mechanisms of brain damage in tropical diseases had not yet been elucidated and Dr. Shimoke thought that there was a possibility to mediate ER stress-induced apoptosis in brain damage from tropical diseases. Fortunately, there is a marker protein, termed glucose-regulated protein 78 (GRP78) on ER stress. I and Indonesian collaborators hoped that we could discover the molecular mechanism (see Fig. 8).

Dr. Shimoke and Ms. Sasaya discussed details of the plan with Prof. Dr. Satyanegara at University of Indonesia on the first day of visit, and he kindly coordinated medical doctors in Airlangga University. Then, we visited Airlangga University to perform our plan of research. We were welcomed by many doctors, including Head of Hospital, Prof. Dr. Umar Kasan, Dean of Medicine, Prof. Dr. Muhammad Amin, Chairman of the Tropical Disease Center, Prof. Dr. Yoes Prijatna Dachlan, and many others. In order to explain the importance of this project, Dr. Shimoke gave a presentation to the Indonesian doctors on the background of ER stress-induced apoptosis (Fig. 9). Then, the research started according to our plan. However,

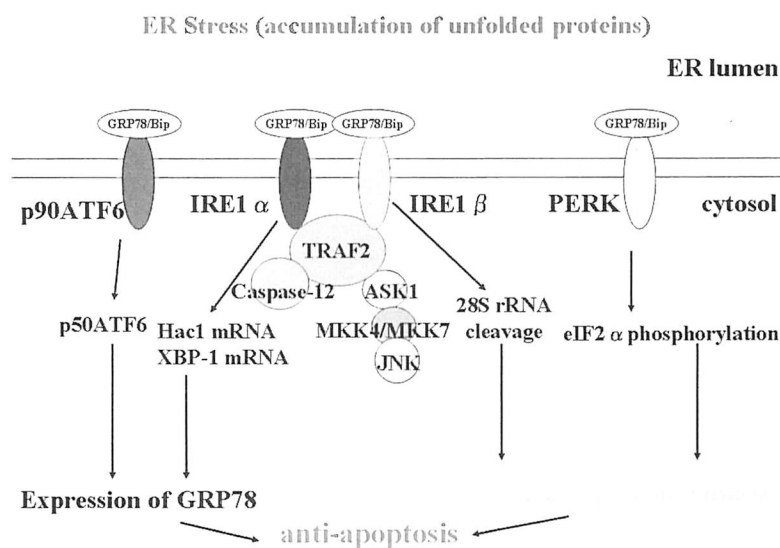


Fig. 8 Molecular mechanism of anti-ER stress-induced apoptosis

difficulties were encountered with the collection of human brain samples. Ms. Sasaya had successfully solved them and obtained the preliminary data during her stay.



Fig. 9 Presentation to Indonesian collaborators at Airlangga University

We wish to thank all doctors associated with this project for their kind cooperation. We will continue to study ER stress-induced apoptosis in the brains of patients with tropical diseases. We hope that we can maintain a good relationship with our Indonesian collaborators and thereby provide new knowledge about tropical diseases in the brain.

(Koji Shimoke)

7. Construction of an “Overseas Practicum” educational system for graduate course students and the International Student Exchange Program between Kansai University and Universiti Sains Malaysia

From 2007, the Graduate School’s Division of Engineering have started an educational program of “Overseas Practicum” for graduate course students. The purpose of this program

is to give the students a chance to conduct a collaborative work in science and technology under the supervision of academic staff in an overseas university, in order to learn new scientific information and experimental skills. This will enhance international communication and provide participants with contact with a different culture. The graduate course students enrolled in this program can get two to six credits for this, corresponding to a period of two to six weeks, mostly during the summer vacation, with additional pre- and post-visit training and workshops in their own countries.

The program committee consisted of a chairman, vice chairman, the educational chief of the Graduate School, three members of the university International Exchange Center, and two special guest professors. We decided to construct a system for sending students to foreign countries, as well as of recognizing credits based upon academic reports proposed not only by the students themselves, but also by their supervisors. In 2007, a total of five students went abroad: to Malaysia, Thailand, U.S.A., and Germany. Working abroad must be an invaluable experience for them.

Prior to the start of this program, in the middle of June, Prof. Shinichi Uesato, Assoc. Prof. Hidehisa Kawahara, and Assoc. Prof. Yasuo Nagaoka and Prof. Tetsuaki Tsuchido visited Penang in Malaysia to discuss with academic staff at Universiti Sains Malaysia about the official process for exchange of a memorandum and about the future plan for mutual international collaboration. The International Center at Kansai University then arranged for exchange of the memorandum on the new program between Kansai University Graduate School's Division of Engineering and Universiti Sains Malaysia's Biological Sciences Faculty. This memorandum is based on the basic agreement and the student exchange program agreement, both of which were exchanged in September 2006. Although the program in 2007 includes uni-directional sending of students from Kansai University to Universiti Sains Malaysia, in the future we wish to advance the program to a substantial student exchange between both institutes.

We would like to thank all the people who have been involved in the construction of this program. Although still at an immature stage, we have been able to set up a bridge of friendship between Kansai University and Universiti Sains Malaysia. May the future bring a more intimate collaboration.

(Tetsuaki Tsuchido, Chairperson of the Division of Engineering, Graduate School)

Acknowledgement

This special project was in part supported by a grant from the Kansai University Special Research Fund, 2007.

References

- 1) R. Gande, L. G. Dover, K. Krumbach, G. S. Besra, H. Sahm, T. Oikawa, and L. Eggeling, "The two carboxylases of *Corynebacterium glutamicum* essential for fatty acid and mycolic acid synthesis", *J. Bacteriol.*, 189, p.5257-5264 (2007).
- 2) R. Gande, K. J. C. Gibson, A. K. Brown, K. Krumbach, L. G. Dover, H. Sahm, S. Shioyama, T. Oikawa, G.S. Besra, and L. Eggeling, "Acyl-CoA carboxylases (accD2 and accD3) together with a

unique polyketide synthase (Cg-pks) are key to mycolic acid biosynthesis in *Corynebacteriaceae* like *Corynebacterium glutamicum* and *Mycobacterium tuberculosis*", *J. Biol. Chem.*, 279, p.44847-44857 (2004).

- 3) H. Tamura, Y. Tsuruta, K. Itoyama, Wannasiri Worakitkanchanakul, Ratana Rujiravanit, S. Tokura, "Preparation of chitosan filament applying new coagulation system", *Carbohydrate Polymer*, 56, p.205-211 (2004).
- 4) H. Tamura, K. Wada, R. Rujiravanit, S. Tokura, "Preservation of Chitosan aqueous gel under neutral conditions", *J. Metals, Materials and Minerals*, 15, p.19-21 (2005).