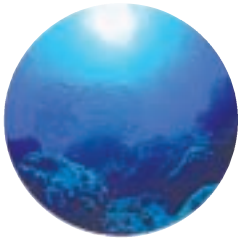
OIST

Okinawa Institute of Science and Technology Promotion Corporation

Annual Report 2005



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Preface

After a long period of incubation the OIST project took an important step forward with the formal initiation of the OIST Promotion Corporation on September 1st 2005. We have two tasks: one is to conduct research and development of high international standard in Okinawa and the second is to prepare for a new graduate institution of science and technology to be located on a site in Onna-son.

In this first annual report of the OIST Promotion Corporation, we summarize the plans so far made for the new campus. We include renovations on the Hakuun-so site, which will serve as the centre for OIST until the new facilities are constructed.

We also report on the scientific work, which is a continuation of the Initial Research Project. The number of units continues to increase and we plan to continue our growth to the capacity of our temporary laboratories in Uruma-shi.

The next year will be particularly active one for us as we engage in new research projects, and in the growth of a research community in Okinawa.

Sydney Brenner
President
Okinawa Institute of Science and Technology Promotion Corporation

I. OIST Events

The Okinawa Institute of Science and Technology Promotion Corporation was established on September 1st of 2005. The corporation was established in order to prepare the opening of the graduate school university. The main events of 2005 were:

September 1, 2005

The Okinawa Institute of Science and Technology Promotion Corporation was established. Dr. Sydney Brenner was appointed the President of the Corporation by the Prime Minister.

October 13, 2005

Tokyo inauguration party was held at Toranomon Pastoral in Tokyo. Attendance included Mr. Hosoda, Chief Cabinet Secretary; Ms. Koike, Minister of State for Okinawa and Northern Territories Affairs; Mr. Tanahashi, Minister of State for Science and Technology; Mr. Omi, former Minister of State for Okinawa and Northern Territories Affairs, and former Minister of State for Science and Technology Policy; Mr. Inamine, Governor of Okinawa Prefecture; and Dr. Kurokawa, Chairman of the Board of Governors, OIST P.C. The number of attendees was approximately 300.

October 14, 2005

Okinawa inauguration party was held at Laguna Garden Hotel in Naha. Attendance included Mr. Inamine, Governor of Okinawa Prefecture; Mr. Omi, former Minister of State for Okinawa and Northern Territories Affairs; Mr. Shikiya, Lord-mayor of Onna-son and Dr. Kurokawa, Chairman of the Board of Governors, OIST P.C. The number of attendees was approximately 250.

November 1, 2005

Design review meeting was held in Tokyo to discuss the Campus Master Plan. The final Campus Master Plan was made public on January 20, 2006.

January 10, 2006

The first official Board of Governors meeting was held in San Francisco (USA).

II. BOG Meeting Summary

The Board of Governors was established under the Corporation Act in 2005. The tasks of the Board are to oversee the Corporation and advise the President. The Board of Governors operates

within Articles 12 and 13 of the Independent Administrative Institution Okinawa Institute of Science and Technology Promotion Corporation Act. In September 2005, Japanese Prime Minister, Junichiro Koizumi, appointed the current Board members.

Board of Governors:

- Dr. Akito Arima - President, Japan Science Foundation; former President, Tokyo University
- Dr. Steven Chu - Director of Lawrence Berkeley National Laboratory; Nobel Laureate (Physics, 1997)
- Dr. Jerome Friedman - Professor, MIT; Nobel Prize Laureate (Physics, 1990)
- Dr. Jean-Marie Lehn - Professor, University Louis Pasteur-Strasbourg; Nobel Laureate (Chemistry, 1987)
- Dr. Hiroko Sho - Professor Emeritus, University of the Ryukyus
- Dr. Susumu Tonegawa - Professor, MIT; Nobel Laureate (Physiology or Medicine, 1987)
- Dr. Torsten Wiesel - Secretary General, Human Frontier Science Program Organization; Nobel Laureate (Physiology or Medicine, 1991)
- Dr. Kiyoshi Kurokawa (Chair) - President, Science Council of Japan

The 1st official Board of Governors meeting of Okinawa Institute of Science and Technology P.C. was held on 10 January 2006 in San Francisco, California. Invited guests included Parliamentary Secretary Takuya Hirai; Director-General Bunshichi Fujioka and Deputy-Director Tomoaki Wada from the Cabinet Office; Honorable Mr. Koji Omi; Dr. Hiroaki Kitano, Mr. John Dickison, Dr. Ken Kornberg and Mr. Takashi Okamoto of the Campus Planning Group; Dr. Robert Baughman. The meeting minutes were duly recorded in accordance with regulations.

The following items were covered in the meeting agenda:

- Opening remarks were made by Parliamentary Secretary Hirai, Minister of State for Okinawa and Northern Territories Affairs designate, on behalf of Minister Koike to express appreciation to all participants for the dedication and contributions to the OIST P.C. project.
- Dr. Kurokawa was approved as Chairman of Board of Governors.
- Sir Martin Rees was approved by the Board as a Board Member.
- Board member regulations and compensation frame (honorarium, etc.) overview.
- Dr. Robert Baughman was appointed by the President to the position of Vice-President of Research and Academic Affairs.
- Status reports:
  - ◆ OIST P.C. Events and Ceremonies (2005)

- IRP and Workshop Research Report
- Research Report - Endo Unit
- Campus Planning and Architecture Report

- Discussion items included:
  - President's comments on a road map of the Medium-term Plan
  - Organizational structuring of OIST P.C.
  - Budgetary, facility planning, research and time issues towards establishing the university
  - Next BOG meeting was agreed to be 26th-29th May with a tentative location of Hawai'i agreed upon

III. Campus Master Plan Status

A kick-off meeting was held on 19th July 2005 to finalize the agreement of the master plan with the architect and civil engineer and began the master planning process. The first phase concentrated on information gathering, reviewing earlier-prepared materials and touring laboratories in America.

The master plan development work proceeded with a series of discussion sessions within the Campus Planning Group. Four master plan schemes were proposed by the master plan architect to BOG members. At the 1st November 2005 master plan review session, it was agreed that the southern area was attractive considering its higher potential for the development of a campus linked to the surrounding community. The question of a 'dispersed' vs. 'concentrated' layout for the laboratory buildings was left open for later discussion. The architect was requested to propose a new 'globally dispersed, locally concentrated' scheme. A new scheme was distributed via e-mail one month later.

After the master plan review session, a meeting was held with Onna-son local representatives and the fisheries association. After presenting the four schemes, there was a consensus with the selection of the southern area.

On 10th January 2006, the master plan architect presented a revised plan at the January 2006 San Francisco BOG meeting and the master plan on OIST campus was approved.

The master plan architect and civil engineer commenced work on the basic design after 1st

November 2005. Based on the choice of the southern area, a more detailed site investigation was ordered to obtain accurate site landform and borehole data.

The basic civil design was completed at the end of January 2006. The basic architectural design was completed at the end of March 2006. The master plan architect prepared the Hakuun-so renovation designs to provide a seminar house and OIST head office. Bidding for contractors was held on 12th December 2005. The renovation work has been completed on 24th March 2006.

IV. Administration and Finance

Management Committee (MACO) and Committee of PIs (COPI) hold monthly meetings. The Management Committee includes the President, Executive Director, Director Budget and Finance, Director Research and Training, and a PI representative. At MACO meetings there are discussions about key issues, reporting on financial matters and progress of campus construction planning. At COPI meetings, there are discussions concerning the recruitment of PIs, future research theme, research collaboration with other institutions, etc. With these two committees, maintaining communication between scientists and administration is ensured.

Concerning general financial oversight, the function of Chief Financial Officer has been established. The Director of Budget and Finance has the responsibility for the implementation of effective budget control. Expenditures and budget are reported at monthly MACO meetings, aiming to narrow the budget deviation. A final financial report cannot be given at this time as the fiscal year has not finished.
To strengthen the administration of research and training programmes a Director of Research and Training has been appointed. The Director is a member of both MACO and COPI committees. Throughout the start-up operations appropriate and effective support has been given to the research area. In particular, there is an emphasis on recruitment for research units and establishing international collaborations.

To improve administrative capabilities, staffs are provided with appropriate training opportunities. For new recruits, one-week training has been given with the assistance of the Japan Science and Technology Agency (JST), RIKEN and other science institutions. Considering the impact of increasing numbers of foreign researchers, administrative staffs were sent to an Immigration Office training course in order to obtain a certificate which improves

immigration procedure handling.

Rules and Regulations governing spending on such matters as travel and other administrative expenses have been stipulated and put into effect.

In order to strengthen management and administrative efficiency, an Enterprise Resource Planning (ERP) system was introduced. Security issues and customization to the work process at OIST PC is nearly completed. The ERP system is expected to be fully operational by the next fiscal year. In order to facilitate the administrative work process, a comprehensive operation manual is being compiled and anticipated to be completed by the end of the year.

The current total of indefinite term employees is fifteen (15), as of March 2006. The total of fixed-term contract employees is 53. The total of regular attending employees is 68. The departmental breakdown is:

Finance, HR	: 9
Research and Training	: 10
Facilities Planning	: 4
Initial Research Project	: 45

In principle, employment of the regular attending staff is tendered, public offerings. The primary channels for researcher recruitment are journals, the OIST homepage, "Nature" and "Science" magazines and recruiters.

Scientific Report

Initial Research Project (IRP)

Initial Research Project (IRP) is one of the precursory activities for the Okinawa Institute of Science and Technology. This project aims at establishing a strong research base in Okinawa by gathering active scientists and providing support for their research and their collaboration with other prominent scientists.

IRP was first announced as OIST Research Funding Program. Invitation of application started in August, 2003. Out of 137 applications from 28 nationals, four proposals have been selected. Four research units (described as below) started in 2004 at the IRP Laboratory and Okinawa Health Biotechnology Research and Development Center in Uruma-shi.

Neural Computation Unit

Principal Investigator: Dr. Kenji Doya

- A Computational Approach to Molecular Mechanisms of the Mind

Unit for Molecular Neurobiology of Learning and Memory

Principal Investigator: Dr. Shogo Endo

- Genetic and Molecular Dissection of Learning and Memory

Electron Microscopy Unit

Principal Investigator: Dr. Akira Tonomura

- Holography Electron Microscopy Dedicated to Nanotechnology

G0 Cell Unit

Principal Investigator: Dr. Mitsuhiro Yanagida

- Cellular Strategy for Starved G0 Arrest and Vegetative Proliferation

On December 1, 2005, a new group, Molecular Neuroscience, was formed consisting of three units. Molecular Neuroscience Units conduct their research at Okinawa Industrial Technology Center.

Molecular Neuroscience Units

Principal Investigator: Dr. Sydney Brenner

- Molecular Genetics

Principal Investigator: Dr. Ichiro Maruyama

- Information Processing by Life

Principal Investigator: Dr. Takayuki Naito

- Single Cell Biochemistry and Molecular Analysis of Brain Functions

V. Neural Computation Unit

Principal Investigator: Kenji Doya
Research Theme: A Computational Approach to Molecular Mechanisms of Mind

Abstract:

The goal of this research is to understand the neurobiological substrate of human mind by combining top-down computational modeling and bottom- up neurobiological experiments. The major progresses of the three groups in FY 2005 are the following:

1) Dynamical Systems Group: Toward developing a novel system identification method for intracellular networks, we implemented probabilistic estimation method for benchmark processes.

2) Systems Neurobiology Group: In order to elucidate circuit and neuromodulatory mechanisms of decision making, we set up multi-electrode recording and microdialysis measurement systems and started recording from rats performing reinforcement learning tasks.

3) Adaptive Systems Group: To explore adaptive mechanisms required for survival and reproduction, we performed embodied evolution experiments of reward functions and meta-parameters for reinforcement learning using Cyber Rodent robots.

1. Participants:

1.1. Individuals:

Adaptive Systems Group:

-Researchers: Eiji Uchibe, Takashi Sato

-Research Technician: Stefan Elfwing

-Graduate Students: Emma Brunskill, Tetsuro Morimura, Takumi Kamioka, Makoto Otsuka

Dynamical Systems Group:

-Researchers: Junichiro Yoshimoto, Atsushi Shinkai (from NEC Software)

-Research Technician: Yasuhiro Inamine (from NEC Software Okinawa)

-Graduate Students: Michiyuki Magono, Takashi Nakano

Systems Neurobiology Group:

-Researchers: Makoto Ito, Katsuhiko Miyazaki, Kayoko Miyazaki, Thomas Strosslin

-Research Assistants: Emiko Asato, Izumi Nagano

1.2. Partner Organizations:

ATR Computational Neuroscience Laboratories

Type: Joint research.

Principal researcher : Dr. Mitsuo Kawato
Theme: "Functional brain imaging study of molecular basis of mind"

Nara Institute of Science and Technology, Graduate School of Information Science
Type: Joint research.
Principal researcher: Prof. Shin Ishii
Theme: "Application of Bayesian method to identification of biological reaction systems"

NEC Software
Type: Joint research.
Principal researcher: Mr. Atsushi Shinkai
Theme: "Parallel implementation of Bayesian estimation methods"

2. Activities and Findings:

2.1. Activities and Findings of the Dynamical Systems Group

1) Bayesian approach to reverse engineering of biochemical reactions

Computer simulation of biochemical kinetics is an essential tool for verifying hypothetical models of unexplained signaling pathways. Since many kinetic parameters used in the simulation are often unidentified or unreliable, development of methods for automatically estimating the parameters and their certainty is in great demand. We developed a Bayesian framework for this estimating problem.

Based on the theory of chemical reaction, we formulated generic biochemical kinetics and their measuring processes as a probabilistic state space model, where the measurement noises are modeled by gamma distributions. This formulation is helpful to make the non-negative constraints of the measurements (i.e. concentrations of substances) explicit and also identical to a usual model with Gaussian noises in an extreme condition. Then, we introduced a prior distribution over all unknown variables (latent state variables and parameters we want to identify). As a result, we derived a basic formula to represent the plausibility of the unknown variables as a posterior distribution, based on the Bayes theorem. Although the calculation of the posterior distribution is generally intractable, we also developed a Metropolis-Hasting method based on a multi-state sampling technique to efficiently approximate this posterior distribution.

To test the performance of our method, we applied it to three benchmark systems: 1) a single binding process with one latent variable and two unknown parameters; 2) a bioreactor system with strongly non-linear kinetics governed by four unknown parameters; and 3) a three-step metabolic pathway with 36 unknown parameters.

The main contribution of this work is to unify the following three issues in the framework of Bayesian inference: 1) estimating the most likely parameters; 2) estimating the levels of measurement noises; and 3) representing the confidence interval of the estimated

parameters. The last two issues are rarely addressed by existing methods based on simulated annealing methods and genetic algorithms. This result was presented in a poster session of PSB 2006.

2) Efficient state representation for reinforcement learning of "Cyber Rodent"

Animals and machines have to monitor their own state in environment in order to make an appropriate action selection. This is more critical when sensory inputs from the environment have uncertainty and/or ambiguity. This can be formulated as a state representation in partially observable Markov decision process in the context of machine learning. Self-localization is one of the most fundamental issues for such a state representation. We addressed the development of efficient self-localization method based on Bayesian inference.

We used our mobile robot Cyber Rodent (CR) in this work. To begin with, we formulated the dynamics of environment around CR as a linear Gaussian state transition model, and approximated the relationship between CR's location and sensory inputs with a probabilistic RBF network model. According to this formulation and a recursive Bayesian technique, we derived a mixture-Kalman-filter algorithm to represent the confidence in the current location of the CR as a posterior distribution. The effectiveness of our method was demonstrated through computer simulations and real experiments.

The accuracy and computational efficiency of our developed methods outperforms those of Kalman filter and Monte Carlo localization, which are now widely-used self-localization methods. Especially, our method works much more robustly in real environment that the others.

2.2. Activities and Findings of the Systems Neurobiology Group

1) Network mechanism of decision making

The aim is to elucidate how the multi-step network of the cortico-basal ganglia loop works for action selection based on the prediction of rewards. Two major questions are 1) where and how the expected reward for action candidates are learned, and 2) where and how stochastic action selection according to the reward expectation is realized. Our working hypothesis is that the striatum is mainly involved in 1) and its downstream, the pallidum, is mainly involved in 2).

We designed a probabilistic reward-based free choice task for rats, by which we should be able to dissociate neural activities for reward prediction and action selection. We fine-tuned the experimental setup, such as cue signal and reward probabilities, so that rats can efficiently learn the task. We also modeled the rats' choice behaviors by reinforcement learning models.

Using the 32-channel electrode recording system we developed last year, we so far recorded about 60 neurons in the four stages of the cortico-basal ganglia circuit: the prefrontal cortex, the ventral striatum, the ventral pallidum, and the MD nucleus of the thalamus. We continue to record from more neurons to clarify the differences in the neural coding in these four areas.

2) Neuromodulator mechanisms in meta-learning

We have a set of working hypotheses that the ascending neuromodulatory systems coordinate the higher-level parameters of the distributed learning networks in the brain. Specifically, we conjecture: 1) dopamine signals the change in predicted reward, 2) serotonin regulates the time scale of prediction, 3) noradrenaline regulates the width of exploration, and 4) acetylcholine regulates the speed of memory update (Doya 2002, Neural Networks). To test these hypotheses, we are running neural recording experiments from the originating nuclei of these neuromodulators, and microdialysis measurement of those neuromodulators in the recipient brain regions.

We started chronic recording from the dorsal raphe nucleus, the origin of serotonergic projection. We found serotonin neurons, with wider spikes, and non-serotonin neurons, with sharp spikes. The major findings so far is that both types of neurons exhibit responses during the delay period before reward delivery. This is a very much encouraging finding in support of the hypothesis that serotonin allows the animal to wait for delayed rewards.

We also measured the concentration of dopamine and serotonin in the nucleus accumbens and the dorsal raphe nucleus using a micordialysis system. We also found increased serotonin release when an increasing delay was incorporated before reward delivery. We continue to improve the behavioral paradigm to clarify the role of serotonergic neurons.

2.3. Activities and Findings of the Adaptive Systems Group

We are running a series of experiments using the Cyber Rodent robots, which have two marked features: capacity for self-preservation by capturing and recharging from battery packs, and capacity for self-reproduction by copying its programs or parameters through infrared communication ports. The aim is to explore variety of adaptive mechanisms necessary for survival and reproduction, or learning and evolution.

1) Learning algorithms

We are developing learning algorithms for basic behaviors, such as capturing batteries and 'mating' with other agants.

We extended our "concurrent learning with importance sampling (CLIS)" so that it is efficiently implemented under real-time constraints. We also developed a new reinforcement learning algorithm that allows quick learning by natural gradient method and robust implementation without matrix inversion that was required in previous methods. We also investigated methods for dealing with multiple rewards and state constraints.

2) Embodied evolution

Embodied evolution is a methodology for evolutionary robotics that mimics the distributed, asynchronous and autonomous properties of biological evolution. The evaluation, selection and reproduction are carried out by and between the robots, without any need for human intervention. We proposed a biologically inspired embodied evolution framework, which

fully integrates self-preservation, recharging from external batteries in the environment, and self-reproduction, pair-wise exchange of genetic material, into a survival system. The individuals are, explicitly, evaluated for the performance of the battery capturing task, but also, implicitly, for the mating task by the fact that an individual that mates frequently has larger probability to spread its gene in the population. We have evaluated our method in simulation experiments and the simulation results show that the solutions obtained by our embodied evolution method were able to optimize the two survival tasks, battery capturing and mating, simultaneously. We have also performed preliminary experiments in hardware, with promising results.

We also showed that the supplementary reward functions for foraging and mating can be evolved to allow appropriate orienting behaviors.

3. Publications

3.1. Journals

Capi, G., Doya, K. (2005). Evolution of neural architecture fitting environmental dynamics. *Adaptive Behavior*, 13, 53-66.

Elfwing S., Doya K., Christensen. H. I., Evolutionary Development of Hierarchical Learning Structures, *IEEE Transactions on Evolutionary Computations*, (in press)

Daw N., Doya K. (2006). The computational neurobiology of learning and reward. *Current Opinion in Neurobiology*, 16, 199-204

Doya K. (2005-2006). Introduction to computational neuroscience, part 1 to 10. *Mathematical Sciences*.

Doya K., Uchibe E. (2005). The Cyber Rodent project: Exploration of adaptive mechanisms for self-preservation and self-reproduction. *Adaptive Behavior*. 13, 149-160.

Hirayama J., Yoshimoto J. & Ishii, S., Balancing plasticity and stability of on-line learning based on hierarchical Bayesian adaptation of forgetting factors, *Neurocomputing* (in press).

Samejima K., Ueda Y., Doya K., Kimura M., Representation of action-specific reward values in the striatum, *Science*, 310, 1337-1340 (2005)

Sugimoto N., Samejima K., Doya K., Kawato M. Hierarchical reinforcement learning: Temporal abstraction based on MOSAIC model, *Journal of IEICE (The Institute of Electronics, Information and Communication Engineers)* (in press, in Japanese)

Uchibe E., Asada M, Incremental co-evolution with competitive and cooperative tasks in a multi-robot environment, *Proceedings of the IEEE*, (in press)

3.2. Book(s) or other one-time publications

Doya K., Ishii S., Pouget A., Rao R. (in press). Bayesian Brain: Probabilistic Approach to Neural Coding and Learning. MIT Press.

3.3. Oral Presentations and Posters

Oral Presentations:

Doya K., Designs of learning robots and reward systems of the brain. *Sony Intelligence Dynamics 2005*, Shinagawa, Japan, April 8, 2005.

Doya K., Motor control and learning in human: Approaches with functional neuroimaging, *The 82nd Annual Meeting of the Physiological Society of Japan*, Sendai, Japan, May 18-20, 2005.

Doya K., Serotonergic modulation of the cortico-basal ganglia circuits for long- and short-term reward prediction. *Neuroscience 2005 The 28th Annual Meeting of the Japan Neuroscience Society*, Yokohama, Japan, July 26-28, 2005.

Doya K., Learning from rewards: in robots and the brain. *Human Forum 2005*, Wako, Japan, September 6, 2005.

Doya K., Mechanisms and Origins of Reward-based Behaviors: neurobiological and Robotic Approaches, *Brain-inspired Information Technology (BrainIT) 2005*, Kitakyushu, Japan, October 7, 2005.

Doya K., Prediction of immediate and future rewards in the cortico-basal ganglia loops and its modulation by serotonin, *First Dunedin Workshop on the Neurobiology of ADHD*, Dunedin, New Zealand, November 28-December 1, 2005.

Doya K., Meta-parameter regulation in adaptive agents and meta-learning in the brain. *Winter Symposium on Cognitive Science*, Tokyo, Japan, December 3, 2005.

Doya K., Prediction of immediate and future rewards in cortico-basal ganglia loops, *Alpine Brain Imaging Meeting*, Champery, Switzerland, January 22-26, 2006.

Doya K., Reinforcement Learning and the Basal Ganglia, *Japan-Germany Symposium on*

Computational Neuroscience, Wako, Japan, February 1-4, 2006.

Elfwing S., Uchibe E., Doya K. and Christensen I. H., Biologically Inspired Embodied Evolution of Survival, *2005 IEEECongress on Evolutionary Computation*, Edinburgh, U.K. September 2-5, 2005.

Kamioka T., Uchibe E., Doya K., Multiobjective reinforcement learning based on multiple value functions. *Neuro-computing Workshop, The Institute of Electronics, Information and Communicate Engineers*, Tokyo, Japan, March 15-17, 2006.

Magono M., Yoshimoto J. & Doya K., (2005) Localization of Cyber Rodent based on mixture Kalman filters (in Japanese), *2005 Annual Conference of the Japanese Neural Network Society (JNNS 2005)*, pp.64-65, Kagoshima, Japan, September 20-22, 2005.

Magono M., Yoshimoto J., Ishii S. & Doya K., (2005) Localization of Cyber Rodent based on mixture Kalman filters, *2005 International Symposium on Nonlinear Theory and its Applications (NOLTA 2005)*, pp.401-404, Bruges, Belgium. October 18-21, 2005.

Morimura T., Uchibe E. and Doya K., Utilizing the natural gradient in temporal difference reinforcement learning with eligibility traces, *The 2nd international symposium on information geometry and its application*, Tokyo, Japan, December 2-16, 2005.

Tokita Y., Nakamura Y., Yoshimoto J. & Ishii S., (2006) Reinforcement learning method to control real robot by switching of multiple controllers, *International Symposium on Artificial Life and Robotics (AROB 11th '06)*, Beppu, Japan. January 23-25, 2006.

Uchibe E., Doya K. Reinforcement learning by interaction of multiple, heterogeneous learning modules. Robotics & Mechatronics Symposium, Kobe, Japan, June 11, 2005.

Uchibe E., and Doya K, Reinforcement learning with multiple heterogeneous modules: A framework for developmental robot learning, *International Conference on Development and Learning*, Osaka, Japan, July 21, 2005.

Posters:

Ito M. & Doya K., Analysis of decision making of rats based on reinforcement learning theory, *The 6th Summer Workshop on Mechanism of Brain and Mind*, Matsushiro, Japan, August 20, 2005.

Ito M. & Doya K., Neural activity in the cortico-basal ganglia loop in a conditional free-choice task, *The 6th Winter Workshop on Mechanism of Brain and Mind*, Rusutsu, Japan, January 11, 2006.

Miyazaki K. and Doya K. Behavioral correlated neural activity in the dosal raphe nucleus of free moving rat, *The 6th Winter Workshop on Mechanism of Brain and Mind*, Rusutsu, Japan, January 10-12, 2006.

Yoshimoto J., Magono M. & Doya K., (2005) Self-localization algorithm of mobile robots using RBF networks and mixture Kalman filters , *The 6th Summer Workshop on Mechanism of Brain and Mind*, Matsushiro, Japan, August 20-21, 2005.

Yoshimoto J. & Doya K., (2006) Bayesian approach to reverse engineering of biochemical reactions, *The Pacific Symposium on Biocomputing 2006 (PSB 2006)*, Maui, U.S.A. January 3-8, 2006.

4. Intellectual Property Rights and Other Specific Products

Patent

Title of the invention: A mixture-Kalman-filter algorithm for identifying hidden states in non-linear dynamical systems
Inventors: Junichiro Yoshimoto, Michiyuki Magono
Registration number: Uunder examination(domestic application)

Title of the invention: Natural policy gradient based on learning algorithm
Inventors: Eiji Uchibe, Tetsuro Morimura
Registration number: Uunder examination(domestic application)

5. Meetings and Events

5.1. Okinawa Computational Neuroscience Course 2005

Date: July 1-10, 2005
Place: Rizzan Sea Park Hotel, Okinawa
Title: Predictions and Decisions
Co-organizers:
Peter Dayan, Gatsby Computational Neuroscience Unit
Kenji Doya, Okinawa Institute of Science and Technology
Masamichi Sakagami, Tamagawa University

Lecturers:
Bernard Balleine (UCLA)
Andrew G. Barto (University of Massachusetts)
Nathaniel Daw (Gatsby Computational Neuroscience Unit, UCL)
Peter Dayan (Gatsby Computational Neuroscience Unit, UCL)
Kenji Doya (Initial Research Project, OIST)
Mitsuo Kawato (ATR, Computational Neuroscience Laboratories)
Minoru Kimura (Kyoto Prefectural University of Medicine)
Daeyeol Lee (University of Rochester)
John O'Doherty (California Institute of Technology)
Anitha Pasupathy (MIT)
Masamichi Sakagami (Tamagawa Universitty)
Stefan Schaal (University of Southern California)
Wolfram Schultz (University of Cambridge)
Reza Shadmehr (Johns Hopkins University)
Leo Sugrue (Stanford University)
Jun Tanji (Tohoku University)

5.2. OIST Initial Research Project Seminars

Date: August 22, 2005(16:00-17:30)
Title: The Contribution of the Striatum to Cortical Function
Speaker: Dr. Jeff Wickens, University of Otago

Date: November 21, 2005 (16:30-17:30)
Title: The Role of Serotonin in Evaluation of Future Rewards
Speaker: Dr. Nicolas Schweighofer, University of Southern California

Date: December 13, 2005 (14:00-15:00)
Title: Attention deficit hyperactivity disorder (ADHD): From identification to management
Speaker: Dr. Gail Tripp, University of Otago

Date: January 30, 2006 (16:00-17:30)
Title: Hippocampal and striatal activity and goal-directed navigation behavior
Speaker: Dr. Sidney Wiener, CNRS-College de France

VI. Unit for Molecular Neurobiology of Learning & Memory

Principal Investigator: Shogo Endo

Research Theme: Molecular and genetic dissection of learning and memory

Abstract:

The brain, the organ of thinking and memory, is supported by the result of the fine-tuned molecular mechanisms in neuronal cells. The functions of neuronal cells are the origin of all of mental activities. One of the fundamental but attractive questions in the field of neuroscience is to understand the molecular mechanisms underlying the functions of neurons and glial cells in the central nervous system. The molecules involved in the cascade are still waiting to be revealed for their roles. More than 15 years, I have studied the molecular and cellular aspect of memory based on the techniques of biochemistry, molecular biology and gene targeting. Since I became Principal Investigator in Initial Research Project in OIST, I continue to attack the biochemical and cellular aspects of the neuronal plasticity and memory to reveal using them as models of brain functions.

1. Participants:

1.1 Individuals:

-Researchers:

Nobuhiko Kojima, Toshiro Sakamoto.

-Research Technicians

Masako Suzuki (Laboratory Manager), Michiko Arai, Tomoko Arasaki, Chihiro Honma, Mika Takiguchi.

-Graduate Student

Yukiko Uechi (University of the Ryukyus Graduate School of Medicine)

-Research Assistant

Shoko Yamakawa

1.2 Partner Organizations:

National Defense Medical College

Department of Biochemistry

Collaborative research, Principal Investigator: Dr. Kunio Takishima.

Research theme: "Generation of the mice with modified ERK2 gene."

RIKEN Brain Science Institute

Laboratory for Memory and Learning
Collaborative research, Principal Investigator: Dr. Masao Ito.
Research theme: "Electrophysiological examination of genetically modified mice."

Laboratory for Behavioral Genetics
Collaborative research, Principal Investigator: Dr, Shigeyoshi Itohara.
Research theme: "Generation of conditional gene knockout mice."

Laboratory for Motor Learning Control
Collaborative research, Principal Investigator: Dr, Soichi Nagao.
Research theme: "Behavioral examination of genetically modified mice."

Shinshu University Graduate School of Medicine
Laboratory of Neuronal plasticity
Collaborative research, Principal investigator: Dr. Tatsuo Suzuki.
Research theme: "Comprehensive isolation of mRNAs localized in synapse"

University of the Ryukyus Graduate School of Medicine
Division of Cell Biology
Collaborative research, Principal Investigator: Dr. Ken-ichi Kariya
Research Theme: "Genetic analysis of cancer-related genes."

University of Tokyo Graduate School of Arts and Sciences
Department of Life Sciences.
Collaborative research, Principal Investigator: Dr. Dai Yanagihara.
Research theme: "Behavioral analysis of genetically modified mice."

2. Activities and Findings:

We are dissecting signal transduction cascade involved in neuronal plasticity and memory in two categories; early phase and long-lasting phase (late phase): The latter requires newly synthesized proteins through gene transcription and translation. We attack the molecular mechanism underlying neuronal plasticity and, learning and memory using a variety of methods including biochemistry, molecular biology, behavioral analysis and also gene manipulation technology.

Memory can be observed only by the examining the behaviors of animals. Even though the neuronal plasticity is believed to be the cellular basis of the memory, the memory itself requires the neuronal network based on the interaction of neuronal cells, the brain and the whole body. To observe the memory we chose the mice as a model animal. The gene manipulation of

mice is studied well and the method for the generation of gene-deficient mice is established. We utilized the genetically-modified mice to examine the molecular mechanisms underlying learning and memory.

2. 1. Main Results

Generation of genetically modified mice

The generation of genetically modified mice is an important part of our project. In our lab, the identification and construction of targeting vector, the homologous recombination in ES cell (embryonic stem cell) has been established. Currently, we are establishing the injection of ES cells to blastocysts, then transfer the injected blastocysts to psedopregnant mice. By establishing this, we will be able to generate the knock-in and knockout mice in OIST.

In addition to the establishing the generation method for knock-in and knockout mice, the device for the injection to generate transgenic mice is being established. Even though the knockout mice using homologous recombination in ES cell increases in its demand, the transgenic mice are still useful tool for the analysis of protein overexpression and promoter activity in the animals.

Test battery of mice behaviors

Comprehensive screening of behaviors is essential as a first step to characterize genetically modified mice for learning and memory because a variety of physiological conditions such as emotional states may affect the ability to learn. For example, the animal can be sensitive to sensory stimulation such as tone or light, then this animal is overreacted to these stimuli in the case of learning paradigm such as the association of tone and electric stimulation. So the evaluation of general behaviors of the genetically modified mice is carried out before the memory-oriented examinations. A series of behavioral examination is called "test battery" which is carried out in the order of the weakness of the effect on the animals. We have established following test battery;

General behavior test battery
24-hr activity measurement (Day 1-4)
Open field test (high light/ low light, Day7-Day 18)
Light-and-dark box test (Day 20)
Startle and prepulse inhibition (Day 21)
Fear conditioning (Day 23-25)

In addition to the behavior test battery mentioned above, we have established the following behavioral measurements; Morris water maze, passive avoidance, rotational rod, eye blink conditioning, hanging wire.

Function of NO (Nitric oxide)-cGMP-PKG (cGMP-dependent protein kinase) pathway in cerebellar LTD.

a) Isolation and characterization of downstream components of PKG in cerebellum

G-substrate was identified as a substrate of PKG by Paul Greengard's group in early 80's. Since then, the molecular cloning of the G-substrate cDNA was not successful despite the numerous efforts (Nairn and Greengard, personal communication). Previously, we carried out following researches: successful cloning of the G-substrate cDNA for rat, mouse and human. generation of a variety of tools such as cDNA probes, antibodies, G-substrate mutants; identification of protein phosphatase inhibitory activity of G-substrate upon phosphorylation of the protein by PKG; shuttling of G-substrate between nuclear and cytosol in Purkinje cells; generation of the G-substrate gene deficient mice.

The homozygote G-substrate gene deficient mice mate normally, and no apparent ataxia was observed. However, the homozygote mice lacked cerebellar LTD. We further characterized the mice for general behaviors and also for cerebellun-dependent learning such as rotational rod test, eye-blink conditioning, and VOR (vestibulo-ocular reflex) and OKR (optokinetic response) adaptation. General behaviors of the mice are normal, however, the long-term adaptation of OKR of eye movement was impaired WITHOUT any effect on short-term adaptation of OKR. G-substrate may be involved in the consolidation of short-term memory to long-term memory. Further examination of, for example, the extinction of OKR adaptation will be necessary to establish the role of G-substrate in the memory.

b) Characterization of nuclear localization signal (NLS) and nuclear export signal (NES) of G-substrate.

The results of the G-substrate gene deficient mice showed the importance of G-substrate in long-term memory in OKR. Long-term memory requires *de novo* protein synthesis, i.e., the transcription and translation of the genes. The results suggest that the G-substrate may directly or indirectly be involved in the gene transcription and translation.

Originally, we have observed the intense staining of G-substrate in some of Purkinje cells in cerebellum (Fig 1). Related to the possible function of G-substrate in transcription and translation, the translocation of G-substrate from nuclear to cytosol was induced by 8-Br-cGMP but not by PKA activator (Fig 2). Furthermore, Leptomycin B caused nuclear accumulation of G-substrate. The results suggest the export of G-substrate from nuclear to cytosol through CRM1-dependent mechanism.

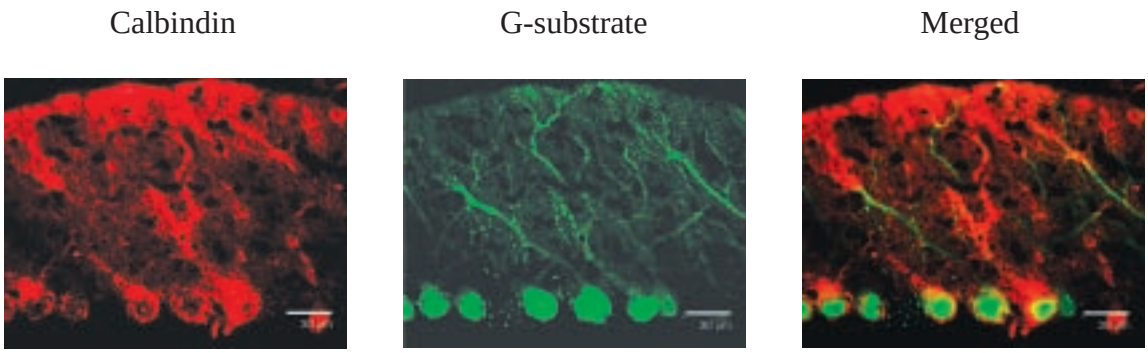


Figure 1. The distribution of G-substrate in mouse cerebellar slice. The slice is doubly stained with the antibodies against the calbindin (red) and G-substrate (green).

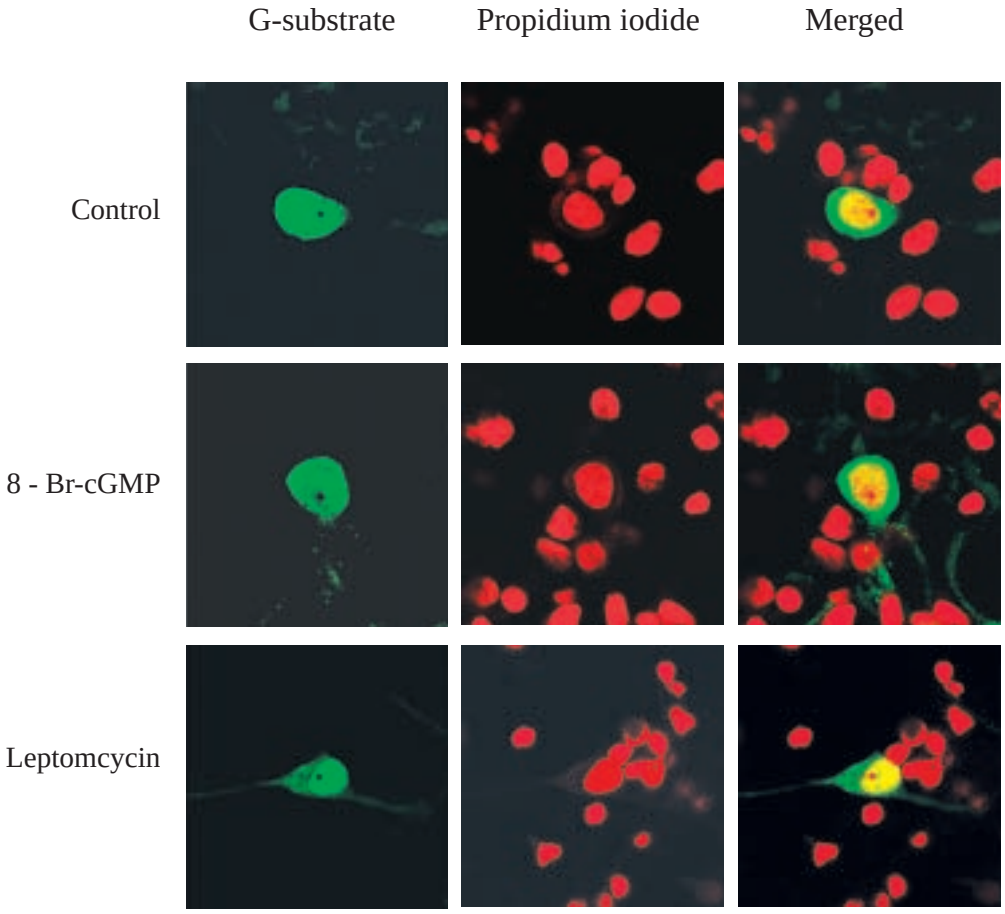


Figure 2. The translocation of G-substrate in cerebellar Purkinje cells in culture. The cells were stained with G-substrate antibody (green) and with propidium iodide for nuclear (red). The cells around Purkinje cell are granule cells.

G-substrate contains potential NLSs and NESs (Fig. 3). Two NLSs (NLS2 and 3 in Fig. 3) are located in the vicinity of two phosphorylation sites (Site 1 and 2) by PKG. The addition of negative charges to The residues by phosphorylation may disturb the function of NLS which constitute of amino acids with positively charged side chains (Arg and Lys).

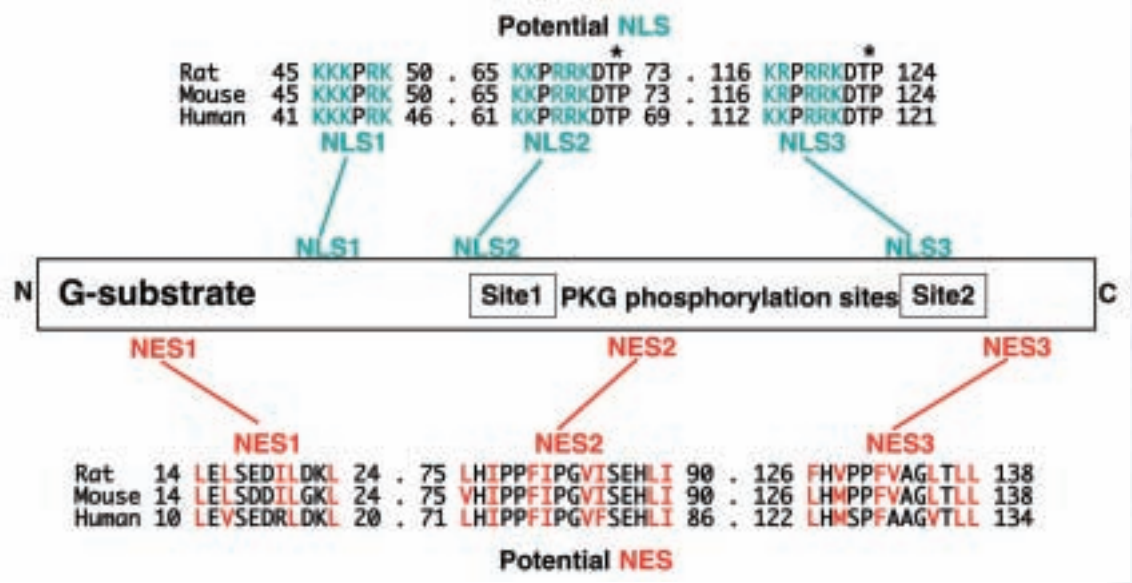


Figure 3. Potential NLSs and NESs in G-substrate. Potential relative positions of NLSs (blue) and NESs (red) are indicated in the G-substrate sequences. The numbers are the amino acid residue numbers of G-substrate in each species. Thr residues with asterisks are the PKG phosphorylation sites.

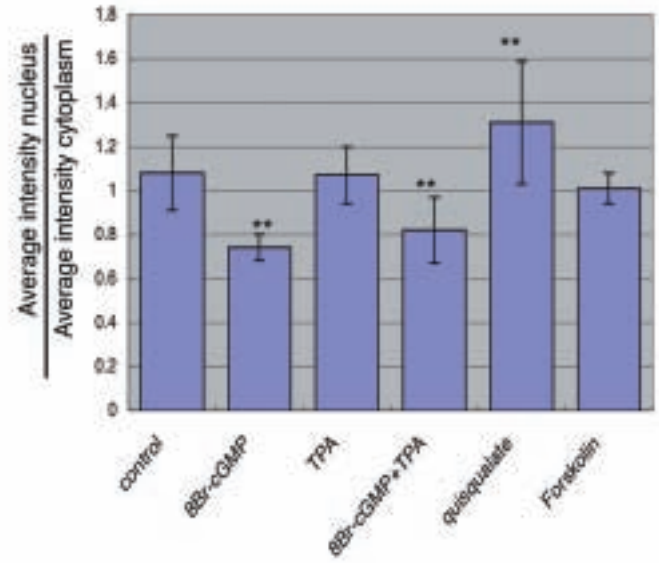


Figure 4. Stimuli-induced alteration of G-substrate distribution was measured in cultured Purkinje cells by the fluorescence due to the staining with G-substrate antibody. **, $p<0.01$; compared with the control.

In addition, the relative ration of the G-substrate localization between nuclear and cytosol can be altered by a variety of stimuli that can cause altered neuronal plasticity in cerebellum (Fig. 4). Though 8-Br-cGMP, a PKG activator caused the major accumulation of G-substrate in the cytosol of Purkinje cells, Forskolin (adenylate cyclase activator), and TPA (PKC

activator) didn't lead to the major alteration of G-substrate distribution. Further examination of the function of NLS and NES of G-substrate in cerebella Purkinje cell is being carried out by the introducing the mutation in NLS and NES of G-substrate. The identification of essential NLS and NES will lead to the further characterization of G-substrate in the cerebellar Purkinje cells and also will reveal role of G-substrate in the nuclear and cytosol of Purkinje cells.

Molecular mechanisms underlying cerebellun-dependent behavior.

One of the well-studied cerebellun-dependent behavior is eye blink conditioning that is classified as classical conditioning. In this conditioning, animal learns the association of tone and air puff (electric shock) to the eye lid. The learned behavior is tone-dependent eye blink. The eye blink conditioning is established in rabbit and the involved neuronal circuit is well characterized. However, the studies in mice are limited despite the availability of genetically modified mice which give us the great chance to look into the molecular mechanisms for the conditioning in detail. Furthermore, there is a long debate on the role of the cerebellar parts in the conditioning. Eye blink conditioning measuring system in mice has been established in OIST. As a first step to characterize mice eye blink conditioning, we examined the role of GABA receptors in cerebellar nuclear. The $GABA_A$ antagonist (picrotoxin) and $GAGA_A$ agonist (muscimol) was injected into the cerebellar deep nuclear through the surgically implanted canulas. The conditioned response (tone-dependent eye blink) was increased gradually during the training session, and plateaued at training session 5-6 (Fig. 5).

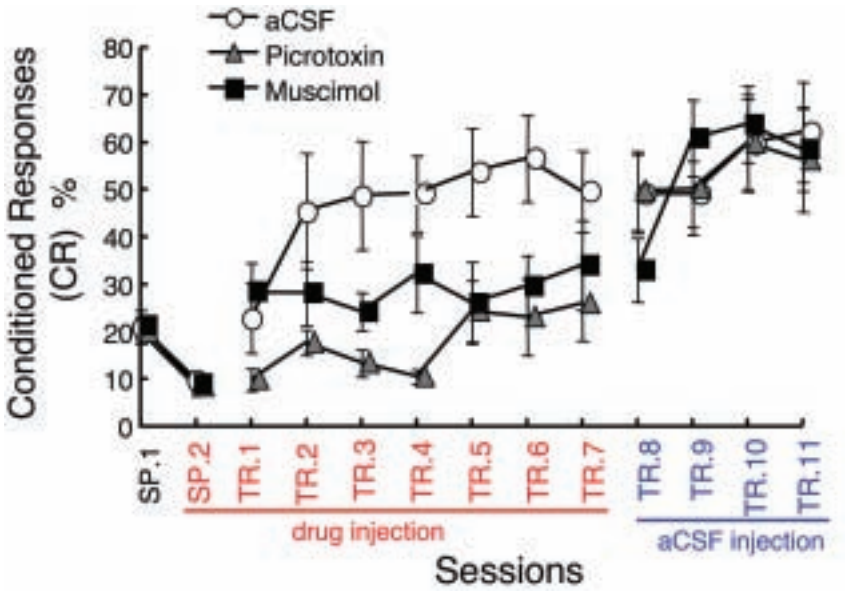


Figure 5. The eye blink conditioning of mice injected $GABA_A$ agonist (muscimol) and antagonist (picrotoxin) into cerebellar nuclear. Either aCSF, muscimol, or picrotoxin was injected into deep cerebellar nuclear during training session (TR.1-7) in addition to 2nd day of habituation session (SP.2). Then, drug was replaced with aCSF during TR. 8-11

Either picrotoxin- or muscimol-injected mice did not show the effective learning (Fig. 5). However, the learning ability of the picrotoxin- or muscimol-injected mice is recovered by the injection of artificial cerebrospinal fluid (aCSF) in training session 8-11. Interestingly, in the case of pictrotoxin-injected mice, the conditioned response obtained in the first day (Session 8) of aCSF was as high as the one of aCSF-injected mice. This result may suggest that in the presence of picrotoxin, the acquisition of conditioned response was normal but the expression of conditioned response was impaired in the presence of the picrotoxin (Session 1-7). On the other hand, muscimol-injected mice seemed to start to obtain the conditioned response after the aCSF-injection was started (training session 8-9). Further examination of effects of two drugs may give the insight into the mechanism and role of the cerebellar nuclear and$GABA_A$ receptor.

3. Publications

3.1. Jaurnals

(1) Wang X., Tian Q-B., Okano A., Sakagami H., Moon I.S., Kondo H., Endo S. and Suzuki T. BAALC 1-6-8 protein is targeted to postsynaptic lipid rafts by its N-terminal myristoylation and palmitoylation, and interacts with α, but not β, subunit of Ca^{2+}/calmodulin-dependent protein kinase II. *J. Neurochem*. **92**, 647-659, 2005.

(2) Suzuki T., Li W., Zhang J-P., Tian Q-B., Sakagami H., Usada N., Kondo H., Fujii T. and Endo S. A novel scaffold protein, TANC, possibly a rat homolog of *Drosophila* rolling pebbles (*rols*), forms a multi-protein complex with various postsynaptic density proteins. *Eur. J. Neurosci*. **21**, 339-350, 2005.

(3) Nakazawa T., Endo S., Shimura M., Kondo M., Ueno S., and Tamai M. Retinal G-substrate, potential downstream component of NO/cGMP/PKG pathway, is located in subtype of retinal ganglion cells and amacrine cells with protein phosphatases. *Brain Res. Mol. Brain Res*. **135**, 58-68, 2005.

(4) Kojima N., Sakamoto T., Endo S., and Niki H. Impairment of conditioned freezing to tone, but not to context, in Fyn-transgenic mice: Relationship to NMDA receptor subunit 2B function. *Eur. J. Neurosci*. **21**, 1359-1369, 2005.

(5) Zhang J-P., Tian Q-B., Okano A., Sakagami H., Kondo H., Endo S., and Suzuki T. p55 protein is a member of PSD scaffold proteins in the rat brain and interacts with various PSD proteins. *Brain Res. Mol. Brain Res*. **135**, 204-216, 2005.

(6) Bae J.-S., Furuya S., Shinoda Y., Endo, S., Schuchman E.H., Hirabayashi, Y., and Jin H.K. Neurodegeneration Augments the ability of bone marrow-derived mesenchymal stem cells to fuse with Purkinje neurons in Niemann-Pick type C mice. *Hum. Gene Ther*. **16**, 1006-1011, 2005.

(7) Satoh Y., Kanda Y., Terakawa M., Obara M., Mizuno K., Watanabe Y., Endo S., Ooigawa H., Nawashiro H., Sato S., and Takishima K. Targeted DNA transfection into the mouse

central nervous system using laser-induced stress waves. *J. Biomed. Optics* **10**, 60501.

(8) Nakazawa T., Shimura M., Endo S., Takahashi H., Mori, N., and Tamai M. N-methyl-D-aspartic acid suppresses Akt activity through protein phosphatase in retinal ganglion cells. *Mol. Vis*. **11**, 1173-1182, 2005.

(9) Sakamoto T., Takatsuki K., Kawahara S., Kirino Y., Niki H., Mishina M.. Role of hippocampal NMDA receptors in trace eyeblink conditioning. *Brain Res*. **1039**, 130-136, 2005.

3.2. Book(s) or other one-time publications

(1) Endo S. Mechanisms of Memory. Vol1, pp254-264. (Neurosurgery GuideBook, Yamaura, A ed), Nakayama Shoten Co., Tokyo, Japan. 2005.

(2) Endo S. Learning and Memory. (Neuroscience Illustrated, Mori et al ed.) Yodosha. Tokyo, Japan. pp 247-256, 2006.

(3) Endo S. An efficient animal facility for the study of learning and memory study using genetically modified animals. LABIO21. In press.

3.3. Oral Presentations and Posters

Oral Presentations:

(1) Sakamoto T. and Endo S. Roles of GABA receptor in cerebellar nuclear for the mouse eye blink conditioning. Annual meeting of Japan Society for Animal Psychology, Oct 8-9, 2005.

Posters:

(1) Kojima N., Sakamoto T., Endo S., Niki H.. Impairment of tone-dependent conditioned fear in fyn-overexpressing mice. Neuroscience 2005 Satellite Symposium Unraveling Higher Brain functions: Recent Progress with Animal Models July 24, 2005, Yokohama, Japan.

(2) Kojima N., Ikeda T., Itohara T., Endo S. ICER acts as a negative regulator for neuronal plasticity and long-term fear memory. Neuroscience 2005 Satellite Symposium Unraveling Higher Brain functions: Recent Progress with Animal Models July 24, 2005, Yokohama, Japan.

(3) Endo S., Ikeda T., Itohara S., Shutoh F., Nagao S., Ito M., Suzuki M. Generation and characterization of G-substrate gene deficient mice. Neuroscience 2005 Satellite Symposium Unraveling Higher Brain functions: Recent Progress with Animal Models July 24, 2005, Yokohama, Japan.

(4) Suzuki M., Ikeda T., Nakazawa K., Tonegawa S., Itohara S., Endo S. Generation of the mice with floxed Junb gene for conditional deletion of the gene. Neuroscience 2005

Satellite Symposium Unraveling Higher Brain functions: Recent Progress with Animal Models July 24, 2005, Yokohama, Japan.

(5) Endo S., Sato Y., Ikeda T., Itohara S., Nakazawa K., Tonegawa S., Takishima K., Suzuki M. Generation of Purkinje cell-specific ERK2 deficient mice using Cre-loxP system. Neuroscience 2005 Satellite Symposium Unraveling Higher Brain functions: Recent Progress with Animal Models July 24, 2005, Yokohama, Japan.

(6) Karachot L., Kitsukawa T., Vigot R., Suzuki M., Endo S., Yamamori T., and Ito M. Inducible transcription factor JunB controls rapid protein synthesis for induction of long-term depression in cerebellar Purkinje cells. Neoroscience 2005, July 26-28, Yokohama, Japan.

(7) Kiyota R., Imamura O., Satoh Y., Endo S., Takishima K., Study on the degradation mechanism of over-expressed ERK2. Annual meeting of Japan Society for Molecular Biology, December 7-10, 2005, Fukuoka, Japan.

4. Intellectual Property Rights and Other Specific Products

Nothing to be reported.

5. Meetings and Events

Nothing to be reported.

VII. Electron Microscopy Unit

Principal Investigator: Akira Tonomura

Research Theme: Holography electron microscope dedicated to nanotechnologies

Abstract:

The aim of this project is to observe and understand the microscopic behaviors of materials including ferromagnets using our coherent electron-wave techniques. Up to now, we developed electron holography techniques to visualize materials and fields with nanometer resolution using the phase information of electrons, which opened a new way to perform fundamental experiments on quantum physics and also to observe quantum phenomena which have begun to appear at many places in microscopic regions. In this project, we develop a dedicated "Nano-mag TEM" which is equipped with a long-focal, small-aberration objective lens, and investigate by using it the response of specimens, such as recording materials, molecular magnets and diluted magnetic semiconductors, to an external magnetic field at various temperatures with high spatial resolution.

1. Participants:

1.1. Individuals:

Researcher : Akira Sugawara (Group leader)

1.2. Partner Organizations:

Hitachi Advanced Research Laboratory

Type of research: Joint research

Principal researcher: Takaho Yoshida

Participating researchers: H. Kasai and Y. Hirayama

Research subjects:

(1) Observation of nanomagnetic materials by using field-emission electron microscopes.

A molecular beam epitaxy (MBE) -grown GaMnAs was examined by 1 MeV FE-TEM using a liquid helium specimen holder. The magnetic domain of weak ferromagnetic films (saturation magnetization = 0.01 - 0.02 T depending on temperature) was observed. The transition of the domain types according to anisotropy change in temperature was confirmed *in-situ (a paper in preparation).*

(2) Development of Nano-Mag TEM and spherical aberration corrector.

The details are described in section 2.2.

Japan Advanced Institute of Science and Technology

Joint Research

Principal researcher: Prof. Tadaoki Mitani

Participating researchers: Y. Tanaka, G. Mizutani, M. Miyake and M. Yamada

Research subjects: Wide-area assembly of nanoparticles/ nanowires/ nanorods by novel synthesis methods and their applications to electronic/magnetic devices.

The self-organized Pt nanowire arrays grown by shadow deposition methods exhibited anisotropic optical second-harmonic generation. The arrays angular dependence was explained in terms of broken symmetry *(a paper in preparation)*. The work was extended to also include growth of copper nanowires *(a presentation will be given at the JPS meeting)*.

2. Activities and Findings:

2.1. Observation of nanoscale magnetic structures by electron microscopy

The recording density of magnetic data storage is increasing rapidly as a result of the development of new materials. The present recording bit size is typically a few hundreds of nanometers but has to be downsized by factor of 10 for 300 Gbit/in^2 technology. Measurement techniques are essential to observe the recording state inside each bit and the crosstalk among neighboring bits. The TEM-based measurements, including electron holography and Lorentz microscopy, are used to determine magnetic structures with high spatial resolution and sensitivity.

We started measurements using 1 MeV and 350 kV electron microscopes. In recent phase contrast analysis that used electron microscopes, various numerical methods were introduced to extend the detection limit. An image acquisition system with wide dynamic range and high linearity is essential. A 4k x 4k high definition slow-scan charge coupled device (CCD) camera has been used to improve the measurement precision and test operation will soon start using the 350 kV electron microscope.

As mentioned in the following section, the development of a new microscope that is being optimized for nanomagnetism observation is also in progress. Once the new microscope is operational, the camera system will be transferred to the new system.

The choice of the material system that exhibits attractive magnetic behavior is important. Most interesting phenomena are found in thin film and nanoparticle materials. To prepare such materials by ourselves we have installed a molecular beam epitaxy system at the Uruma site. We have obtained preliminary results on

Fig. 1 Molecular beam epitaxy system for thin film and self-organized magnetic arrays installed in Uruma Laboratory.

the growth of high-quality metallic multilayers using the system. It will be also used for the growth of self-organized array of ferromagnetic nanoparticles and nanowires.

We also plan to study the effects of thermal fluctuation on magnetic ordering. Magnetic fluctuation is becoming a serious problem for recording media according to the size reduction required of the recording bits. Present technology for recording density is limited by the nature of the magnetic domain formation in continuous films. However, the arrays of the magnetic elements prepared by lithography and chemical synthesis will be used for future recording media. In such materials, the thermal fluctuation of magnetization determines lifetime of the memory. We will perform in-situ TEM observation by using a specimen heating holder to elucidate the problem of the thermal fluctuations. The research subjects include, for example, patterned magnetic recording media, whose shape and size is precisely controlled, and a nanoparticle system prepared by wet chemical processes, which are capable of mass producing of uniform nanostuructured materials.

Another challenging issue is to determine the magnetic structures of materials with small magnetization. Traditional metallic magnetic materials have a high signal-to-noise ratio that results from large magnetizations of the materials. Novel materials of half-metal-like electronic structures have recently been investigated for their application in recording heads. Although these materials do not always exhibit large magnetization, they exhibit giant magneto-resistance due to their large spin polarization factors. The micromagnetics of these materials is also expected to change from that of large magnetization materials because the contribution of the anisotropy energy is greater than that of the magnetostatic energy. Using holography/ Lorentz microscopy We investigate materials those do not produce a large phase change of electrons. The analysis will enable us to map anisotropy-controlled two-dimensional magnetization distribution and to observe magnetic ordering in the domain with high-resolution. The obtained results are expected to lead to a better understanding of the giant magneto-resistance of the novel materials.

An example of a novel material is a diluted magnetic semiconductor, such as GaMnAs. This material behaves as a ferromagnetic at low temperatures (typically below 50 K), even though it is still a semiconductor, and has a magnetization is typically 0.02 T, which is a hundred times smaller than that of iron. Thick specimens require to use of an ultra high voltage electron microscope to detect such small magnetization. The experiment is in being undertaken by using a H-1000FT 1 MeV electron microscope.

2.2. Nano-Mag TEM development: an optimized electron microscope to observe nanomagnetism

2.2.1 Motivation and necessity for Nano-Mag TEM development

We intend to observe both material structures and magnetic structures in nano-scale materials using advanced electron microscopy, including electron holography. To achieve this,

we first developed a TEM that is enhanced to observe nano-magnetism.

Existing TEMs have already been one of the powerful measurement instruments used to study microscopic magnetism. Novel electron microscopy techniques such, as electron holography and Lorentz microscopy, have recently been used to reveal the magnetic structure and behaviors at the nano level. However, in conventional TEM, the specimen has to be placed in the strong magnetic field (~1 Tesla) of an objective lens to be observed with spatial resolution better than 0.2 nm.

For our purpose, we want to observe nano-magnetic specimens in variable weak magnetic fields to understand their magnetic structure and behavior since they are often sensitive to external magnetic fields. When we want to observe the specimen in a zero or weak magnetic field, the objective lens has to be turned off. We also need to observe their atomic structure with a high spatial resolution of 0.3 nm. As mentioned above, obtaining a magnetic free environment and observing high resolution images at the same time is difficult with conventional TEM. In this project we develop a new electron microscope, Nano-Mag TEM, that is capable of high-resolution imaging in a magnetic field free environment.

2.2.2 Nano-Mag TEM outline

The specification of the Nano-Mag TEM has been determined and will be equipped with following components.

(1) 300 kV field emission electron gun (FEG)

The bright and coherent electron beams obtained from a 300 kV FEG are indispensable to observe the nano-magnetic structure, especially when we use the electron holography method.

(2) Electron biprisms

They are also necessary for the electron holography observations. Three electron biprisms will be installed in the Nano-Mag TEM and they will be selected according to the observation condition.

(3) Three dimensional magnetic field chamber (3D MFC)

This is the device to supply the magnetic field with variable amplitude and direction onto the specimen. The residual magnetic stray field from the objective lens is designed to be smaller than 10^{-4}T at the specimen position. In-field observation will be possible in the magnetic field up to 0.01 T and 0.05 T in the horizontal and vertical directions.

(4) Aberration corrector (Long focal Cs corrector)

A nano-magnetic specimen will be located in the 3D MFC, about 10 cm from the objective lens. In such a the long focal-length configuration, a spatial resolution will be severely lowered because of the spherical aberration (Cs) of the long-focal (objective) lens. To obtain the high resolution image with a long focal configuration, a special aberration corrector to compensate the Cs of the objective lens at a resolution of 0.3 nm is being developed.

(5) 4k x 4k pixels CCD camera system

Electron micrographs are digitally acquired by this high resolution and sensitive CCD camera system.

(6) Unified TEM control

Users can control most functions of the TEM through a unified control system. They will be also able to operate the TEM by a user defined control script/program

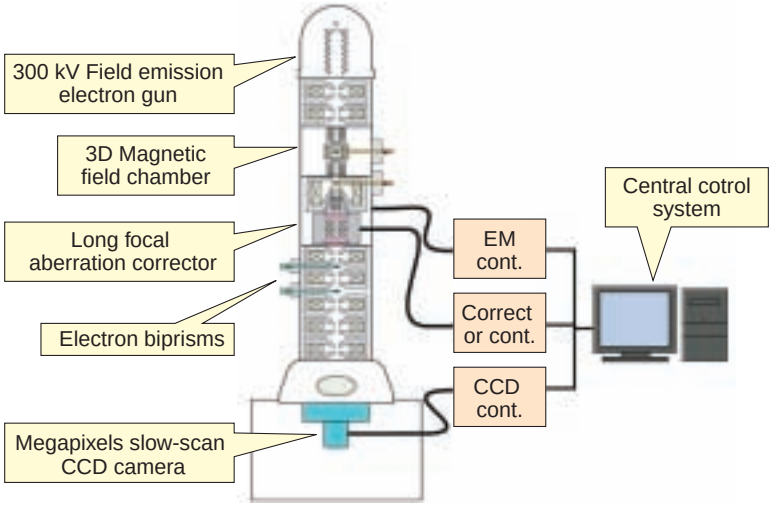


Fig. 2 Nano-Mag TEM concept

2.2.3 Nano-Mag TEM development plan

The development schedule is summarized in a chart shown in Fig. 3

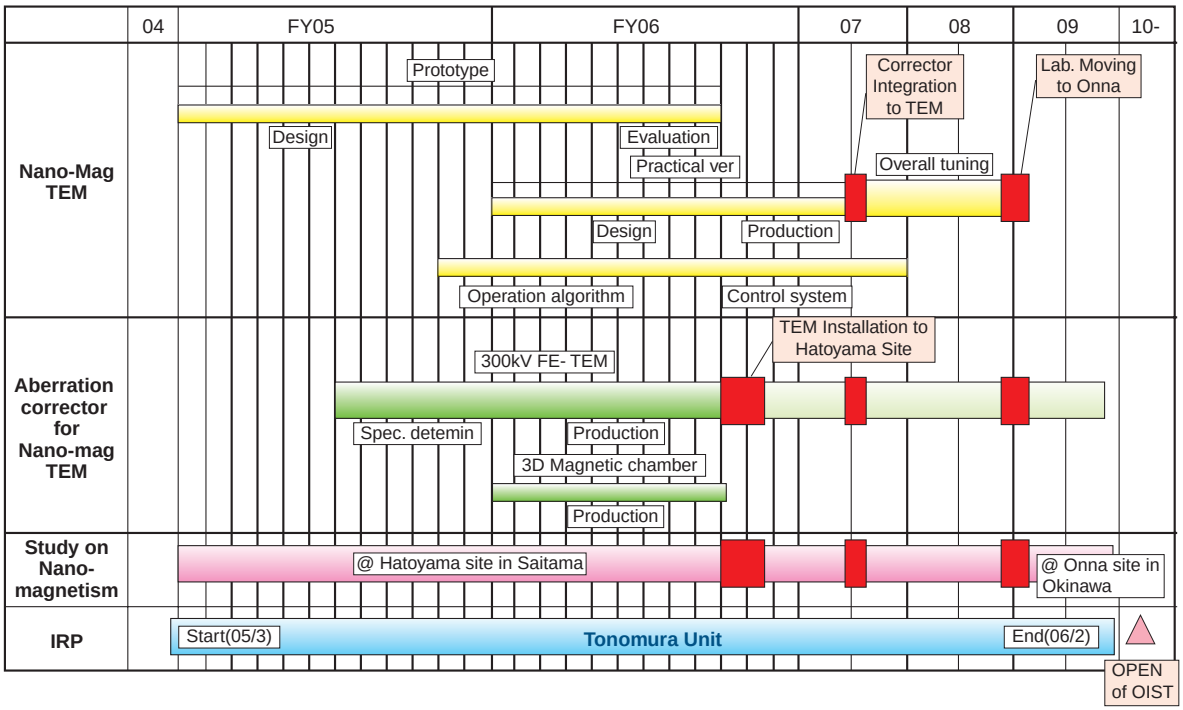


Fig. 3 Nano-Mag TEM and its aberration corrector development schedule

2.2.4 Development status of long focal Cs corrector for Nano-Mag TEM

(1) With the aid of an electron optical simulation software, we determined the fundamental electron optics of the corrector system. (see Fig. 4).

(2) The schematics of a prototype apparatus have been developed to complete the electron optic requirements derived from the simulated production of the prototype Cs corrector started in March, 2006. Components manufactured with a precision better than 5 micro-meters are expected by May, 2006 and the apparatus will be assembled by July, 2006. The basic performance of the prototype Cs corrector will be evaluated in late 2006.

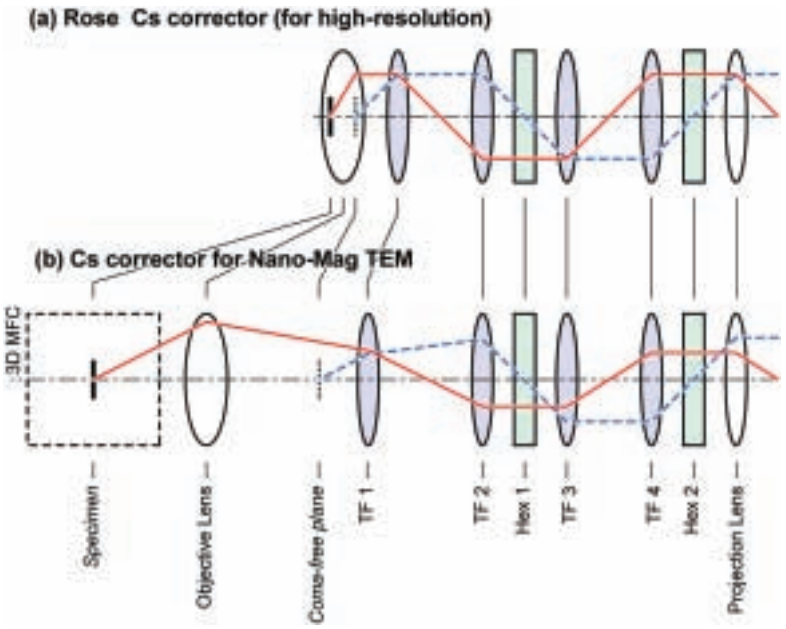


Fig. 4 Electron Optics of (a) Rose's Cs corrector and (b) our long-focal Cs corrector for Nano-Mag TEM (TF: transfer lens, Hex: hexpole lens)

3. Publications

3.1. Journals

Tonomura A., "The Aharonov-Bohm effect and its applications to electron phase microscopy", *Proc. Japan Academy, Ser. B* **82**, No. 2, 45-58 , 2006

3.2. Book(s) or other one-time publications

Nothing to be reported.

3.3. Oral Presentations and Posters

Oral Presentations:

- Tonomura A., "Quantum Phenomena Observed Using Electrons", Quantum Physics of Nature, Vienna, May 20-26, 2005

● Tonomura A., "Quantum Phenomena Observed Using Electrons", 50th Anniversary, of Erwin Mueller, Atomic Resolution Microscopy, Penn State, USA, June 15-17, 2005

● Tonomura A., "The Quantum World Observed by Electron Waves", International Conference Recent Challenges in Novel Quantum Systems, Camerino, Italy, July, 6-8, 2005

● Tonomura A., "Imaging the Quantum World using the Phase Electron Waves", IEEE-Nano 2005, Nagoya, Japan, July 11-15, 2005

● Tonomura A., "Dynamic Observation of Vortices in High-Tc Superconductors by Lorentz Microscopy" Microscopy & Microanalysis, Hawaii, USA, August, 2-5, 2005

● Tonomura A., "Observation of Quantum Phenomena Using Electrons", Looking to the Next 100 Years of Physics and Its Impact on Engineering, Life Science and Technology, Singapore, August 10-12, 2005

● Tonomura A., "Quantum Phenomena Visualized Using Electron Waves" , The 8th International Symposium on Fundamentals of Quantum Mechanics in the Light of New Technology, Saitama, Japan, August 22-25, 2005

● Tonomura A., "Quantum Phenomena Observed using Electron Waves", The 5th International Conference on Materials Engineering for Resources, Akita, Japan, October 20-22, 2005

● Tonomura A., "Dynamic Observation of Vortices by Lorentz Microscopy", JST-CREST Nano-Virtual-Labs Joint Workshop on Superconductivity, Awaji, Japan, December, 19-21, 2005

● Tonomura A., "The Quantum World Unveiled by Electron Waves" Innovative Nanoscale Approach to Dynamic Studies of Materials, Okinawa, Japan, January 9-14, 2006

● Locharoenrat K., Sugawara A., Takase S., and Mizutani G., "Fabrication of Copper Nanowires on the NaCl(110) Faceted Surface", The Physical Society of Japan 2006 Annual (Spring) Meeting, Matsuyama, Japane, Mar. 27-30, 2006

4. Intellectual Property Rights and Other Specific Products

Nothing to be reported.

5. Meetings and Events

Symposium: "Roadmap toward future ultimate electron microscopy"

Date: Oct 14, 2005

Place: Laguna Garden Hotel (Ginowan, Okinawa)

Speakers: Hideki Ichinose (Hokkaido Univ.), Sumio Iijima (Meijo Univ, AIST), Kazutomo Suenaga (AIST), Tsukasa Hirayama (JFCC), Kei-ichi Namba (Osaka Univ.), Yoshio Matsui (NIMS), and Akira Tonomura (OIST, HARL, RIKEN)

VIII. G0 Cell Unit

Principal Investigator: Mitsuhiro Yanagida

Research Theme: Cellular Strategy for Maintaining Starved G0 Arrest and Promoting Vegetative Proliferation

Abstract:

The aim of our research is to identify the genes and their functions that maintain the G0 cell state of *S. pombe*. If such genes are conserved, we will examine whether the same is true in human G0 cells. The *S. pombe* G0 cell may be an ideal model to understand why muscle, heart and neuronal differentiated cells do not divide and remain in the state of G0. The *S. pombe* G0 is defined as the state of cells under nitrogen source starvation. Most cells contain 1C DNA. Cell shape becomes round, and cells never divide. Cells are viable for months by metabolizing glucose and intracellular nitrogen source. Our principal effort this year has been to demonstrate that these never-dividing G0 cells are actually metabolically active and not in the state of dormancy. With the aide of classic and modern genetics and the microarray as well as proteomic approaches, we begin to find the names of genes that may be implicated in maintaining the G0 state. Various approaches have been taken to tackle the problems. (1) Microarray approach to identify transcripts that greatly alter (increase or decrease) in the transition from the G0 state to vegetative state. (2) Proteomic approaches to identify proteins that greatly change in their amounts or state of protein modifications between the G0 and vegetative cell states. (3) Genetic approach to disrupt the genes that are thought to be candidate genes for entering, maintaining or exiting the G0 state. (4) DNA damage repair mechanism in G0 state in comparison with that in vegetative cells. (5) Role of proteolysis machinery in the G0 state maintenance. (6) Search a ts collection (employed in the Mandala project) for identifying mutations that are defective in the maintenance of G0 state at the restrictive temperature. (7) Search chemicals and drugs that inhibit the G0 maintenance.

1. Participants:

1.1. Individuals:

Okinawa Group

-Researchers: Mizuki Shimanuki (Group Leader), Mitsuko Hatanaka, Satoru Mochida (until July 2005), Koji Nagao, Kojiro Takeda

-Research Technicians : Sakura Kikuchi, Ayaka Mori, Aya Kokubu, Lisa Uehara, Tomas Pluskal

-Research Assistant : Tomomi Teruya

Kyoto Group

-Researchers : Chikashi Obuse (part time), Takeshi Hayashi

-Research Assistant : Yukari Matsushita

1.2. Partner Organizations:

Kansai Advanced Research Center, National Institute of Information and Communications Technology

Collaboration: Principal researcher: Yasushi Hiraoka, Researcher: Yuji Chikashige

Theme: "cDNA microarray analysis of *S. pombe* during re-entry into proliferation from G0-like stage"

Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo

Technical Help: Professor: Masayuki Yamamoto, Lecturer: Kayoko Tanaka

Theme: "Gene disruption analysis of *S. pombe*"

2. Activities and Findings:

To pursue the molecular switch problem of cell division and arrest, we are using the fission yeast cells under nitrogen starvation as a model of G0 cells. The state of arrested cells under nitrogen source starvation is defined as G0. It was found that whether G0 cells contain 1C or 2C DNA depended on the way of exit from vegetative state (Mochida and Yanagida, 2006). This was an important finding as 1C and 2C containing G0 cells show many similar phenotypes, but are very different in the modes of damage repair and the timing of first replication after nitrogen replenishment. Round G0 cells that never divide are viable for months by metabolizing glucose and intracellular nitrogen source. We were able to demonstrate that the fission yeast G0 cells are metabolically active and efficient in damage repair. We are at present engaged in three types of approaches: 1) Comprehensive analyses on transcriptome and proteome between G0 and proliferating states. 2) Screening/searching for key molecules/pathways for the G0-control using gene disruption and temperature-sensitive mutants. 3) Detailed investigations on the focused biological processes such as DNA damage repair, ubiquitin-mediated protein degradation, transcription etc in the G0 phase. It was found that transcriptome is globally altered during the transition from the arrested G0 state to the state of proliferation. Gene disruption for a number of altered transcripts that may maintain the G0 state has been initiated. We have succeeded to develop the technique of quantitative analysis on proteome using mass spectrometry, and detected ~800 proteins so far with their quantitative data at G0 and vegetative state. Approaches to key regulator genes/pathways are ongoing using gene disruption, mass ts mutant analyses and chemical screening that cause the toxicity to the G0 cells. In our initial analyses, proteasome and probably also the *tor2*$^{+}$ gene were found to be required for maintaining the G0 state at the restrictive temperature. Localization of proteasome was distinct from

vegetative cells and under the control of exportin/Crm1. In addition, ts cells defective in the *tor2*$^+$ gene entered the G0 like stage at the restrictive temperature even in the nutrient medium. Analysis of DNA damage repair showed that the modes of DNA repair were distinct between G0 and vegetative states, and between 1C- and 2C-containing G0 cells. In G0 cells, Chk1 was not required, whilst Rad3/ATR and Crb2/53BP1 were needed for damage repair.

2.1. Searching for key regulators/pathways for the G0 control

Gene disruption of the candidate regulators

A number of genes that showed significant changes in the transcript levels upon nitrogen replenishment (see below) are chosen for disruption. Some thirty genes have been disrupted. Disruption phenotypes will be observed under the nitrogen starvation.

Screening mutants that show the G0 like arrest in the rich medium

We tried to obtain fission yeast temperature-sensitive (ts) mutants that are arrested in the cell cycle progression with 1C DNA content at the restrictive temperature. First we selected about 100 mutants from the ts mutant library, whose cell sizes were small at the restrictive temperature. Plasmids that could rescue the ts phenotypes had been already cloned from the genomic DNA library. Then DNA contents of these mutants at the restrictive temperature were investigated by FACS flow cytometry. Three mutants among them, H393, H523 and H287, displayed 1C peak as well as 2C peak after 6 hrs at 36°C, whilst the wild type cells displayed only 2C peak. Genetic analysis established that H287 contained the mutation in the *tor2* gene (Figure 1), which encoded a phosphatidylinositol kinase that played an important role in the nutrient-signaling pathway. One of multicopy-suppressor genes encoded a putative tRNA methyltransferase, suggesting the function of Tor2 in translation. Analyses on the other two mutants, H393 & H523, are now ongoing.

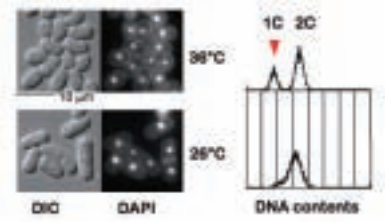


Figure 1, Phenotypes of tsH287 mutant, defective in *tor2* gene

2.2. Analyses of the focused biological processes in G0 state

DNA damage response in G0 cells

We show that G0 cells contain an efficient DNA damage repair system by evaluating whether the S. pombe cells arrested under nitrogen starvation can repair the damages made by irradiation of UV, gamma-ray and DNA damaging drugs. We found that, while DNA damages were efficiently repaired in G0 cells, the modes of DNA damage repair were very distinct between vegetative and G0 cells. As expected, the

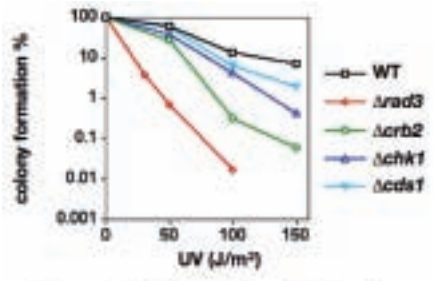


Figure 2, Cell viability of G0 cells defective in rad3, crb2

checkpoint kinase Chk1 was not required for the damage response in the arrested G0 cells, but other checkpoint proteins Rad3 (ATR-like) and Crb2 (53BP1-like) were needed (Figure 2 and 3). The double strand break (DSB) repair in 1C-containing G0 cells requires Pku80, one of non-homologous end joining (NHEJ) proteins. In addition, we found by pulsed field gel electrophoresis that UV-induced DNA damages were converted into double strand breaks (DSBs) in an UVDE-nuclease dependent manner both in vegetative and G0 cells. The repair of such DSB repairs in the 1C G0 cells might be error-prone and often lead into lethality.

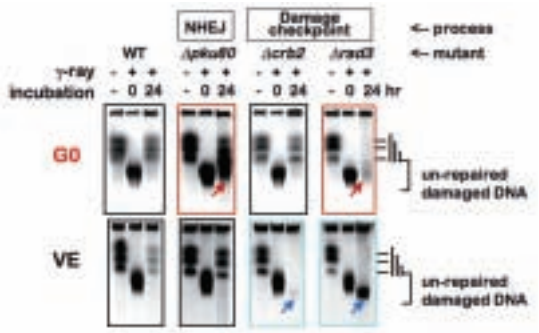


Figure 3, Distinct Modes of DNA Damage Response in *S. pombe* G0 and Vegetative Cells

Ubiquitin/proteasome dependent proteolysis in G0 cells

Though it is well known that ubiquitin dependent proteolysis plays a pivotal role for regulating vegetative cell division, its role in the G0 state is little understood. To gain information whether proteasome is essential in G0, we employed proteasome mutant and examined cell viability of proteasome-deficient mutants in G0. Temperature sensitive *mts3-1* and *pad1-932* mutants of the 19S proteasome complex were found to lose the viability in G0 phase under nitrogen starvation. Indeed poly-ubiquitinated proteins are accumulated in the mutant cells (Figure 4). Proteasome thus seems to be required for maintaining the G0 cells.

26S proteasome consists of 19S regulatory and 20S core complex in vegetatively growing cells. It was unknown, however, whether proteasome forms the same 26S complex in G0. Immunoprecipitation and following mass spectroscopic (LC/MS/MS) analysis of purified proteasome were performed. Our results clearly demonstrated that the proteins compositions of G0 proteasome were identical to those of vegetative proteasome.

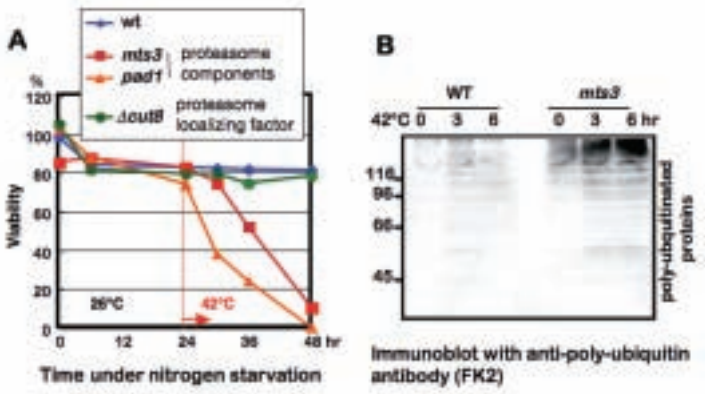


Figure 4, Ub/Proteasome dependent proteolysis is essential in G0.

Intracellular localization of proteasome in G0 cells, however, was found to be different from that in vegetative cells (Figure 5). In vegetative dividing cells, proteasome is mainly localized to the nucleus and the nuclear periphery. This nuclear enriched localization in vegetative cells requires a specific protein called Cut8 that is important for the normal

progression of mitosis and facilitates the efficient destruction of mitotic regulator proteins, Cdc13/cyclin and Cut2/securin that are abundantly present in the nucleus. Interestingly, the localization pattern of proteasome dynamically changes from the nucleus to the cytoplasm (and also nuclear envelope and ER) in the G0 cells upon nitrogen starvation. Cut8 protein level was reduced coincidently with the change of proteasome localization. On the exit from G0, proteasome becomes concentrated again to the nucleus in parallel with re-appearance of Cut8 in vegetative growth. Proteasome and its localization change seem to be a key phenomenon in the transition between G0 and proliferating stages.

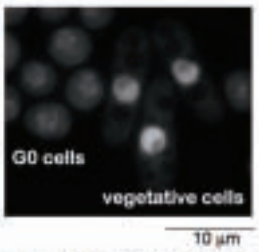


Figure 5, Distinct localizations of GFP-labeled proteasomes in vegetative and G0 cells.

2.3. Comprehensive analyses

Transcripts analysis

We examined the global transcription program during the course from the G0 arrest to vegetative cell cycle, using cDNA microarray. About 55% of the whole genes were up or down regulated more than 2 fold in the transcript level. The altered patterns of the transcripts were analyzed by the Cluster 3.0 software (*Bioinformatics* **20**, 9: 1453-1454, 2004) and resulting gene clusters were classified into "continuous", "transient" "transient (weak)" and "delayed" classes (Figure 6). The shift in transcriptome occurs twice, 1 hr and 2-3.5 hr after replenishment of the nitrogen source. The latter timing corresponds to the start of cell size increase. The patterns between the transcriptome at R3.5/G0 and R6/G0 were similar to those at VE/G0. In Table 1, gene ontology is shown for the gene clusters with similar alteration patterns by the implicated biological processes and cellular components (Table 1). Gene for amino acid biosynthesis, RNA processing, ribosome biogenesis, translation, phosphate and sulfur metabolism, protein folding and targeting, secretion, intracellular transport, microtubule organization, are found in the up-regulated groups, while transcripts for sexual differentiation, autophagy, proteolysis, stress-response, sugar transport, glycolysis, TCA cycle, respiration, signal transduction, negative regulation of CDK, actin cytoskeleton, lipid biosynthesis are found in the down regulated groups. These results are

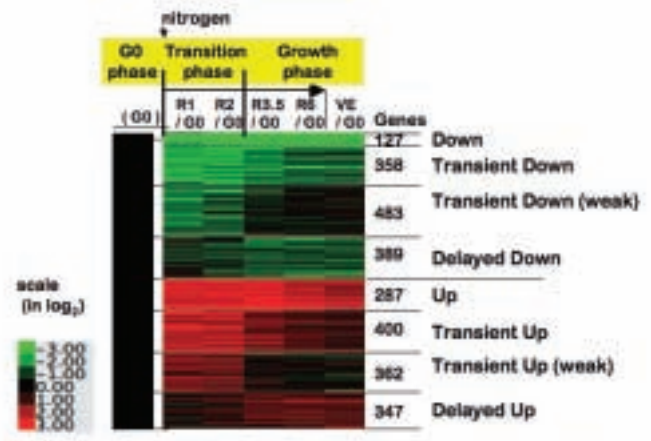


Figure 6, Clusters of genes with similar alterations in transcripts

Table 1, Significant features enriched in the Gene clusters

	Gene Ontology: Biological Process	Gene Ontology: Cellular Component
Down	polyamine catabolism, amino acid transport, disaccaride catabolism, conjugation, meiosis, response to stress	-
Transient Down	carbohydrate (hexose, polyol, and alcohol) catabolism, hexose transport, glycolysis, TCA cycle, autophagy, stress response, retrotransposon	vacuole
Transient Down (weak)	carbohydrate catabolism, pentose-phosphate shunt, oxidative phosphorylation, mitochondrion organization and biosynthesis, ubiquitin-dependent protein catabolism, vesicle docking, lipid biosynthesis, response to stress, DNA repair, regulation of actin filament length, water soluble vitamin metabolism	mitochondrion proteasome core complex endoplasmic reticulum
Delayed Down	conjugation, meiosis, proteolysis, signal transduction, cofactor transport, actin nucleation, negative regulation of CDKs,	endosome, membrane, spindle pole body
Up	rRNA processing, ribosome biogenesis, transcription from RNA pol I promoter, translation, amino acid biosynthesis and salvage, sulfur metabolism, amine (spermidine) metabolism, protein folding,	cytosolic ribosome, eukaryotic 43S preinitiation complex, DNA-directed RNA polymerase I
Transient Up	ribosome biogenesis and assembly, RNA processing (tRNA and rRNA), translation initiation, amino acid biosynthesis (branched chain family, hydrophobic), nucleotide biosynthesis, transcription from RNA pol III promoter, cell wall biosynthesis	nucleolus, nucleus, small nucleolar ribonucleoprotein complex, nucleus, nucleolar preribosome, exosome (RNase complex),
Transient Up (weak)	tRNA processing, translation (peptidyl-histidine biosynthesis), ribosome biogenesis, phosphate transport, establishment/maintenance of cell polarity	site of polarized growth, integral to plasma membrane, nucleus
Delayed Up	secretion, intracellular transport (protein, nucleotide-sugar, Golgi vesicle, mitochondrial matrix), translation, protein glycosylation, protein targeting to membrane, protein folding, vitamin B6 biosynthesis, acetyl-CoA biosynthesis, inner mitochondrial membrane organization, microtubule nucleation and organization	mitochondrial ribosome, mitochondrial matrix, endoplasmic reticulum, coated vesicle, Golgi apparatus,
Low Alteration	mRNA processing, DNA metabolism, transcription, cell cycle, vesicle mediated transport, transcription from RNA pol II promoter, secretion, ubiquitin cycle, lipid modification, di- tri-valent inorganic cation homeostasis, aerobic respiration,	nucleoplasm, chromosome, chromatin, transcription factor complex, spliceosome complex, proteasome complex, replisome, chromatin remodeling complex, vesicle coat, nuclear pore, mitochondrial ribosome, mitochondrial electron transport chain,

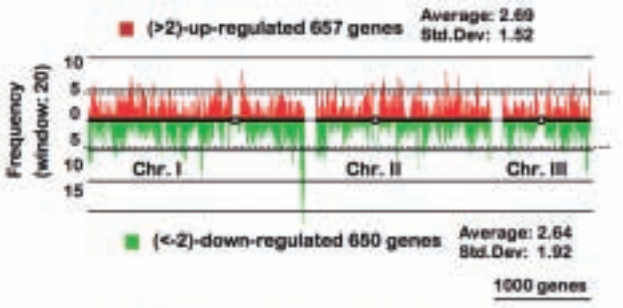


Figure 7, Correlation between transcriptional regulation and chromosomal position

reminiscent of the features of mammalian transcripts from G0 to proliferation. These transcripts are also useful for searching the genes that may be required for the maintenance of or the exit from G0 state. A higher order regulation of transcription may occur as the cluster genes with similar functions exist along distinct regions of the chromosomes (Figure 7).

Proteome analysis

We carried out proteome analysis to compare protein compositions between G0 and vegetative cells. In contrast to the transcriptome analysis, which can provide information only of the relative amount of mRNAs, we can get the information about the modifications and compositions of the proteins in addition to the amount, from the proteome analysis combined with

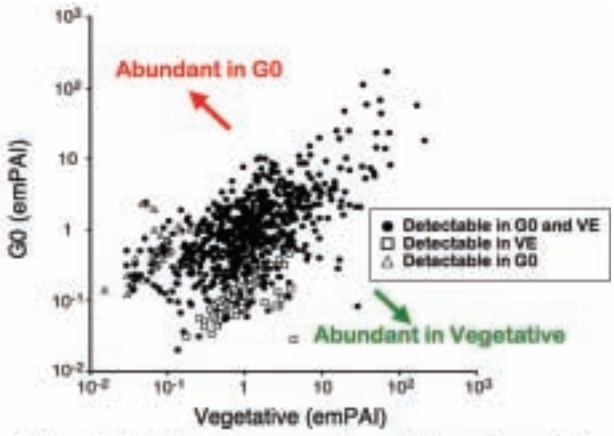


Figure 8, Proteomic comparison of phosphoprotein-enriched fractions between G0 and VE

fractionation of cell extracts. For this end, we have developed the strategy and the software to identify proteins with semi-quantification, using LC-MS (liquid chromatography-mass spectrometry). For example, we enriched phosphoproteins from the G0 and vegetative cell extracts, and analyzed them by LC-MS (Figure 8). About 800 proteins have been so far identified from the cell extracts. Those were then semi-quantified by emPAI (exponentially modified protein abundant index). We found several proteins whose abundances were different between G0 and vegetative cells. This approach can be expected to reveal the regulation for maintenance of G0 state by protein kinase (s) and/or phosphatase (s) .

Searching for chemicals and drugs that are inhibitory to the maintenance of G0 state

Never dividing G0 cells are generally thought to be resistant to drugs, and that is the basis for cancer chemical therapy. Our aim is to search chemicals and drugs that would cause the decrease of cell viability in G0. For its first step, the minimum nutrients for G0 cells to be maintained were tested. Viability of G0 cells in the medium that excluded glucose, vitamin, and/or minerals in addition to the lack of nitrogen source dropped earlier than that in the medium lacking only nitrogen source. These results are consistent with the previous paper by Su et al. We next tested 2-deoxy-glucose (2-DG), an inhibitor of glycolysis that is phosphorylated by hexokinase but not further metabolized. Viability of G0 cells decreased to <10% after 24 hr in the medium containing 2-DG as the sole carbon source (Figure 9). If, however, 2DG and glucose were both added, viability was still high. G0 cells were then treated with various inhibitor drugs (leptomycin B, staurosporine, camptothecin, trichostatin A, cycloheximide). No drug tested so far decreased the viability of G0 cells. Because the drug effect of cycloheximide and LMB has been clearly observed in G0 cells, it was unlikely that all the drugs did not enter the G0 cells. We will further examine many drugs. These results are consistent with a hypothesis that glucose metabolism is essential for maintaining the G0 cells, while many drugs are not effective at all to kill G0 cells as they do not divide.

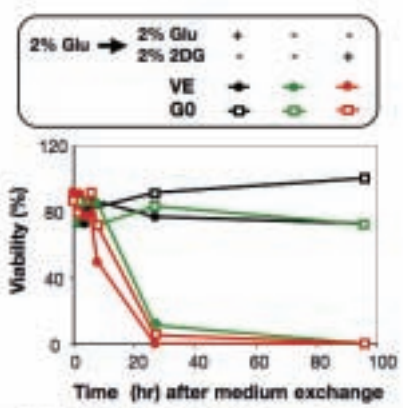


Figure 9, Lethal effect of 2-deoxy-D-glucose

2.4 An additional study: Role of securin for stabilization and inhibition of separase

The securin-separase complex promotes sister chromatid separation in mitotic anaphase. Upon the onset of anaphase, separase is activated by degradation of its inhibitory partner, securin. Separase then cleaves cohesin, which holds sister chromatid together, thus allowing sister chromatid separation. In addition to the inhibition, securin acts as a chaperon for stabilization of separase. However, how securin regulates separase activity mechanistically is

still elusive. We found that securin can have a separase cleavage site by substitutions in the domain required for stabilization and inhibition of separase. These results suggest that securin may regulate the separase activity as a pseudosubstrate of separase (Figure 10).

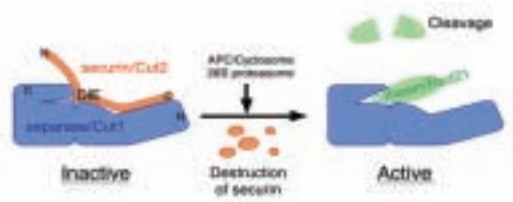


Figure 10, A model how securin inhibits separase protease

3. Publications

3.1. Journals

Takeda, K. and Yanagida, M. Regulation of Nuclear Proteasome by Rhp6/Ubc2 through Ubiquitination and Destruction of the Sensor and Anchor Cut8. *Cell*, 122. 393-405(2005)

Mochida, S. and Yanagida, M. Distinct Modes of DNA Damage Response in *S. pombe* G0 and Vegetative Cells. *Genes Cells*, 11. 13-27(2006)

Nagao, K. and Yanagida, M. Securin can have a separase cleavage site by substitution mutations in the domain required for stabilization and inhibition of separase. *Genes Cells*, 11. 247-260(2006)

3.2. Book(s) or other one-time publications

Mochida, S. and Yanagida, M. Cell cycle and mechanisms of checkpoint activation. *Experimental Medicine*, 24. 335-338(2006) *in Japanese*

3.3. Oral Presentations and Posters

Oral Presentations:

Takeda, K. and Yanagida, M. "Regulation of Nuclear Proteasome by Rhp6/Ubc2, Ubiquitin-Conjugating Enzyme, through Degradation of Cut8, the Sensor and Anchor of Proteasome", *Symposium on the research of life inheritance and its future*, Nara, Japan, May. 20, 2005

Shimanuki, M., Chung, S-Y., Kawasaki, Y., Hatanaka, M., Nagao, K., Chikashige, Y., Hiraoka, Y. and Yanagida, M. "Post-genomic approach to the regulation of G0 phase in fission yeast", *The 38th Yeast Genetics Society of Japan*, Kashiwa, Japan Sep. 5-7, 2005

Yanagida, M. "Genome-wide Mandala gene-network and G0 cell maintenance in *S. pombe*" *A seminar at the Sanger Institute*, Hinxton, UK, Sep. 15, 2005

Nagao, K., Adachi Y. and Yanagida, M., "Separase-mediated Cleavage of Cohesin Is Required for DNA Repair", *The 2nd Shizuoka Bio Science Symposium*, Shizuoka, Jan. 13, 2006

Yanagida, M. "On the fission yeast G0 cell project in Okinawa", *Cell Regulations in Division and Arrest Workshop*, Okinawa, Japan, Mar. 6-9, 2006

Nagao, K. and Yanagida, M. "The Domain of Securin Required for Stabilization and Inhibition of Separase Can Have a Separase Cleavage Site by Substitutions", *European Fission Yeast Meeting*, UK, Mar. 16-18, 2006

Nagao, K., Yuasa, T., Adachi, Y., Ikai, N., Hayashi, T., Katayama, T., Kitagawa, D., Takahashi, K., Nakaseko, Y. and Yanagida, M. "The roles of separase-securin complex for DNA damage repair and the regulation of separase by Cdc48/p97/VCP", *COE Closing Symposium 'Maintenance and Inheritance of Diverse Life by Chromosome Dynamics'*, Kyoto, Japan, Mar. 29-30, 2006

Posters:

Mochida, S., and Yanagida, M. "Analysis of dormant G0 state defines active DNA repair function of checkpoint protein in fission yeast", *The international Symposium on STUDY OF LIFE INHERITANCE*, Nara, Japan, May. 20, 2005

Shimanuki, M., Chung, SY., Kawasaki, Y., Hatanaka, M., Chikashige, Y., Hiraoka, Y., Obuse, C., Mochida, S., Nagao, K., and Yanagida, M. "Post-genomic approach to the mechanism of the G0-state control in fission yeast", *The international Symposium on STUDY OF LIFE INHERITANCE*, Nara, Japan, May. 20, 2005

Shimanuki, M., Chung, SY., Kawasaki, Y., Hatanaka, M., Nagao, K., Chikashige, Y., Hiraoka, Y. and Yanagida, M. "Post-genomic approach to the regulators of G0/division control in Fission Yeast", *The 28th Annual Meeting of the Molecular Biology Society of Japan*, Fukuoka, Japan, Dec. 7-10, 2005

Takeda, K. and Yanagida, M. "The intracellular localization of 26S Proteasome in fission yeast G0-like cells", *The 28th Annual Meeting of the Molecular Biology Society of Japan, Fukuoka*, Japan, Dec. 7-10, 2005

Hatanaka, M. and Yanagida, M. "A screening for mutants involved in exit from cell cycle and maintenance of G0 phase in the fission yeast, *Schizosaccharomyces Pombe*", *Cell*

Regulations in Division and Arrest Workshop, Okinawa, Japan, Mar. 6-9, 2006

Mochida, S., and Yanagida, M. "DNA damage responses are efficient in the *S. pombe* G0 state", *Cell Regulations in Division and Arrest Workshop*, Okinawa, Japan, Mar. 6-9, 2006

Mori, A., Nagao, K., and Yanagida, M. "A search for drugs that are inhibitory to *S. pombe* G0 cells", *Cell Regulations in Division and Arrest Workshop*, Okinawa, Japan, Mar. 6-9, 2006

Shimanuki, M., Chikashige, Y., Chung, SY., Kawasaki, Y., Hatanaka, M., Nagao, K., Uehara, L., Tsutsumi, C., Hiraoka, Y. and Yanagida, M. "Transcriptional Program of Fission Yeast G0 Cells in Response to Nutritional Replenishment", *Cell Regulations in Division and Arrest Workshop, Okinawa*, Japan, Mar. 6-9, 2006

Takeda, K., Kikuchi, S., Nagao, K., Kokubu, A. and Yanagida, M. "The essential role and the distinct localization of the fission yeast 26S Proteasome in G0 and vegetative cells", *Cell Regulations in Division and Arrest Workshop*, Okinawa, Japan, Mar. 6-9, 2006

4. Intellectual Property Rights and Other Specific Products

Nothing to be reported

5. Meetings and Events

Cell Regulations in Division and Arrest Workshop

Date: March 6th-9th, 2006

Place: Hotel Grand Mer, Okinawa Health Biotechnology Research & Development Center

Organizer:

Mitsuhiro Yanagida (OIST, Japan)

Invited Speakers:

Jürg Bähler (Sanger Institute, Hinxton, UK)
Yuji Chikashige (Kansai Advanced Research Center, Japan)
Aaron Ciechanover (Technion-Israel Institute of Technology, Israel)
Andrea Ciliberto (European Institute of Oncology, Italy)
David Glover (Cambridge University, UK)
Tim Hunt (Cancer Research UK, UK)
Takumi Kamura (Nagoya University, Japan)
Jianhua Liu (Genome Institute of Singapore, Singapore)
Sergio Moreno (University of Salamanca, Spain)
Keiichi Nakayama (Kyushu University, Japan)

Keiji Nishida (Rikkyo University, Japan)
David Pellman (Harvard Medical School, USA)
Mari Shimura (International Medical Center of Japan, Japan)
Toshio Suda (Keio University, Japan)
Takashi Takeuchi (Mitsubishi Kagaku Institute of Life Sciences, Japan)
Fuyuhiko Tamanoi (University of California, Los Angeles, USA)
Takeshi Tomonaga (Chiba University, Japan)
Fumiko Toyoshima (Kyoto University, Japan)
Masayuki Yamamoto (University of Tokyo, Japan)
Hiro Yamano (Marie Curie Institute, UK)
Mitsuhiro Yanagida (IRP, OIST/ Kyoto University, Japan)
Minoru Yoshida (Riken, Wako Institute, Japan)
Anders Zetterberg (Karolinska Institute, Sweden)

IX. Molecular Neuroscience Unit

Principal Investigators: Sydney Brenner, Ichiro Maruyama, and Takayuki Naito

Research Theme: Molecular neuroscience towards an understanding of the development, function and evolution of neurons, brains and nervous systems.

Abstract:

On December 1, 2005, a new group, Molecular Neuroscience, consisting of three units was established. The units occupy the western half of the third floor of the Okinawa Industrial Technology Center, Uruma, Okinawa. Since then, active recruitment of researchers and Research technicians has been carried out, and purchase of common major equipment has also been completed.

1. Participants:

1.1. Individuals:

-Researchers: Takashi Murayama, Michael Chandro Roy .

-Research Technicians: Mayuki Fujiwara, Hiraku Miyagi, Sayaka Arai .

1.2. Partner Organizations:

Joint Genome Institute, DOE, USA.

Joint research

Principal researcher: Sydney Brenner

Theme: "Sequencing of octopus genome"

X. Education and Training Activities

Collaboration with Universities

We have concluded ACADEMIC EXCHANGE AGREEMENT with Nara Institute of Science and Technology (NAIST) with a view to promoting exchange between the two institutes in the fields of education and research. Several graduate students are participating in the IRP in accordance with this agreement.

In order to use radio isotope facility and library we have concluded JOINT RESEARCH AGREEMET with University of the Ryukyus. Under this agreement some research units of IRP are carrying out their research activities using the radio isotope facility and library of University of the Ryukyus. We have a plan to extend the relationship with University of the Ryukyus coming fiscal year.

International Workshops

International Workshops, as well as IRP, are one of the precursory activities for the Okinawa Institute of Science and Technology. The purposes of International Workshops are to establish close relations with domestic and foreign scientists through exchanging scientific knowledge, to spread the research result of IRP and to provide opportunities to study advanced natural science for young researchers who have interest in OIST.

In FY2005 following two workshops were held;

OCNC (Okinawa Computational Neuroscience Course) 2005

Outline: The aim of OCNC2005 was to provide opportunities for young researchers with theoretical backgrounds to learn up-to-date neurobiological findings, and those with experiment backgrounds to have hands-on experience in computational modeling. The special topic for this year's course was "Predictions and Decisions."

Date: June 1- 10, 2005

Organizer: Kenji Doya (OIST)
Peter Dayan (Gatsby Computational Neuroscience Unit, University College London)
Masamichi Sakagami (Tamagawa University)

Place: Rizzan Sea-Park Hotel, Tancha-Bay (Onna-son)

Participants: Organizer+ lectuer 15, Tutor 10, Student 35

Discussion

Group picture

Workshop "Cell Regulations in Division and Arrest"

Outline: The aim of Workshop "Cell Regulations in Division and Arrest" was to provide opportunities for researchers interested in how cells maintain the state of arrest or division and what would be the key molecules and cellular events to determine the fates of cells in regard with division and arrest. Part of the workshop was held as an open session focusing on the cancer research.

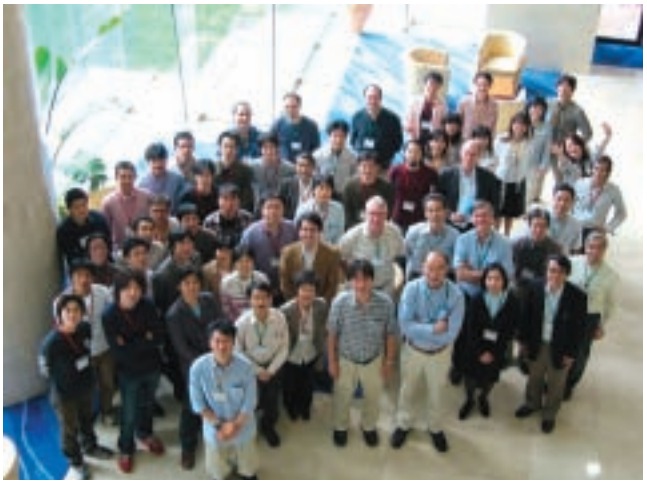
Date: March 6-9, 2006

Organizer: Mitsuhiro Yanagida (OIST)

Place: Hotel Grand Mer (Okinawa-shi), Okinawa Health Biotechnology Research and Development Center (Uruma-shi)

Participants: Organizer+ Speakers 22, Participants 33

Open session talk by
Dr. Aaron Ciechanover



Group photo of workshop participants

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