Okinawa Institute of Science and Technology Promotion Corporation

Annual Report
Fiscal Year 2006

Okinawa Institute of Science
and Technology
Promotion Corporation

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Preface

This year has seen the rapid development of the research activities of OIST. We have appointed a further 6 PIs and, during next fiscal year when all have taken up their positions in Okinawa, the majority of our scientists will be non-Japanese thus fulfilling the plan to make OIST an international research organization. The successful remodeling of the Seaside House has given us an excellent facility for workshops, our headquarter activities and space to house developments in Mathematical & Computational Biology.

In today's global world of science, new institutions such as ours have to find some special focus in order to achieve excellence and international recognition. Some may wish us to populate wider areas of basic science more quickly, but this would spread us far too thinly at this stage of development. Our coherence and unity of purpose depends on everybody working together and this is the only basis for building the future University.

A significant feature of our progress is the constant and strong participation of our scientists in the running and development of OIST both as individuals and through COPI (Committee of Principal Investigators). My thanks go to them and indeed to all of our staff who have helped us through this first full year of operation.

I must also acknowledge the help of our Special Advisors: Dr. Robert Baughman (Academic Affairs), Dr. Chris Tan (International Relations) and Dr. Hiroaki Kitano (Mathematical & Computational Biology).

Sydney Brenner
President
Okinawa Institute of Science and Technology Promotion Corporation

I. Events

The Okinawa Institute of Science and Technology Promotion Corporation was established on September 1st, 2005. The Corporation was established to prepare the opening of the graduate school university.

The main events in FY2006 were as follows:

April 24, 2006
Headquarter of the Corporation moved to the OIST Seaside House (formerly, "Hakuun-so") in Onna Village.

May 25, 2006
Opening Ceremony for the OIST Seaside House was held at the Seaside House with attendees including Mr. Kakazu, Senior Vice-Minister of State for Okinawa and Northern Territories Affairs; Mr. Inamine, Governor of Okinawa Prefecture; Mr. Shikiya, Mayor of Onna Village.

May 27, 2006
The second Board of Governors meeting was held in Tokyo.

December 11, 2006
The third Board of Governors meeting was held in Tokyo.

II. BOG Meeting Summary

The Board of Governors (BOG) was established under the Corporation Act in 2005. The tasks of BOG are to oversee the Corporation and advise the President. BOG operates within Articles 12 and 13 of the Corporation Act. In September 2005, Japanese Prime Minister, Junichiro Koizumi, appointed the current Board members.

From December 2006, Dr. Arima and Dr. Wiesel are co-chairs of the Board.

<Members of the 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 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	Nobel Laureate (Physiology or Medicine, 1987) Professor, MIT
Dr. Torsten Wiesel*	Secretary General, Human Frontier Science Program Organization Nobel Laureate (Physiology or Medicine, 1991)
Dr. Kiyoshi Kurokawa	Special Cabinet Adviser Former President, Science Council of Japan

* Co-chairs

[The 2nd BOG meeting]

The 2nd BOG meeting was held on May 27, 2006 in Tokyo. Attending Board members were: Dr. Akito Arima, Dr. Jerome Friedman, Dr. Kiyoshi Kurokawa (Board Cheiu), Dr. Hiroko Sho, Dr. Susumu Tonegawa, and Dr. Torsten Wiesel. Invited guests included Senior Vice-Minister Chiken Kakazu; Director-General Bunshichi Fujioka and Deputy Director-General Tomoaki Wada from the Cabinet Office; Honorable Mr. Koji Omi.

The following items were covered in the meeting agenda:

- Senior Vice-Minister Kakazu conveyed message from Minister Koike.
- The President welcomed members of the Board to this meeting, and emphasized that this meeting was critical to progress to the next stage of development of OIST.
- The President reported annual plan, medium-term plan revision, scientific program/recruitment, new initiative, workshop, education & training activities and financial reports.
- The Project Manager highlighted the recent events related to the Campus Master Plan, including review with each member of the Board about the Basic Design Plan.
- Roles of the Board of Governors were discussed. A conclusion was not reached at the time. Follow up discussion would be held at later stage.

[The 3rd BOG meeting]

The 3rd BOG meeting was held on December 11, 2006 in Tokyo. Attending Board members were: Dr. Jerome Friedman, Dr. Kiyoshi Kurokawa (then co-chair), Dr. Hiroko Sho, Dr.

Susumu Tonegawa, Dr. Torsten Wiesel (co-chair). Invited guests included Dr. Ichiro Kanazawa, President, Science Council of Japan; Director-General Shoji Harada and Deputy Director-General Takehiro Fukui from the Cabinet Office.

Following the assumption of Special Cabinet Adviser, Dr. Kurokawa announced that he would step down from the chairpersonship while Dr. Arima and Dr. Wiesel would be co-chairs.

The following items were covered in the meeting agenda:

- Director-General Harada conveyed message from Minister Takaichi.
- The President expressed that he intends to remain in his office until his task is accomplished despite the recent news coverage. Board members unanimously agreed that they will continue to support the efforts of the President.
- The President reported on the progress of the PI recruitment, which has made better progress than planned.
- Status of Campus Construction was reported which included: (a) Progress of design and preliminary works, (b) Land acquisition, contracts planed and executed, (c) Plan for financing the residential village and its completion.
- Cabinet Office reported on the Budget request for FY2007 and the Evaluation Committee of OIST PC.
- Report was given about the academic activities with focus on the workshops.

III. Campus Design and Construction Status

[Campus design]

The 2006 Fiscal Year began in April with the basic design for the campus of the 50 PI phase of the university completed and awaiting approval from the Board of Governors, and the renovation of Hakuun-so (now, Seaside House) essentially complete and ready for the OIST administration team to move into. An opening event was held at the Seaside House on May 25.

Several Board Members met with the design team on May 26 to review the basic design materials. This session was followed by a BOG meeting in Tokyo on May 27 where the Board approved the basic design as submitted, and authorized work to proceed on developing the detailed design of the campus and facilities, in an integrated manner and to a level of quality that will support the 'best in the world' objective of the project. Target completion dates were set for the construction of the campus as follows: Lab 1 and the Central Building by March 2009, Lab 2

by September 2009, and Lab 3 by 2012 at the latest, with housing and other village facilities to be developed in parallel with the labs to suit the growing population of OIST.

A further Board Meeting was held in Tokyo on December 11. During this meeting the progress of the campus design work was reviewed by the BOG members. They indicated general satisfaction with the design being developed.

Once the detailed design of the lab zone buildings was completed at the end of January, a detailed design review session was held on February 19 in Okinawa with representatives of the Campus Planning Group(CPG), to confirm that the final drawings accurately incorporated the input that the CPG groups had provided over the preceding months.

With the completion of the building works' detailed design in January, the architects' attention shifted to prepare a detailed cost estimate for the construction works, in advanee of the bidding of the building works packages, which will get underway early in Fiscal Year 2007.

[CPG working group activities]

To provide input to the architects on the technical requirements for the labs, vivarium and other shared support facilities, CPG was established with the participation of specialists from universities and research institutions in Japan and the USA. The vivarium working group met first on June 29, then again on July 31. At this meeting the decision was reached to construct a higher-order center detached from the rodent and small animals vivarium, to avoid potential contamination and interference between the two functions, and enable optimal facilities. It was also decided to provide a separate zebrafish aquarium. The group met again on September 15 and two further working sessions were held in the US in October.

The shared facilities working group meanwhile met first on August 1 then again on September 15. At this later date, the CPG members reviewed the design solutions proposed by the architects and recommended that these be forwarded to OIST President for confirmation. He reviewed the proposals on September 22 and gave his approval with some provisos. Development of all aspects of the lab zone design continued, with the architects submitting model laboratory layouts for biology, organic chemistry, molecular chemistry or physics, and computational activities in October. The design for the main cafeteria and administrative areas was developed in parallel with consultation with OIST staff.

[PFI matter]

The Cabinet Office began investigating the potential to engage the private sector to develop the campus village – the housing and related facilities – and in September selected Private Finance

Initiative (PFI) consultant to carry out a feasibility study on this matter. Their findings were submitted in December, and indicated that a PFI approach could be workable, provided that their proposed prerequisite targets for variables such as rents and other charges, facility utilization rates, construction costs and finance charges were achieved.

[Environmental impact assessment]

Work on the Environmental Impact Assessment (EIA) continued throughout the year. The Okinawa Prefecture Environmental Assessment Committee visited the site in April. Comments from the Governor were received in August, and after taking all interested parties' comments into account, the EIA application was formally submitted on December 04. The EIA was duly approved by the Prefecture on schedule on February 13.

[Civil works]

Detailed design of the civil works was commissioned in June and was largely completed in October, although bridge design work is scheduled to continue for several more months. Discussions subsequently commenced with Okinawa Prefecture concerning the application for the site Development Permit, which is required before permanent works can begin on site. The permit, which was originally targeted for issue on February 15, 2007, was in fact received on March 02.

Preparations for the procurement of the construction works got underway in July with the project manager preparing a paper on possible bidding strategies to encourage open, competitive procurement of the construction works. The strategy was finalized in October. It was decided to apply the Comprehensive Evaluation Bid Process for the first time in Okinawa for major elements of the work, to ensure that bids would be fairly evaluated on their technical merits as well as on price. An initial small package of temporary road and bridge works was awarded on January 29. This was followed by the first phase of the site development works on February 16 and construction of the access tunnel and elevator shaft on March 15.

IV. Administration and Finance

[Overall matters]

The Office of the President has been established and all planning activities have been conducted there as individual projects. Four Special Advisers have been appointed so far to be in charge of the following matters.

Dr. Robert Baughman (Senior Adviser)	Planning and implementation of research and training program
Dr. Hiroaki Kitano	Development of the project in Mathematical and Computational Sciences
Dr. Chris Tan	Building up relations with universities and research institutions in Asia-Pacific countries
Dr. Mamoru Tamura	Development of the project in imaging facility

Committee of PIs (COPI) had regular monthly meetings, where especially the recruitment exercise was conducted and various scientific planning was discussed. The members of COPI include President, Special Advisors, PIs and the Director of Research and Training Division.

Management Committee (MACO) had occasional meetings, where there are discussions about key issues, reporting on financial matters and progress of campus construction planning. MACO includes President, Executive Director, Directors, and a PI representative.

With COPI and MACO, communication between scientists and administration has been ensured.

[Finance]

The total budget for FY2006 was 7.678 billion yen, which was approximately 50% increase from the previous year, 5.089 billion yen. The budget increase was primarily attributable to the budget for construction.

<Budget comparison for FY2005 and FY2006> **Million Yen**

Year / Breakdown	2005 Budget			2006 Budget
	Cabinet Office*	OIST PC	sum	
Facilities Improvement Subsidy	397	605	1,003	3,530
1) Land Development	-	275	275	903
2) Construction	-	-	-	1,323
3) Infrastructure Improvement	-	-	-	650
4) Land Acquisition	-	-	-	654
5) Hakuun-so Renovation	-	330	330	-
6) Hakuun-so Purchase	397	-	397	-
Operation Subsidy	1,534	2,553	4,087	4,148
1) Research Expenses, Workshops etc	1,130	2,044	3,173	3,461
2) General Administrative Expenses	404	510	914	687
Grand Total	1,931	3,158	5,089	7,678

Note: Budget for FY2005 is made up of two parts: one is the budget for the Cabinet Office during April 2005 to August 2005; the other is the budget for OIST PC during September 2005 to March 2006.

[Operation]

The Enterprise Resource Planning (ERP) system introduced in the previous fiscal year became fully operational. In order to improve the system operation, orientation sessions were held. Following the comments and requests submitted from the users at such sessions, the programs were updated with more functions to achieve more efficiency and effectiveness, especially in the Travel System.

[Rules and regulations]

After recieving legal counsel, some of the rules and regulations, etc. have been revised. Review work continues to improve the current rules and regulations so that they are flexible enough for conducting research while securing compliance.

[Personnel affairs]

The total number of full-time employees (exclusive of officers) is 103 as of March 1, 2007. Of these, 84 are fixed-term employees.

<Breakdown of employees>

Office of the President	1
General Affairs	6
Finance	4
Research and Training	15
Facilities Planning	5
Initial Research Project	72
Total	103

[Staff training]

To improve administrative capabilities, staff menbers are provided with appropriate training opportunities. Notably, we have established English classes for Japanese researchers, technicians and administrative staff to better realize one of the basic concepts of OIST "International" Japanese classes were organized to meet the request of increasing foreign researchers and technicians.

<Training programs in FY2006>

Dates	Program Theme	Organized by	Participants
April 11, September 22, 2006	Grants-in-Aid for Scientific Research	MEXT	1
May 8-June 1, 2006	Presentation Technique	OIST PC	35
June 14, 22, 27, 2006	Sexual Harassment	OIST PC	97
July 25 - September 6, 2006	Presentation Technique	OIST PC	35
September 26-29, 2006	Intellectual Property	National Center for Industrial Property Information and Training	2
October 5-6, 2006	Fire Prevention Management	Okinawa Fire Protection Equipment Maintenance Association	1
November 3, 2006	Mental Health	Occupational Health Promotion Foundation	1
November 14, 2006	Information Disclosure and Individual Information Protection	Kanto District Administrative Evaluation Bureau	1
January 26, 2007	Legal Matters and Actual Practice of Personnel Affairs	Nihon Keizai Shimbun	2
January 29, 2007	Information Disclosure and Individual Information Protection	Ministry of Internal Affairs and Communications	1
All year round	Japanese (language)	OIST PC	13
July 31, 2006-January 31, 2007	English (language)	OIST PC	62
September 29, 2006 January 30-31, 2007	Accounting work	OIST PC	25

[IT infrastructure]

In order to promote a better understanding of the OIST initiative and research, two official websites, that of the Corporation and that of the Initial Research Project (IRP) were consolidated into one official website of the Corporation (http://www.oist.jp/).

As part of the functional development of the OIST Seaside House and the improvement of the overall IT infrastructure, information network was broadened to include IRP and OIST Seaside House. Also a demonstration was carried out to broadcast a workshop taking place at the OIST Seaside House so that the audience at the IRP can see the workshop live.

In order to ensure more efficient administrative procedures, some measures were taken including the establishment of the internal website, establishment of a new groupware with English-Japanese interface and upgrading the e-mail server.

Scientific Report

During 2006 fiscal year, the number of research units has increased from seven to thirteen.

The new research units are as follow:

1. Mathematical Biology Unit

Principal Investigator: Robert Sinclair

Establishment date: June 2006

2. Developmental Neurobiology Unit

Principal Investigator: Ichiro Masai

Establishment date: October 2006

3. Theoretical and Experimental Neurobiology Unit

Principal Investigator: Klaus Stiefel

Establishment date: November 2006

4. Neurobiology Research Unit

Principal Investigator: Jeff Wickens

Establishment date: January 2007

5. Human Developmental Neurobiology Unit

Principal Investigator: Gail Tripp

Establishment date: January 2007

6. Brain Mechanisms for Behaviour Unit

Principal Investigator: Gordon Arbuthnott

Establishment date: January 2007

Our progress can be summarized as follows:

Of the 13 research units, six(6) of the principal Investigators are foreigners.

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 2006 are the following:

1) Dynamical Systems Group: We constructed a computer model of the signaling cascade for dopamine-dependent synaptic plasticity of cortico-striatal synapses. We also developed a Bayesian parameter estimation software for biological networks defined by SBML.

2) Systems Neurobiology Group: We recorded neural activities of the basal ganglia while rats performed a decision making task and found neurons encoding values of candidate actions. We also showed by neural recording and microdialysis measurement of dorsal raphe nucleus that the serotonergic system is activated while rats waited in expectation of delayed rewards.

3) Adaptive Systems Group: We developed a unified learning and evolution framework by which Cyber Rodent robots learned behaviors for survival and reproduction using the reward functions and meta-parameters optimized by evolution.

1. Participants:

1.1. Dynamical Systems Group:

-Researchers: Junichiro Yoshimoto, Atsushi Shinkai (from NEC Software)
-Technician: Yasuhiro Inamine (from NEC Software Okinawa)
-Graduate Students: Michiro Magono, Takashi Nakano

Systems Neurobiology Group:

-Researchers: Makoto Ito, Katsuhiko Miyazaki, Kayoko Miyazaki, Thomas Strosslin
-Graduate Students: Takehiko Yoshida, Alan Rodrigues

Adaptive Systems Group:

-Researchers: Eiji Uchibe, Takashi Sato, William Alexander
-Technician: Stefan Elfwing
-Graduate Students: Viktor Zhumatiy, Tetsuro Morimura, Takumi Kamioka, Makoto Otsuka

Secretary:

-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 system"

NEC Software

Type: Joint research.

Principal Researcher: Mr. Atsushi Shinkai

Theme: "Parallel implementation of Bayesian estimation methods"

Honda Research Institute Japan

Type: Joint research.

Principal Researcher: Dr. Hiroshi Tsujino

Theme: "Biological modeling of basal ganglia in behavioral learning"

2. Activities and Findings:

2.1. Activities and Findings of the Dynamical Systems Group

1) Biochemical model of dopamine-dependent cortico-striatal synaptic plasticity

The dopamine-dependent plasticity of cortico-striatal synapses is supposed to play a critical role in normal behavioral learning as well as in pathology like addiction. Based on a wealth of literature on the signal transduction pathway linking the dopamine input from the midbrain and the glutamate input from the cortex, we built a computer model using GENESIS/kinetikit. Simulation of the model replicated three major features of the cortico-striatal synapses: i) long-term depression (LTD) when cortical input is given without dopamine input; ii) long-term potentiation (LTP) when cortical input is associated with dopamine input, iii) LTP when a strong cortical input causes a very large increase in intracellular calcium ion concentration.

Furthermore, by simulation the knockout of DARPP-32 (dopamine and c-AMP regulated phospho protein), we showed that the positive feedback dynamics realized by this molecule plays an essential role in dopamine-dependent LTP. This model will be a basis for our future exploration of the mechanisms of the timing dependence of glutamate and dopamine inputs for synaptic plasticity.

2) Bayesian system identification of biological networks

Bayesian system identification paradigm can provide coherent solutions to a hierarchy of problems in biological modeling, namely, i) hidden variable estimation, ii) parameter search, iii) model selection, and iv) experiment design. Marked features are that the "solution" is not a point, but a probability distribution, and that the assumptions of a modeler is made explicit in a form of prior distribution.

Based on the Bayesian parameter estimation framework for biochemical reaction that we derived last year, we developed a general-purpose parameter estimation tool for models described by SBML (system biology markup language). We verified its performance in a number of benchmark problems and are now combining the parameter estimation engine with a graphic user interface for easy operation by biologists.

We also developed a Bayesian hidden variable estimation framework based on stochastic molecular reaction dynamics and particle filtering. We implemented the method using MPI, a standard library for parallel computers, and verified its basic performance. We will further optimize its performance and combine it with our Bayesian parameter estimation system described above.

2.2 Activities and Findings of the Systems Neurobiology Group

1) Action and action value coding in the basal ganglia network

In order to understand the mechanisms of action selection by prediction of rewards, we performed neural recording from the striatum and the pallidum while rats learned a probabilistic reward-based free choice task. We found neurons encoding the selected action or the resultant reward, but also those encoding reward expected for a candidate action, both in the striatum and the pallidum.

We further modeled the rats' choice behaviors by a generalized reinforcement learning model and Bayesian hidden-variable and parameter estimation method. The estimated model could predict the animals' choice better than the best higher-order Markov model did. We then performed a regression analysis of striatal and pallidal neuron firing during one second before and after action initiation. The result showed that there are both action coding and action-value coding neurons both in the striatum and pallidum, both before and after action initiation. From this result, we are considering a new model of action selection in the cortico-basal ganglia network.

2) Role of serotonin in delayed reward expectation

In order to test our hypothesis that serotonin regulates the time scale of future reward prediction, we performed neural recording and microdialysis measurement from the dorsal raphe nucleus, the major source of serotonergic projection to the cortex and the basal ganglia.

By microdialysis experiments, we found that the level of serotonin release is significantly elevated when a delay is introduced before reward delivery in a food-water

navigation task. Such increase in serotonin release was not seen when the rewards were omitted in two out of every three trials. These results are consistent with our hypothesis that serotonin facilitates behaviors for delayed rewards, but not consistent with another hypothesis that serotonin encodes expectation of punishment or omission of expected reward.

Further, by multiple electrode recording from the dorsal raphe nucleus, we found a marked increase of serotonin neuron firing while the rat stayed at the food or water dispenser in expectation of reward delivery. When the rat abandoned to wait, it was often preceded by diminished firing of serotonin neurons. These findings also support the hypothesis that activation of serotonin is necessary for behaviors directed for delayed rewards.

2.3. Activities and Findings of the Adaptive Systems Group

We aim to understand computational mechanisms for satisfying two critical requirements for life: self-preservation and self-reproduction. We are exploring the roles of learning, evolution, and communication in adaptive behaviors including survival and reproduction. We are running a series of experiments using the Cyber Rodent robots, which can realize self-preservation by capturing battery packs and self-reproduction by copying its programs or parameters by infrared communication.

1) Embodied evolution of reward functions and meta-parameters

While standard genetic algorithms assume a central manager who selects best individuals and sets up the next generation, embodied evolution is realized by distributed agents that select each other and reproduce by local interactions. We developed an embodied evolution framework for Cyber Rodents, in which the visual rewards for orienting to targets and meta-parameters of reinforcement learning (e.g., learning rate) of battery-capturing and mating behaviors are optimized by evolution. 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. We verified in simulation that the visual reward functions matched the physical feature of the environment and that the evolved meta-parameters indeed accelerated learning. We also verified the method in hardware experiments.

2) Learning to communicate and cooperate

The origin of learned communications, including languages, is a highly intriguing problem. Previous computational studies of the origin of language investigated how lexicons or grammars can emerge, but under the implicit assumption that the agents use linguistic communication. We asked a more fundamental question: in what condition agents start to use their excess degrees of action freedom for communication, and how the dual mappings from situation to signaling and from perceived signals to appropriate reactions can concurrently be learned. We developed a simple two-agent game in which each agent is rewarded by stepping into another's territory but punished upon a collision, which could be avoided by appropriate

visual signaling. By use of a standard reinforcement learning algorithm, agents with action and sensing capability for visual signaling could learn to map different behaviors (go ahead or back up) to different signals (light on or off). Interestingly, we found variety of "dialects" in signal-meaning mapping and also asymmetric role taking, such as a leader and a follower.

3) Robust and efficient learning algorithms

We also developed a number of novel reinforcement learning algorithms:

a) a method for dealing with both soft and hard constraints in reinforcement learning.

b) a novel natural policy gradient learning algorithm that resolves instability from matrix inversion.

c) a max-min reinforcement learning algorithm for robust learning of multiple object tasks.

d) a new method for task-dependent state coding by neighborhood component analysis.

These have been verified in simulation and will be tested in robotic experiments.

3. Publications

3.1. Journals

Bapi, R.S., Miyapuram, K.P., Graydon, F.X. & Doya, K. (2006) fMRI investigation of cortical and subcortical networks in the learning of abstract and effector-specific representations of motor sequences. Neuroimage, 32(2), 714-727.

Daw, N. & Doya, K. (2006). The computational neurobiology of learning and reward. Current Opinion in Neurobiology, 16(2), 199-204.

Doya, K. (2005-2006). Introduction to computational neuroscience, part 1 to 14. Mathematical Sciences (in Japanese).

Elfwing, S., Doya, K., Christensen, H. I., Evolutionary development of hierarchical learning Structures, *IEEE Transactions on Evolutionary Computations,* (in press).

Hirayama, J., Yoshimoto, J. & Ishii, S. (2006). Balancing plasticity and stability of on-line learning based on hierarchical Bayesian adaptation of forgetting factors. Neurocomputing, 69(16–18), 1954–1961.

Kawawaki, D., Shibata, T., Goda, N., Doya, K. & Kawato, M. (2006) Anterior and superior lateral occipito-temporal cortex responsible for target motion prediction during overt and covert visual pursuit. Neuroscience Research, 54(2), 112-123.

Morimoto, J. & Doya, K. (2007) Reinforcement learning state estimator. Neural Computation, 19(3), 730-756.

Ogasawara, H., Doi, T., Doya, K. & Kawato, M. (2007) Nitric oxide regulates input specificity of long-term depression and context dependence of cerebellar learning. PLoS Computational Biology, 3(1), e179.

Sato, T., Uchibe, E., & Doya, K. (in press), Emergence of communication and cooperative behavior by reinforcement learning agents. Transactions on Mathematical Modeling and its Applications (in Japanese).

Schweighofer, N., Shishida, K., Han, C.E., Okamoto, Y., Tanaka, S.C., Yamawaki, S. & Doya, K. (2006) Humans can adopt optimal discounting strategy under real-time constraints. PLoS Computational Biology, 2(11), e152.

Sugimoto, N., Samejima, K., Doya, K. & Kawato, M. (2006) Hierarchical reinforcement learning: Temporal abstraction based on MOSAIC model, *Journal of IEICE (The Institute of Electronics, Information and Communication Engineers)* (in Japanese).

Tanaka, S.C., Samejima, K., Okada, G., Ueda, K., Okamoto, Y., Yamawaki, S. & Doya, K. (2006) Brain mechanism of reward prediction under predictable and unpredictable environmental dynamics. Neural Networks, 19(8), 1233-1241.

Uchibe, E. & Asada, M. (2006) Incremental co-evolution with competitive and cooperative tasks in a multi-robot environment, *Proceedings of the IEEE*.

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

Doya, K., Ishii S., Pouget, A., Rao, R. P. N. (2007). Bayesian Brain: Probabilistic Approach to Neural Coding. MIT Press.

Sato, T., & Hashimoto., T. (2007). Dynamic social simulation with multi-agents having internal dynamics. In A. Sakurai, et al (Eds.), New Frontiers in Artificial Intelligence: Joint Proceeding of the 17th and 18th Annual Conferences of the Japanese Society for Artificial Intelligence. LNCS, Springer Verlag.

3.3. Oral Presentations

Doya, K., Analysis of brain functions by learning model-based estimation of hidden variables. The Annual Meeting of the Medical and Biological Engineering, Fukuoka, Japan, May 17, 2006 (in Japanese).

Doya, K., Reward prediction in basal ganglia and its modulation by serotonin. Keck Center for Theoretical Neurobiology, University of California, San Francisco. May 30, 2006.

Doya, K., Action values and action selection in the cortico-basal ganglia loops. Symposium on Reward and Decision Making in Cortico-Basal Ganglia Networks. Arrowhead, California, June 3, 2006.

Doya, K., Computing neurons: an introduction. Okinawa Computational Neuroscience Course 2006. Okinawa, June 26, 2006.

Doya, K., Designs of reward systems in animals, humans and robots. Symposium: What makes a reward worth? 5th Forum of European Neuroscience, Vienna, July 10, 2006 (in Japanese).

Doya, K., Bayesian brain: Bayesian inference for functional modeling and data analysis of the brain. Institute of Statistics and Mathematics, Tokyo. November 8, 2006.

Doya, K., Learning model-based analysis of neuroimaging data. Symposium on Advances in Anatomical, Functional, and Computational Brain Imaging, East Asian Biophysics Symposium, Okinawa, Japan. November 15, 2006.

Doya, K., What is happening in the brain during motor learning? 33rd Workshop on Sports Psychology, Okinawa, Japan. December 7, 2006 (in Japanese).

Doya, K., Learning how and what to learn. Honda International Symposium on Creating Brain-Like Intelligence, Germany. February 3, 2007.

Doya, K., Data-driven modeling and model-based analysis in computational neuroscience. Inauguration Symposium of International Neuroinformatics Coordination Foundation. Stockholm, Sweden. February 15, 2007.

Doya, K., Research projects of Neural Computation Unit. Okinawa-Korea Workshop: Neuroscience and Beyond. Okinawa, Japan. February 22, 2007.

Doya, K., Neural implementation of reinforcement learning. 10th Tamagawa-Riken Dynamic Brain Forum. Hakuba Village, Japan. March 5, 2007.

Ishii, S. & Yoshimoto, J. Bayesian reproduction of biological data. Okinawa Computational

Neuroscience Course 2006. Okinawa, July 4, 2006.

Kamioka, T., Uchibe, E. & Doya, K. (2006). Multi-objective reinforcement learning based on multiple value functions. Neurocomputing Technical Group, IEICE, NC 2006, Vol.105, No.658, p127-132). Univ. Tamagawa, Tokyo, Mar.15-17, 2006.

Otsuka, M., Uchibe, E. & Doya, K., Acquisition of action-oriented state representation by neighborhood component analysis. Neurocomputing Technical Group, Institute of Electronics, Information and Communication Engineers. Tokyo, March 2007 (in Japanese).

Sato, T., Uchibe, E. and Doya, K. Emergence of communication and cooperative behavior by reinforcement learning agents. The 13th MPS Symposium, Nagoya, October. 24-25, 2006.

Sato, T., Uchibe, E. & Doya, K. Learning how, what, and whether to communicate: emergence of protocommunication in reinforcement learning agents. The 12th Internal Symposium on Artificial Lifeand Robotics (AROB2007), Oita, February 25-27, 2007.

Uchibe, E., & Doya, K. (2006). Reinforcement learning under constraints generated by multiple reward functions. Neurocomputing Technical Group, Institute of Electronics, Information and Communication Engineers, NC2006-22, 1-6 (in Japanese).

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Posters:

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Ito, M., & Doya K., Dynamics of decision making and learning, Tougou-nou meeting, Tokyo, December 18, 2006 (in Japanese).

Ito, M. & Doya, K. Activity of the basal ganglia in a decision making task, Workshop on

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Miyazaki, K. & Doya, K. (2007). Neural activity of the dorsal raphe nucleus of freely moving rats during reward delay period. The 7th Winter Workshop on Mechanism of Brain and Mind Workshop, Rusutsu, January 10, 2007 (in Japanese).

Miyazaki, K., Miyazaki, K.W. & Doya, K. Neural activity of the dorsal raphe nucleus of freely moving rats during reward delay period. 5th Forum of European Neuroscience, Vienna, July 11, 2006.

Miyazaki, K.W., Miyazaki, K. & Doya, K. Serotonin efflux in the dorsal raphe nucleus of freely moving rats during reward delay period. 5th Forum of European Neuroscience, Vienna, July 11, 2006.

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Yoshimoto, J. & Doya, K., Bayesian system identification of biochemical signaling pathways, The 7th International Conference on Systems Biology (ICSB-2006), FI61, Yokohama, October 9-11, 2006.

4. Intellectual Property Rights and Other Specific Achievements

JSTS Prize to Kenji Doya for "A Computational Approach to Network and Molecular Mechanisms of the Brain", awarded by Japan Society for the Promotion of Science, March 2007.

5. Meetings and Events

5.1. OIST Workshop: Future Directions in Primate Brain Research

Date/Place: May 14 - 16, 2006, Marriott Resort, Nago, Okinawa

Speakers:

Keiji Tanaka (RIKEN)
Ichiro Fujita (Osaka U)
Manabu Tanifuji (RIKEN)
Jun Tanji (Tamagawa U):
Shintaro Funahashi (Kyoto U)
Toshio Iijima (Tohoku U)
Masamichi Sakagami (Tamagawa U)
Kenji Matsumoto (RIKEN)
Yasuhi Kobayashi (Osaka U)
Masahiko Takada (Metro. Institute of Neurology)
Kazuto Kobatashi (Fukushima Med. U)
Kiyoshi Nakahara (Tokyo U)
Tadashi Isa (National Institute of Physiology)
Shigeru Kitazawa (Juntendo U)
Naotaka Fujii (RIKEN)

5.2. Symposium on Reward and Decision Making in Cortico-Basal Ganglia Networks

Date/Place: June 1 - 4, 2006, UCLA Arrowhead Seminar House, California, USA

Co-organizers:

Bernard Balleine, University of California, Los Angeles
Kenji Doya, Okinawa Institute of Science and Technology
John O'Doherty, California Institute of Technology
Masamichi Sakagami, Tamagawa University

26 Invited Speakers

5.3. Neurocomputing and Bioinformatics Joint Workshop

Date/Place: June 15 - 16, 2006, OIST Seaside House

Co-sponsors:

Institute of Electronics, Information and Communication Engineers
Information Processing Society of Japan

5.4. Okinawa Computational Neuroscience Course 2006

Date/Place: June 26 - July 7, 2006, OIST Seaside House

Title: Computing Neurons - What neurons compute; How we know by computing -

Co-organizers:

Upinder Bhalla, National Center for Biological Sciences, India

Kenji Doya, Okinawa Institute of Science and Technology

Shinya Kuroda, University of Tokyo

Nicolas Le Novère (European Bioinformatics Institute)

Lecturers:

Upi Bhalla (NCBS)

Haruhiko Bito (U Tokyo)

Sydney Brenner (OIST)

Yang Dan (UC Berkeley)

Erik DeSchutter (U Antwerp)

Kenji Doya (OIST)

Bard Ermentrout (U Pittsburgh)

Geoff Goodhill (U Queensland)

David Holcman (Weizmann institute of Science)

Shin Ishii (NAIST)

Shinya Kuroda (U Tokyo)

Nicolas Le Novere (European Bioinformatics Institute)

Roberto Malinow (Cold Spring Harbor Lab)

Ion Moraru (U of Connecticut)

Felix Schuermann (EPFL)

Terry Sejnowski (Salk Institute)

Susumu Tonegawa (MIT)

Jeff Wickens (U Otago)

5.5. Summer Workshop on the Mechanisms of the Brain and Mind

Date/Place: August 25, 2006, Sapporo, Japan

Speakers:

David Heeger (New York University)

Masao Tachibana (University of Tokyo)

Koji Kurata (University of Ryukyus)

5.6. Symposium on Advances in Anatomical, Functional, and Computational Brain Imaging

Date/Place: November 15, 2006, Okinawa Convention Center

Co-sponsors:

East Asian Biophysics Society

Biophysics Society of Japan

Speakers

Seong-Gi Kim, University of Pittsburgh

Susumu Mori, Johns Hopkins University

Mitsuo Kawato, ATR Computational Neuroscience Laboratories

Doris Tsao, University of Bremen

Kenji Doya, OIST

5.7. Winter Workshop on the Mechanisms of the Brain and Mind

Date/Place: August 25, 2006, Hokkaido Koseinenkin Hall

Speakers:

Peter L. Bossaerts (Cal Tech)

Alan Sanfey (U Arizona)

and seven other speakers

5.8. OIST Seminars

Date: June 21, 2005

Title: Structural changers resulting from the loss of dopamine in striatum

Speaker: Dr. Gordon Arbuthnott, University of Otago

Date: January 12, 2007

Title: Risk and reward perception in the human brain outside and inside financial markets

Speaker: Dr. Peter Bossaerts, California Institute of Technology

VI. 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 are required to maintain, enter, or exit from the arrested G0 cell state of the fission yeast *Schizosaccharomyces pombe*. The *S. pombe* G0 cell might be an ideal model to understand why muscle, heart, and neuronal differentiated cells do not divide, but remain in G0. The *S. pombe* G0 state is defined as the state the cells enter under nitrogen source starvation. *S. pombe* G0 cells are round, never divide, and most of them contain 1C DNA. They are viable for months by metabolizing glucose and an intracellular nitrogen source. We demonstrated that these G0 cells are metabolically active, rapid to repair genomic DNA damage, and require proteasome machinery to be viable. This year we were successful in dramatically expanding our approach to identify the genes involved in the maintenance of, and exit from, G0. We are also pleased to report that a detailed paper describing cell biologic, transcriptomic, and gene disruption experiments for those G0 and G0-exiting cells is now in press titled, "Two-step, extensive alterations in the transcriptome from G0 arrest to cell division in *Schizosaccharomyces pombe*" (Shimanuki et al). We also made substantial progress in proteomic analysis, by which the changes in the levels of more than 2500 protein species from G0 to proliferation were quantified. Proteins designated 'Degs' whose levels are reduced immediately after the addition of a nitrogen source are of considerable interest, and gene disruption experiments to evaluate the function of these proteins are ongoing. Through screening 1000 temperature-sensitive (ts) strains that were originally isolated as vegetative growth-defective, we identified ~70 strains that lose cell viability in the G0 state. G0 cellular phenotypes of mutants in these "super-housekeeping" genes are currently under intensive investigation. We identified certain chemicals and drugs that disrupt G0 maintenance that will be useful in our future studies. Finally, mutants of proteasome and Tor (target of rapamycin) kinase have been investigated with regard to their G0 phenotypes.

1. Participants:

1.1. Individuals:

Okinawa Group

-Group Leader: Mizuki Shimanuki

-Researchers: Koji Nagao, Kojiro Takeda

-Research Assistant: Kenichi Sajiki (Graduate Student of Nara Institute of Science and

Technology, on partnership with NAIST)

-Technical Staff: Sakura Kikuchi, Ayaka Mori, Aya Kokubu, Risa Uehara, Tomas Pluskal

-Research Administrator: Tomomi Teruya

Kyoto Group (Laboratory of Molecular Inheritance, Graduate School of Biostudies, Kyoto University)

-Researchers: Takeshi Hayashi, Mitsuko Hatanaka

-Research Administrator: 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*"

Chemical Genetics Laboratory, Discovery Research Institute, Wako Institute, Riken

Collaboration: Chief Scientist: Minoru Yoshida; Researcher: Shinichi Nishimura

Theme: "Search for chemicals and drugs that are inhibitory to the maintenance of G0 state"

Division of Molecular Life Science, Bioinformation and Molecular Science Course, Graduate School of Life Science, Hokkaido University

Collaboration: Professor: Chikashi Obuse

Theme: "Proteomic analysis of *S. pombe* G0 cells"

Laboratory of Molecular Inheritance, Graduate School of Biostudies, Kyoto University

Collaboration: Researcher: Takahiro Nakamura

Theme: "Metabolomic analysis of *S. pombe* cells using LC-MS mass spectrometer"

Laboratory of Molecular Inheritance, Graduate School of Biostudies, Kyoto University

Collaboration: Researcher: Yohta Fujita

Theme: "Identification of novel kinetochore components using LC-MS mass spectrometer"

Unit for Molecular Neurobiology of Learning & Memory, OIST

Collaboration: Principal Investigator: Shogo Endo; Researcher: Nobuhiko Kojima (Present: Associate Professor, Gunma University Medical School, Japan)

2. Activities and Findings:

2.1. From comprehensive transcriptome to functional analysis: Identification of genes required for maintenance of and/or to exit from the G0 state

We previously reported the results of microarray analysis indicating that the G0 cell transcriptome is largely different from that of growing and dividing cells. Many genes (approximately 20%) of the genome (~5000 genes) were up- or down-regulated after the nitrogen source was added to the G0 cells. Global changes in the transcriptome occurred in two steps from the G0 state before DNA replication to the recovery of the transcriptome of the proliferating state. Simultaneously, during the second wave of global transcriptome alteration (around 3 h from nitrogen replenishment), elongation/growth was initiated in the arrested cells (Fig. 1a). The intracellular protein amount in G0 cells was approximately one-sixth that of the average in proliferating cells. On the other hand, the volume of G0 cells, calculated from the size of the cells, was approximately one-third that of proliferating cells (Fig 1b). A classification ontology analysis of the genes indicated that the patterns of transcriptional regulation of certain gene groups are similar (see examples in Fig. 1c).

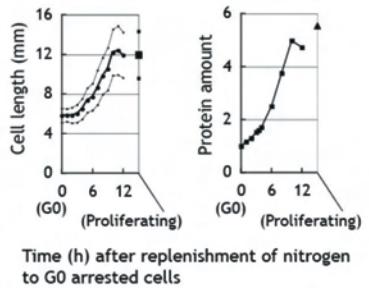


Figure 1a,b Alterations in cell length and protein content during resumption of proliferation from G0 arrest

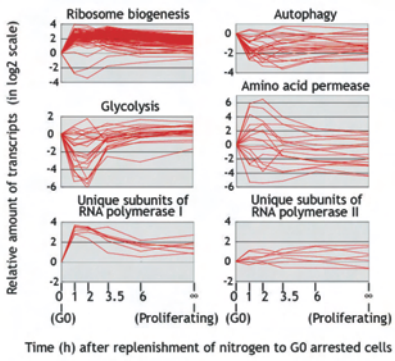


Figure 1c Transcript level alteration profiles for the functionally classified genes.

To obtain functional insight into whether the genes that are highly down-regulated during growth recovery might be involved in maintaining the G0 cell state, we initially studied 43 genes using gene disruption. In addition, we also studied 10 other genes without regard to their transcriptional regulation. These genes included those for enzymes, putative transcription factors, protein kinase- or phosphatase-related factors, cyclins, G-protein-related factors, and chromatin regulators. The resulting 53 deletion strains were examined to determine whether they are required to maintain cell viability in the vegetative and/or G0 state. Fifty-two strains had mostly normal vegetative growth, but 10 strains lost cell viability faster than wild-type at 42°C in the G0 state. The viability defect of those 10 strains was further confirmed by long-term incubation at 26°C in the G0 state. The results of the viability analysis are

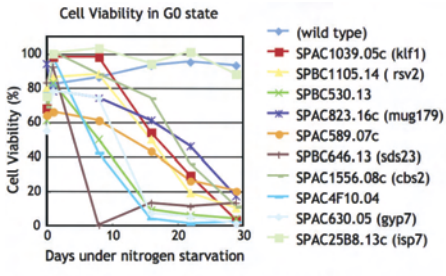


Figure 2 Mutants that lost viability in the G0 phase.

shown in Fig. 2. Nine gene-disruptant strains lost viability much faster than the wild-type strain.

Of these 9 genes, 3 are implicated in transcriptional regulation. Two C2H2 type zinc-finger factors (SPAC1039.05c and SPBC1105.14) seem to support the G0 state. SPBC530.13 contains a cyclin domain and is implicated in transcription as it is bound to a kinase that appears to hyper-phosphorylate the C-terminal repeat domain (CTD) of the largest subunit of RNA polymerase II. Two genes (SPAC823.16c and SPAC589.07c) are similar to each other, and are probably involved in autophagy, in which proteins are degraded in the vacuoles. The two CBS (cystathionine-beta-synthase) domain proteins, SPBC646.13/Sds23/Psp1/Moc1, and a putative subunit of AMP-activated protein kinase (AMPK), SPAC1556.08c (designated Cbs2), are required for maintaining cell viability in G0, probably through their energy-sensing signal transduction functions. SPAC630.05 might be a GTPase-activating protein involved in vacuole transport. SPAC4F10.04 is a putative activator of protein phosphatase. These 9 gene products shed light on the genetic control of the G0 state in *S. pombe*. This study is now in press (Shimanuki et al., 2007).

2.2 From proteome analysis by LC/MS/MS to identification of candidate regulator proteins by gene disruption

Both transcriptome and proteomic analyses are necessary to understand the systematic cellular changes that occur when cells shift from G0 to proliferation as the transcriptome does not provide information on the post-transcriptional regulation that occurs during the cellular transitions. We performed proteome analysis of the G0 cells and cells shifting towards the proliferating state upon nutritional replenishment using a semi-quantifiable liquid chromatography-mass spectrometry (LC-MS) method. This year we made a great technical progress towards improving the detection sensitivity, reproducibility, and quantification of cellular proteins. As a result, more than 2000 proteins were identified and quantified using total cell extracts. The levels of approximately 120 proteins that are abundant in G0 were significantly decreased in the vegetative phase (Fig. 3). The levels of a subset of these proteins diminished immediately after release from G0. We hypothesized that some of these proteins might serve to antagonize the progression of G0-released cells toward proliferation. To test this hypothesis, approximately

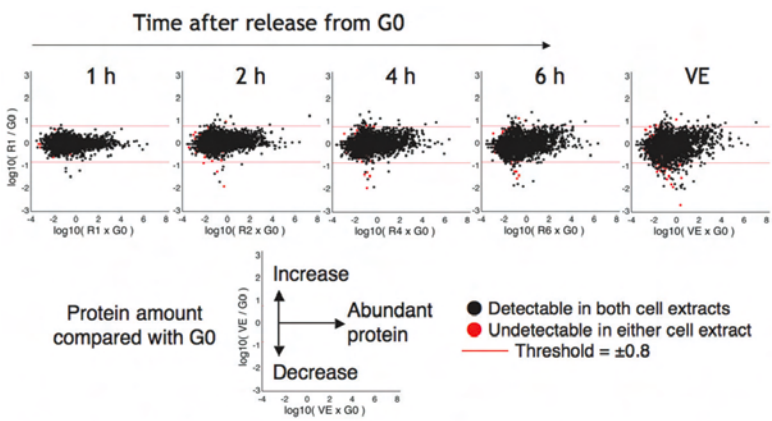


Figure 3 Overview of fission yeast proteome at the G0-vegetative phase transition

15 uncharacterized genes were selected (the level change of an example protein is shown in Fig. 4). Analyses of the function, construction, and phenotype of the gene-disrupted strains and identification of their physically interacting proteins are ongoing.

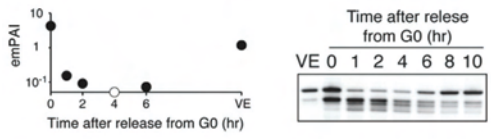


Figure 4 Example of a candidate protein. The level is quantified by LC-MS (left), then confirmed (right).

2.3 Screening of temperature-sensitive (ts) mutant collection for identifying essential 'housekeeping' genes to maintain the G0 state

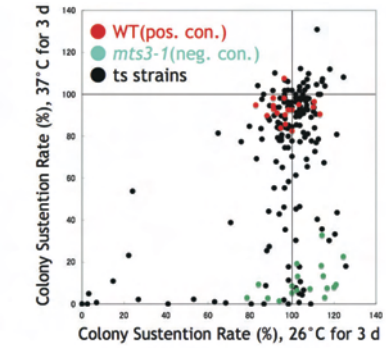


Figure 5 Temperature sensitivity of the mutants

As one way to identify genes essential for maintaining the G0 state of *S. pombe*, we screened a 1000-ts mutant library. Because the genes responsible for the ts mutations are likely to be essential mainly in the vegetative phase, those identified by this approach were considered to be essential 'housekeeping' genes in both G0 and proliferation. Cell viabilities of each strain were measured under three conditions: 24 h after nitrogen deprivation, 3-d nitrogen-free culture at 26℃, or at 37℃. The results, presented in Fig. 5, display the collected data of ~250 strains as a percentage of cells surviving at 26 and 37°C. Cell viability in G0 cells was reduced to less than 50% in 70 strains. The next step will be to identify the genes responsible for those mutations. Because the Mandala project has identified the mutant genes by mass cloning, we will be able to determine all of the mutant genes and also high-copy suppressors within a relatively short period of time. Some mutant genes identified are novel and are apparently involved in RNA-processing or intracellular protein trafficking.

2.4 Ubiquitin/proteasome system is required to maintain the G0 phase

We found that ubiquitin/proteasome system (UPS) was essential for the long-term survival of G0 cells. Though the essential role of the protein-degrading UPS that maintains cell proliferation is well known, its actual 'housekeeping' role in G0 is not well understood. We therefore examined the detailed phenotype of a proteasome mutant in G0 and found that the nucleus was severely deformed (Fig. 6). Transmission electron microscopy revealed abnormal electron-dense materials, membranous

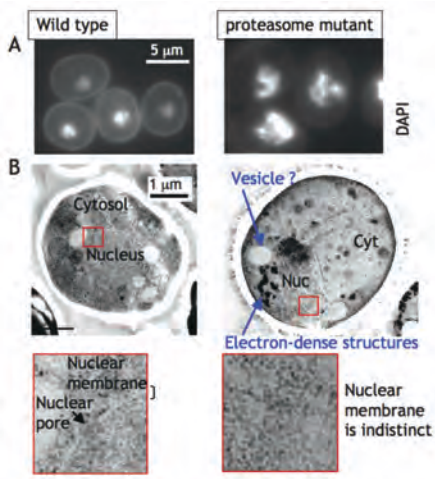


Figure 6 Phenotype of a proteasome mutant in the G0 phase

structures inside the nucleus, and frequent disruption of the nuclear membrane. How UPS is needed to maintain nuclear integrity is under investigation.

Polyubiquitination of proteins require three steps: ubiquitin activation by E1 enzyme, conjugation by E2, and ligation by E3. *S. pombe* has 1 E1, 11 E2, and several E3 enzymes (Fig. 7). As expected, E1 is essential for G0 maintenance. We then examined whether any of 11 E2 mutants lost their viability during G0. Two of them (ubc7 and ubc8) significantly lost cell viability in G0. A homologue of Ubc7 in budding yeast is involved in protein quality control in the endoplasmic reticulum, whereas a Ubc8 homologue regulates glycolysis. We are currently identifying their E3s and substrates that should be needed to maintain cell viability in G0.

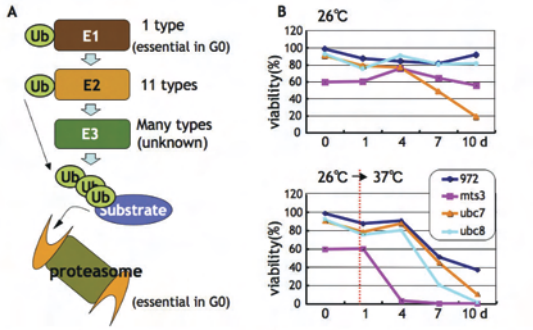


Figure 7 Essential factors of the Ub/proteasome pathway in G0 phase

2.5 Isolation of tor2 mutant that shows the G0-like phenotype under the conditions for vegetative proliferation

By screening *S. pombe* ts strains, we isolated a mutant that displayed the G0-like phenotype without nitrogen starvation (Fig. 8a). The mutant contained a single amino acid substitution in the catalytic domain of Tor2, an inositol kinase-like enzyme essential for sensing nutrients and required for cell growth. The mutant was hypersensitive to rapamycin (Fig. 8b), revealing a G0-like defective phenotype. *S. pombe* has two Tor genes and it was uncertain whether *S. pombe* spTor2 was the counterpart of budding yeast Tor1 or Tor2, therefore we used the LC/MS/MS approach to identify the bound proteins. A total of 12 proteins were bound to *S. pombe* Tor1 and/or Tor2. Our results strongly suggested that spTor2 is like S. cerevisiae Tor1, as spTor2 binds to the putative homologues of Kog1and TCO89, but not to the homologues of Avo1, Avo3, and BIT61. In contrast to *S. cerevisiae* Tor2, spTor1 binds to Avo1, Avo3, BIT61, and Lst8 homologues, but not to Kog1 and TCO89. These results demonstrate that spTor1 and spTor2 are distinct in their protein composition, and, consequently, their functions.

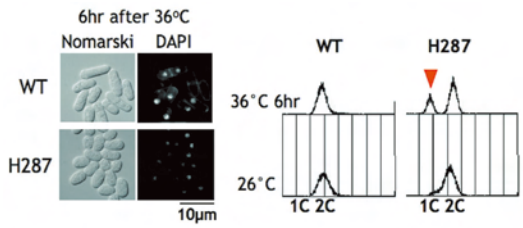


Figure 8a H287 had a small cell size and arrested with 1C DNA content at the restrictive temperature

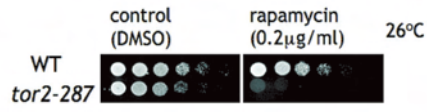


Figure 8b *tor2-287* mutant shows hypersensitivity to rapamycin.

2.6 Search for drugs that cause lethal effects on G0 cells

To identify drugs that inhibit G0 cells, we examined a number of chemicals and drugs that might reduce cell viability in G0. We previously tested various drugs (leptomycin B, staurosporine, camptothecin, trichostatin A, cycloheximide), but none reduced G0 cell viability. We therefore tested 95 other drugs in the SCADS inhibitor kit (a gift from the Screening Committee of Anticancer Drugs supported by Grant-in-Aid for Scientific Research on Priority Area "Cancer" from The Ministry of Education, Culture, Sports, Science, and Technology, Japan). Bleomycin (an inducer of DNA double-strand breaks), actinomycin D (an inhibitor of DNA polymerase), manumycin A (an inhibitor of farnesyl transferase), damnacanthal, and tamoxifen reduced cell viability in G0 cells (Fig. 9a). In particular, damnacanthal and tamoxifen were more toxic to cells in the G0 state than to cells in the vegetative state, though the targets of those drugs in *S. pombe* cells are unknown. The cells exposed to those two drugs had similar phenotypes with an abnormal nuclear structure (Fig. 9b). Detailed observation through an electron microscope revealed that the intracellular membranes were abnormal (Fig. 9c). These drugs will be useful for investigating how G0 cells are maintained.

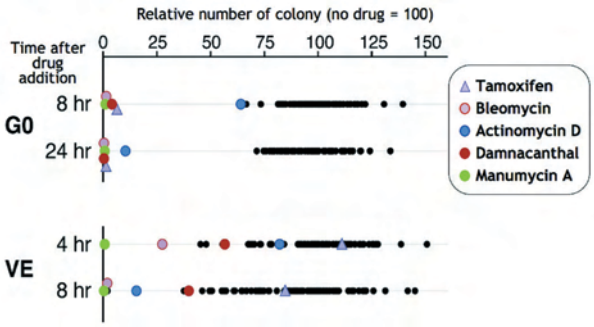


Figure 9a Five drugs decreased viability of G0 cells

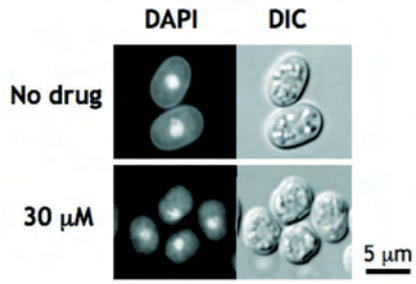


Figure 9b G0 cells 1 h after adding Tamoxifen have deformed nuclei

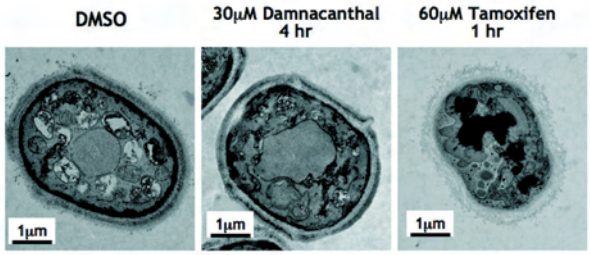


Figure 9c Abnormal membranes are observed in G0 cells after adding Tamoxifen and Damnacanthal

2.7 Toward metabolomic analysis of G0 cells in comparison with proliferating cells

One aim of the metabolomic approach is to assay the quantitative compositions of metabolites in G0 versus vegetative cells, and to understand the physiologic significance of such changes, if any. The abundance of key metabolites was estimated using high performance liquid chromatography electrospray ionization mass spectrometry (HPLC-ESI-MS). An HPLC system combined with a Fourier-Transform mass spectrometer was installed. We tested several LC separation methods using C18, PGC, HILIC-Silica, and ZIC-pHILIC columns. The ZIC-pHILIC method provided the best separation of our compounds of interest and good chromatogram peak

shapes, even for polar molecules like ATP (Fig. 10). This method allowed us to successfully separate and detect pure compounds in the mixtures in picomolar amounts. We investigated the quantification ability of the LC-MS method by injecting pure compound mixtures at various concentrations and measuring the chromatogram peak areas (Fig. 10, upper panel). For most of the compounds, we were able to distinguish a 10-pmol difference in the injected amount, with a 20-pmol difference being distinguishable for all compounds. We are now developing a method for extracting the intracellular metabolites of *S. pombe*, using cold methanol for quenching (rapid cessation of cellular metabolism), liquid nitrogen for breaking the cellular wall, and centrifugation and filtration through a 5-KDa cut-off filter. Using this method, we expect to be able to monitor the levels of hundreds of cellular metabolites.

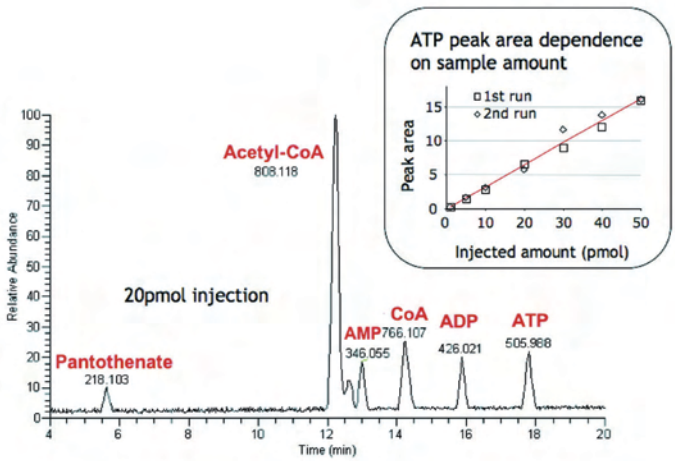


Figure 10 Chromatogram of a mixture of standard compounds

3. Publications

3.1. Journals

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)*

Fujita, Y., Hayashi, T., Kiyomitsu, T., Toyoda, Y., Kokubu, A., Obuse C., and Yanagida, M. Priming of centromere for CENP-A recruitment by human hMis18alpha, hMis18beta, and M18BP1" *Dev Cell. 12, 17-30 (2007)*

Shimanuki, M., Chung, S-Y., Chikashige, Y., Kawasaki, Y., Uehara, L., Tsutsumi, C., Hatanaka, M., Hiraoka, Y., Nagao, K. and Yanagida, M. Two-step, extensive alterations in the transcriptome from G0 arrest to cell division in *Schizosaccharomyces pombe. Genes Cells, in press*.

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*

Takeda, K. and Yanagida, M. How does fission yeast Cut8 regulate the nuclear localization of proteasome? *Tanpakushitsu Kakusan Koso.* Aug;51(10 Suppl):1241-4 (2006) *in Japanese*

Hayashi, T., Fujita, Y. and Yanagida, M.
Mechanism of Centromeric Chromatin Formation. *Cell Technology. 25, 502-505(2006) in Japanese*

3.3. Oral Presentations and Posters

Presentations:

Yanagida, M. "On the Fission Yeast G0 Project in Okinawa", *The First International Workshop on Cell Regulations in Division and Arrest*, Okinawa, Mar. 7, 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

Yanagida, M. "Why yeast has been so useful" Symposium on the Yeast Post-Genome and Bio-resource, Osaka, May 6, 2006

Yanagida, M. "On the Mandala Project A and G0 Proteomics" *Yeast Genome Conference KSBMB, Seoul*, May 26, 2006

Yanagida, M. "On Dividing and Non-dividing cells" *The Summer School of Japanese Bioinformatics Society*, Kyoto, Aug. 21, 2006

Yanagida, M. "On the Difference between Dividing and Arrested Cell States", *Molecular*

Cardiovascular Conference, Hokkaido, Sep. 8, 2006

Yanagida, M. "Genetic Control of Non-dividing Cell State", Mount Sinai School of Medicine, New York, Jan. 16, 2007

Yanagida, M. "Genetic Control of the G0 Cell State", *The Second International Workshop on Cell Regulations in Division and Arrest*, Okinawa, Mar. 25-29, 2007

Posters:

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

Nagao, K., Obuse, C., Kokubu, A., Hatanaka, M., and Yanagida, M. "Proteomic analysis of fission yeast cells at the G0-vegetative phase transition", *The 29th Annual Meeting of the Molecular Biology Society of Japan*, Nagoya, Dec. 6-9, 2006

Takeda, K., Kikuchi, S., Yoshida, T., and Yanagida, M. "The mutation of 26S Proteasome causes abnormality in the nuclear structure in fission yeast G0 cells", 2006 forum of Molecular Biology Society of Japan, Nagoya, Japan, Dec. 6-9, 2006

Mori, A., Nagao, K., Yoshida, T., and Yanagida, M. "A screening for drugs that inhibit the

maintenance of Fission Yeast G0 state", *The 24th Chromosome Workshop*, Saga, Japan, Jan. 31-Feb. 2, 2007

Nagao, K., Obuse, C., Kokubu, A., and Yanagida, M. "Proteomic analysis of fission yeast cells at the G0-vegetative phase transition", *The 24th Chromosome Workshop*, Saga, Japan, Jan. 31-Feb. 2, 2007

Shimanuki, M., Chung, SY., Chikashige, Y., Kawasaki, Y., Uehara, L., Tsutsumi, C., Hatanaka, M., Hiraoka, Y., Nagao, K. and Yanagida, M. "A search for candidate regulators of Fission Yeast G0 phase induced by Nitrogen Starvation", *The 24th Chromosome Workshop*, Saga, Japan, Jan. 31-Feb. 2, 2007

Takeda, K., Kikuchi, S., Yoshida, T., and Yanagida, M. "The mutation of 26S Proteasome causes abnormality in the nuclear structure in fission yeast G0 cells", *The 24th Chromosome Workshop*, Saga, Japan, Jan. 31-Feb. 2, 2007

Hayashi, T., Hatanaka, M., Nakaseko, Y., Kokubu, A., Ebe, M., Nagao, K. and Yanagida, M. "Identification of fission yeast Tor2 and/or Tor1 binding proteins by LC/MS/MS". *The Second International Workshop on Cell Regulations in Division and Arrest*, Okinawa, Japan, Mar. 25-29, 2007

Mori, A., Nagao, K., Yoshida, T., and Yanagida, M. "A screening for drugs that inhibit the maintenance of *S. pombe* G0 state", *The Second International Workshop on Cell Regulations in Division and Arrest*, Okinawa, Japan, Mar. 25-29, 2007

Nagao, K., Obuse, C., Kokubu, A., and Yanagida, M. "Proteomic analysis of fission yeast cells at the G0-vegetative phase transition", T*he Second International Workshop on Cell Regulations in Division and Arrest*, Okinawa, Japan, Mar. 25-29, 2007

Sajiki, K., and Yanagida, M. "Screening of temperature-sensitive (ts) mutant collection for identifying essential 'housekeeping' genes to maintain the G0 state", *The Second International Workshop on Cell Regulations in Division and Arrest*, Okinawa, Japan, Mar. 25-29, 2007

Shimanuki, M., Chung, SY., Chikashige, Y., Kawasaki, Y., Uehara, L., Tsutsumi, C., Hatanaka, M., Hiraoka, Y., Nagao, K. and Yanagida, M. "A search for candidate regulators of Fission Yeast G0 phase: From transcriptome analysis", *The Second International Workshop on Cell Regulations in Division and Arrest*, Okinawa, Japan, Mar. 25-29, 2007

Takeda, K., Kikuchi, S., Yoshida, T., and Yanagida, M. "The mutation of 26S Proteasome causes abnormality in the nuclear structure in fission yeast G0 cells", *The Second International Workshop on Cell Regulations in Division and Arrest*, Okinawa, Japan, Mar. 25-29, 2007

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

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)

The Second International Workshop on Cell Regulations in Division and Arrest

Date: March 25th-29th, 2007

Place: OIST Seaside House, Onna, Okinawa

Invited Speakers:

Jürg Bähler (Wellcome Trust Sanger Institute)

Rey-Huei Chen (Academia Sinica IMB)

Peter Fantes (University of Edinburgh)

David Glover (University of Cambridge)

Gohta Goshima (University of California SF)

Mohan Gupta (DFCI/Harvard Medical School)

Tony Hyman (Max Planck Institute)

Shinichiro Imai (Washington University)

Ken Inoki (University of Michigan)

Stephen Kearsey (University of Oxford)

Sue Lin-Chao (Academia Sinica IMB)

Satoru Mochida (Cancer Research UK)

Sergio Moreno (University of Salamanca)

Masashi Narita (Cancer Research UK)

Randy Poon (Hong Kong Univ. of Science and Technology)

Fuyuhiko Tamanoi (University of California LA)

Anders Zetterberg (Karolinska Institute)

Atsushi Hirao (Kanazawa University)

Hiroshi Kondoh (Kyoto University)

Tomohiro Matsumoto (Kyoto University)0

Masayuki Miura (University of Tokyo)

Akihiko Nakano (RIKEN Institute)

Keiichi Nakayama (Kyushu University)

Yoshinori Ohsumi (National Institute for Basic Biology)

Yoshikazu Ohya (University of Tokyo)

Takashi Takeuchi (Mitsubishi Kagaku Institute of Life Science)

Takeshi Tomonaga (Chiba University)

Fumiko Toyoshima (JST/Kyoto University)

Masayuki Yamamoto (University of Tokyo)

Katsumi Yamashita (Kanazawa University)

Mitsuhiro Yanagida (Okinawa Institute of Science and Technology)

Minoru Yoshida (RIKEN Institute)

VII. Unit for Molecular Neurobiology of Learning & Memory Learning & Memory

Principal Investigator: Shogo Endo

Research Theme: Molecular and Genetic Dissection of Learning and Memory

Abstract:

The brain, the organ of memory and cognition, is supported by the result of the fine-tuned molecular mechanisms in neuronal cells. The functions of neuronal cells are the origin for all mental activities including memory. One of the fundamental 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 intracellular cascades are still waiting to have their roles revealed and fully characterized. I have studied the molecular and cellular aspect of memory based on the techniques of biochemistry, molecular biology and gene targeting. We continue to attack the biochemical and cellular aspects of the neuronal plasticity and memory as models of brain functions to reveal intimate machinery of the brain.

1. Participants:

1.1 Individuals:

-Researchers

Gilyana Borlikova, Ph.D.

Toshiro Sakamoto, Ph.D.

-Technicians

Masako Suzuki, M.Sc. (Laboratory Manager)

Michiko Arai, B.Sc

Tomoko Arasaki, M.Sc

Chihiro Honma, M.Sc

Mika Takiguchi, M.Sc

-Graduate Student

Yukiko Uechi, M.Sc. (University of the Ryukyus Graduate School of Medicine)

-Assistant

Ms. 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."

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."

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"

Tokyo University

Laboratory for Neurophysiology

Collaborative research, Principal Investigator: Dr. Dai Yanagihara.

Research theme: "Behavioral analysis of genetically modified mice."

Harvard Medical School

Neuroregeneration Laboratory

Collaborative research, Principal Investigator: Dr. Ole Isacson

Research theme: "Roles of cGMP-PKG pathway in dopaminergic neurons"

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 created 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 neuronal cell interaction, the brain and the whole body. We chose the mice as a model animal for the study on memory. The gene manipulation of mice is well established and it is possible to obtain gene-deficient mice and transgenic mice in our lab from scratch. We utilized the gene-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. Further, the injection of ES cells into blastocysts, then transfer of the injected blastocysts to psedopregnant mice has been established. By establishing these steps, we are now 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. We are working on the establishing the transgenic mice generation in our lab.

Mice behavioral analysis

We have established comprehensive screening of behaviors in genetically modified mice that is essential as a first step to characterize the mice for learning and memory because a variety of physiological conditions can affect the ability to learn. In addition, we have established a variety of behavior analysis systems in OIST. One of the difficulties of mice behavioral experiment is to establish reliable operant conditioning paradigms which require labor intensive and lengthy shaping process and restriction of food or water. We are establishing this paradigm in our lab. Classical conditioning such as fear conditioning requires strong stimuli such as electric shocks. On the other hand, operant conditioning uses motivation of the animals, and paradigm is usually associated with rewards such as food and water. We extensively pursue the operant conditioning as it is rather close to the learning in daily life. Available behavioral tests in the Unit are as follows;

1) General behavior test battery

24-hr activity measurement, Open field test (low light and high light), Light-and-dark box test, Startle and pre-pulse inhibition, Fear conditioning

2) Other behavioral analysis

Elevated plus maze, Eye blink conditioning, Eye movement measurement, Forced swimming test, Hanging wire test, Hot plate, Morris water maze, Operant conditioning, Optokinetic response measurement, Passive avoidance test, Rotorod test

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

Previously, we have observed the importance of G-substrate in long-term memory in OKR using G-substrate gene deficient mice. 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 be directly or indirectly involved in the gene transcription and translation. Related to the possible function of G-substrate in transcription and translation, we observed the translocation of G-substrate from a nuclear induced by stimuli including 8-Br-cGMP and the export of G-substrate from nuclear through CRM1-dependent mechanism.

G-substrate contains several nuclear localization signals (NLS) and nuclear export signal (NES) (Fig. 1). We have introduced mutation in each of NLS and NES. Then, we have generated the expressing vectors containing mutants of any combination of these NLS and NES. Furthermore, we have cloned PKGI cDNA library. A stable cell line expressing PKGI was established for the co-expression of PKGI and G-substrate in the cell. Using this cell line and G-substrate containing mutations in NLS and NES, we are currently characterizing the role of each NES and NLS of G-substrate.

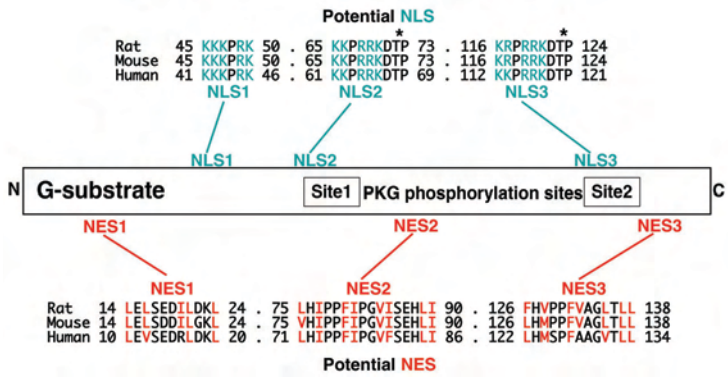


Fig. 1 Potential NLSs and NESs in G-substrate. Potential relative positions of NLSs (blue) and NESs (red) are indicated in the G-substrate sequences. The numbers are the amino acid residue numbers of G-substrate in each species. Thr residues with asterisks are the PKG phosphorylation sites.

Molecular mechanisms underlying cerebellar-dependent behavior.

One of the well-studied cerebellar-dependent behaviors 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 of the conditioning in detail. Furthermore, there is a long debate on the role of the cerebellar parts in the conditioning. We have established a measuring system for eye blink conditioning in mice and examined the role of GABA receptors in cerebellar nuclear.

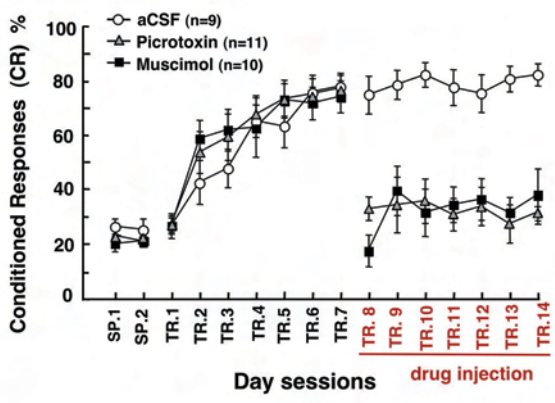


Fig.2 The injection of GABA agonist and antagonist impaired the acquired eyeblink conditioning.

Further, we are characterizing the role of cerebellar nuclear by injecting a $GABA_A$ antagonist (Picrotoxin) and a $GABA_A$ agonist (Muscimol) into the deep cerebellar nuclear through the surgically implanted canulae. We have previously observed that the injection of these drugs impaired the eye blink conditioning. To further characterize the role of GABA receptor in detail, we examined the role of the receptor in the expression of eye blink conditioning after the acquisition of memory (Fig 2). After the conditioning reached a plateau, artificial cerebrospinal fluid (aCSF), picrotoxin or muscimol was injected. The expression of acquired memory was disrupted by the injection.

The cerebellum controls collateral side of the body in many mammals. To characterize the laterality of the eye blink conditioning in mice, either pictotoxin or muscimol was injected after the conditioning bilaterally, ipsilaterally, or contralaterally while eye blink was measured from left eye (Fig. 3). The expression of eye blink conditioning was impaired by the injection of picrotoxin or muscimol (TR8-11, 14-15, 17-18) and was normal between drug injections (TR 12-13, 16, 19). There might be some interactive communication between left and right hemisphere of cerebellum in eyeblink conditioning.

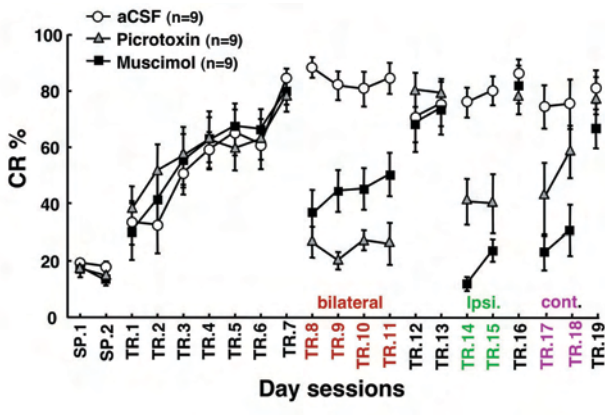
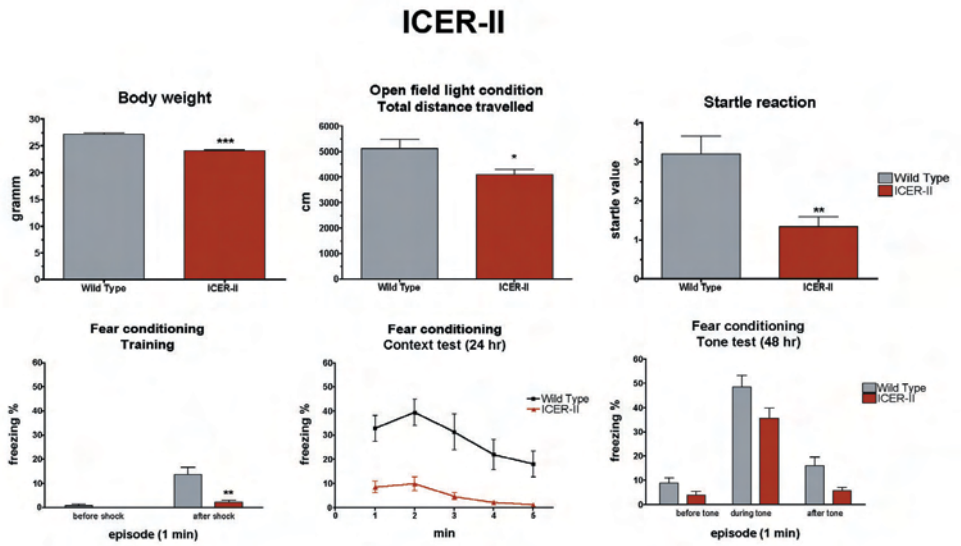


Fig.3 The injection of GABA agonist or antagonist into cerebellar nuclear after conditioning was acquired.

Characterization of ICER-II overexpressing mice.

We have observed previously that the expression of inducible cAMP early repressor (ICER), an immediate early gene product, increased after the fear conditioning. It is assumed that ICER competes with CREB for CRE in the gene to prevent the excessive gene transcription induced by CREB activation. We have generated the ICER gene deficient mice and overexpressing mice and extensively characterized these lines of mice. ICER-II is a subtype of ICER gene that is expressed from CREM gene. We have characterized the ICER-II expressing mice using behavior test battery.

Figure 4. ICER-II overexpressing mice in comparison to non-transgenic littermates had reduced body weight, and were less active in the open field under the light condition. They demonstrated lower startle reaction. Fear conditioning experiment: during conditioning ICER-II mice displayed significantly lower freezing immediately after shock; 24 hr later these mice showed very low freezing to the context comparing to control littermates; at the tone test that was run in a different context 48 hr after the training transgenic mice showed overall reduced freezing during both no-tone and tone time-bins.



In the behavior test battery, no major difference was found except for fear conditioning between ICER-II overexpressing mice and wildtype littermate. However, in fear conditioning major differences were observed as follows; lower freezing immediately after shock and before tone presentation during tone test, lower freezing during 24 hr context test, no deficit in freezing in response to tone during the test conducted 48 hr after the conditioning (Fig. 4). We are further characterizing these mice at behavioral level and molecular biological level.

Identification of mRNA species localized in post synapse.

The local protein synthesis in postsynaptic cells underlies essential mechanism for the neuronal plasticity and learning and memory. The inhibition of the postsynaptic protein synthesis prevents the formation of memory, in particular, long-term memory. Thus, the comprehensive identification of mRNA in post synapse (postsynaptic density fraction, PSD) is of importance to understand the mechanism underlying neuronal plasticity. This project has been carried out in collaboration with Dr. Tatsuo Suzuki of Shinshu University.

We found a variety of mRNA in PSD fraction including several novel mRNA species and mRNA with unknown function. We extracted approximately 1900 mRNA species which are

concentrated in PSD fraction. From the cDNA library constructed using mRNA from PSD fraction, we randomly sequenced 1152 randomly chosen cDNAs. Please find the entire list of mRNA species observed in post synapse in (Suzuki et al, in press). We have picked a few genes and currently generating the gene deficient mice to characterize the role of these proteins.

3. Publications:

(1) S. Endo, Y. Satoh, K. Shah and K. Takishima
A single amino-acid change in ERK1/2 makes the enzyme susceptible to PP1 derivatives. Biochem. Biophys. Res. Commun. 341, 261-265, 2006.

(2) Q.B. Tian, T. Suzuki, T. Yamauchi, H. Sakagami, Y. Yoshimura, S. Miyazawa, K. Nakayama, F. Saitoh, J.P. Zhang, Y. Lu, H. Kondo and S. Endo.
Interaction of LDL receptor-related protein 4 (LRP4) with postsynaptic scaffold proteins via its C-terminal PDZ domain-binding motif, and its regulation by Ca^{2+}/calmodulin-dependent protein kinase II. Eur. J. Neurosci. 23, 2864-2876, 2006

(3) F. Du, F. Saitoh,Q.B. Tian, S. Miyazawa, S. Endo, T. Suzuki
Mechanisms for association of Ca^{2+}/calmodulin-dependent protein kinase II with lipid rafts. Biochem. Biophys. Res. Commun. 347, 814-820, 2006.

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

(1) Endo S. Learning and Memory. pp247-256 (Neuroscience Illustrated, Mori et al ed.) Yodosha. Tokyo, Japan, 2006.

(2) Endo S. An efficient animal facility for the study of learning and memory study using genetically modified animals. LABBIO21. April 18-24, 2006

(3) Sakamoto T. Physiological Basis of Behaviors (pp20-29), Learning (pp100-104), (General Remark of Psychology, Okaichi and Suzuki ed.), Nakanishiya Publishers. 2006

3.3. Oral Presentations and Posters

Oral Presentations:

None to report

Posters:

(1) Sakamoto T. and Endo S. GABA receptors in the deep cerebellar nuclei are essential for mouse eyeblink conditioning. Annual Meeting of the Japan Neuroscience Society, July 19-21, 2006, Kyoto, Japan.

(2) Tian QB, Suzuki T., Kuromitsu J., Kawai T., Endo S. Characterization of mRNA species that are associated with postsynaptic fraction by gene chip microarray analysis. Annual Meeting of the Japan Neuroscience Society, July 19-21, 2006,

Kyoto, Japan.

(3) Maruyama E, Ogawa K., Endo S., Tsujimoto M., Nabetani T, and Tsugita A. GABA stimulation induces tyrosine phosphorylation and nuclear translocation of a 28kDa protein: Involvement of Src tyrosine kinase activity. IUBMB International Congress of Biochemistry and Molecular Biology, June 18-23, 2006, Kyoto, Japan

(4) Miyamoto, K., Hirabayashi Y., Osuka S., Kuwamura M., Okada T., Endo S. Itohara S., Ikeda T. and Kato K. The effect of Siat 4c deficiency on brain function of the adult mouse. IUBMB International Congress of Biochemistry and Molecular Biology, June 18-23, 2006, Kyoto, Japan

(5) Honma C, Endo S. and Yamada K. The effects of bedding on defensive covering behavior in mice strains. Annual Meeting of Japan Society for animal Psychology. Oct 14-15, Kyoto Japan.

4. Intellectual Property Rights and Other Specific Products

Method and device for drug delivery to cells by pulse-laser system (Japan Patent pending 2006-113384) Obara M, Terakawa M, Ohtsuka R, Sato S, Satoh Y, Takishima K, Watanabe Y, Endo S, Mizuno K, Nawashiro H, Ooigawa H.

5. Meetings and Events

None to report

VIII. Electron Holography Unit

Principal Investigator: Akira Tonomura
Research Theme: Holography Electron Microscopy Dedicated to Nanotechnology

Abstract:

Electron phase microscopy provides interesting research opportunities to investigate electromagnetic field inside/outside of various types of materials in nanometer resolution. Employing electron holography and Lorentz microscopy, we examined (1) magnetic domain structures in gallium manganese arsenide (GaMnAs) and its temperature variation, (2) magnetic domain in self-organized undulating iron film dominated by strong in-plane uniaxial anisotropy, and (3) magnetic coupling in chain rings of Ni nanoscale spheres, which cannot be analyzed other conventional magnetic measurements.

We also continued development of a spherical aberration corrector for long-focal objective lens, which is to be integrated into our new 300KV field-emission transmission electron microscope. This unique combination will provide new insights into the relation between magnetic ordering and materials structure in atomic resolution. We built up a prototype corrector, and tested its performance with a 100kV conventional electron microscope. Validity of electron optical design is being confirmed experimentally.

1. Participants:

1.1. Individuals:

-Research advisor: Keiichi Namba (Osaka University, since Oct. 1, 2006-)

-Researchers: Akira Sugawara, Kei-ichi Fukunaga

1.2. Partner Organizations:

Advanced Research Laboratory, Hitachi Ltd., (HARL)

Joint research

Principal researcher: Akira Tonomura (OIST-IRP) and Takaho Yoshida (HARL)

Theme: "Holography electron microscope dedicated to nanotechnology"

2. Activities and Findings:

2.1. Activities and Findings on Nanomagnetism observation

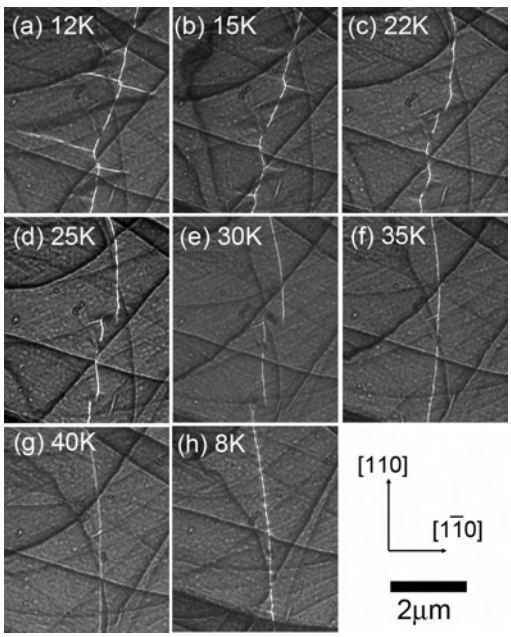
The recording density of magnetic data storage is increasing rapidly as a result of the development of new materials. The present recording bit size is typically a few hundreds of nanometers but has to be downsized by factor of 10 for 300 Gbit/in^2. Measurement techniques are essential to observe the recording state inside each bit and the crosstalk among neighboring bits.

The TEM-based phase microscopy including electron holography and Lorentz microscopy enables us to map two-deimensional distribution of magnetic induction on nanometer resolution. Reliable electron phase maps were obtained through numerical reconstruction on quantitative electron intensity distribution acquired using a 4k x 4k CCD camera that was delivered in March 2006. In this fiscal year we studied (1) magnetic domain structures in gallium manganese arsenide (GaMnAs), (2) magnetic domain in self-organized undulating iron film, and (3) magnetic coupling in rings of Ni nanoscale spheres.

2.1.1 Magnetic domain structures in gallium manganese arsenide (GaMnAs)

Gallium Manganese arsenide is a typical diluted magnetic semiconductor, of which magnetic functionality is expected to be integrated in high-speed III-V semiconductor devices. Although deep understanding of micromagnetic properties such as magnetic domain formation and anisotropy is essential for future application for spintronics, the electron phase microscopy has not been applied to investigate the domain structures of the material. Reasons for this are the difficulties of working with very small magnetizations (typically 1% of ferromagnetic metals), and of preparation of uniform-thick electron transparent specimens. We succeeded in the domain observation by Lorentz electron microscopy. The keys for the success were instrumental advantage of 1MeV field emission electron microscope which enables us to detect small phase change of thick specimens, and a specifically designed multilayer specimen provided from Prof. Gallagher's group at University of Nottingham, UK.

Our observation revealed magnetic domain structures of GaMnAs in submicron resolution, which has not been achieved in previous magneto-optical imaging studies by other groups. We found complex segmented domain structures of submicron scale, and spontaneous domain wall reorganization on temperature. i,e, uniaxial anisotropy and 180°-like walls are



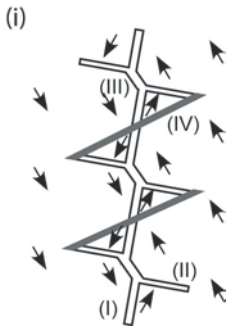


Fig. 1 (a)-(h) Lorentz micrographs of a segmented wall as a function of temperature under zero-field conditions. The specimen temperature was raised from 12 K to 40 K and then quenched to 8 K. (i) magnetization configuration of segmented domain structure corresponding to (a) and (b)

dominant at high temperature, whist biaxial anisotropy and 90°-like walls are dominant at low temperature. The variation of observed image contrast was well explained in terms of temperature-dependent magnetic anisotropy, which was also confirmed by our Landau-Lifshitz-Gilbert micromagnetic simulation (one research paper is under refereeing).

In addition we recently succeeded in domain observation also by electron holography that provides quantitative information about magnetization rotation in each magnetic domain (One research paper is in preparation).

2.1.2 Magnetic domain in self-organized undulating iron film

Magnetic anisotropy is a key parameter in determining the switching mode and reversal field of a magnetic recording medium and head. It is, however, difficult to control in-plane magnetic anisotropy when a polycrystalline film is used for in-plane magnetic devices. We deposited polycrystalline iron films on faceted NaCl(110) with periodic ridge-and-valley (corrugated) surface using a molecular beam epitaxy system installed in Uruma site, and succeeded in generating in-plane uniaxial anisotropy. We studied magnetic domain structure in detail by electron phase retrieval using both electron holography and transfer-of-intensity-equation (TIE) method applied to Lorentz electron micrographs. Although the magnetic hysteresis loops exhibited strong in-plane anisotropy with easy axis parallel to the ridges. Observed walls were based on the intersecting cross-tie structure with magnetization slightly deviated (approx. 15°) from ridge direction in each domain, i.e. complete antiparallel 180° wall formation did not occur. We also found that the wall width was unusually small, suggesting that each domain wall was confined in a single corrugation segment. The features of the observed wall structure were reproduced also by Landau-Lifshitz-Gilbert micromagnetic simulations, indicating that the roughness-induced uniaxial anisotropy dominates the micromagnetics of the particular shaped specimen (one research paper is under refereeing).

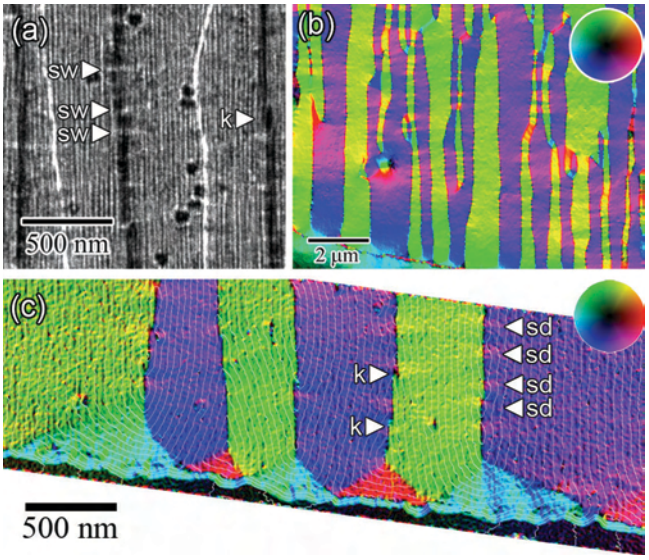


Fig. 2 (a) Lorentz micrograph of an self-organized undulating Fe film,(b) Magnetic induction mapping obtained by TIE analysis over large filed of view, and (c) high-resolution magnetic induction mapping derived from electron phase determined by electron holography.

2.1.3 Magnetic coupling in rings of Ni nanoscale spheres

Chemical synthesis of polymer-coated metallic nanoparticles is extensively investigated recently. This kind of "bottom-up"-type fabrication is expected to contribute to improvement of magnetic storage performance, because their well-controlled sizes guarantee uniform magnetization reversal field. We studied magnetic structure of self-organized ring chains of 30nm-diameter nickel nanospheres provided from Prof. Kitagawa's group at Kyushu University, Japan. We observed long-range magnetic ordering along the ring induced by dipole-coupling between neighboring Ni particles. Such spontaneous closure magnetic ordering has to be measured only under zero magnetic field. Since net magnetization is not produced if particles are dipole-coupled and behaves like magnetic rings, such ordering cannot be investigated by other magnetic measurements such as SQUID magnetometry. The electron phase microscopy is the only method that is capable of determining the local magnetic structure under zero magnetic field in high spatial resolution. We also measured order parameter as a function of temperature between 120K to 500K, which was determined as a result of competition between dipole coupling and thermal fluctuation. The detailed analysis of the temperature-dependent micromagnetics is in progress. (One research paper is in preparation)

2.2. Activities and Findings on development of spherical aberration corrector

2.2.1 Outline of Nano-Mag TEM development

We started to develop Nano-Mag TEM, which was a transmission electron microscope optimized to study nano-magnetism (magnetic structure or phenomena in nano-scale). The Nano-Mag TEM will equip with followings.

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

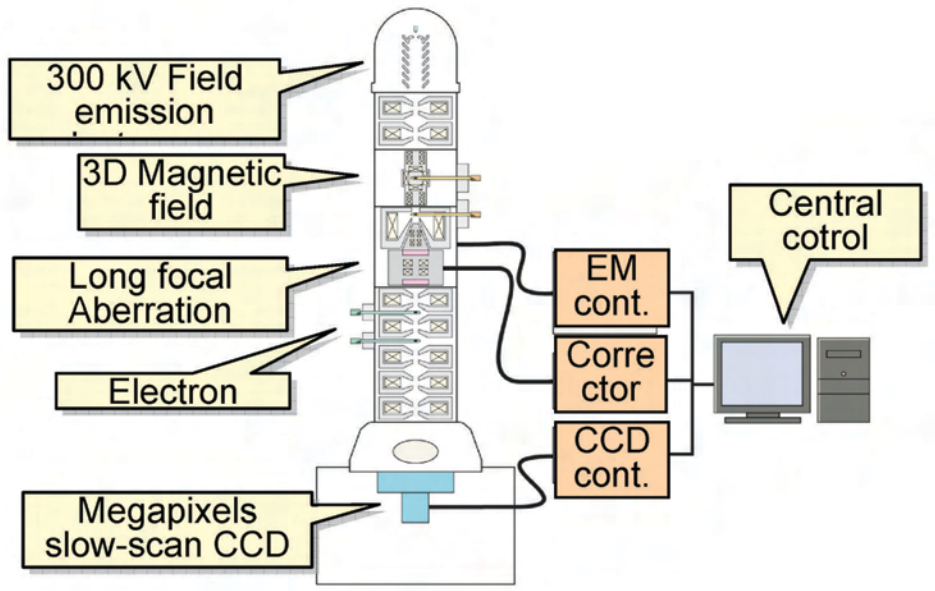


Fig. 3 Concept of Nano-Mag TEM

(2) 3 Electron biprisms for electron holography

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

(4) Aberration corrector (Long focal Cs corrector)

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

A Schematic diagram of Nano-mag TEM is summarized in Fig. 3.The 300kV field emission transmission electron microscope (FE-TEM), Hitachi HF3300X was purchased as a base machine of Nano-mag TEM. The aberration corrector suitable for nano-magnetism study is in development in collaboration with Advanced Research Laboratory, Hitachi Ltd.

2.2.2 Manufacture and tests of proto-type aberration corrector for Nano-Mag TEM

For the magnetic observation using Nano-mag TEM, the specimen is located in the 3D magnetic chamber distant from the objective lens. In such an electron optical condition, i.e. with long focal objective lens, the spatial resolution is severely lowered due to the spherical aberration of the objective lens. Therefore, to avoid such resolution lowering, we decided to develop the aberration corrector specially designed for Nano-mag TEM.

A. Electron optical and mechanical design of long focal aberration corrector

The aberration corrector for Nano-mag TEM was specially designed as

(1) To correct the spherical aberration in nano-magnetism observation (Nano-mag mode), i.e. specimen was located away from the objective lens, and therefore the objective lens had the long focal length more than several centimeters

(2) To correct the spherical aberration in high resolution observation (High-res. mode), i.e. specimen was located in the objective lens, and therefore the objective lens had the short focal length ~2 mm

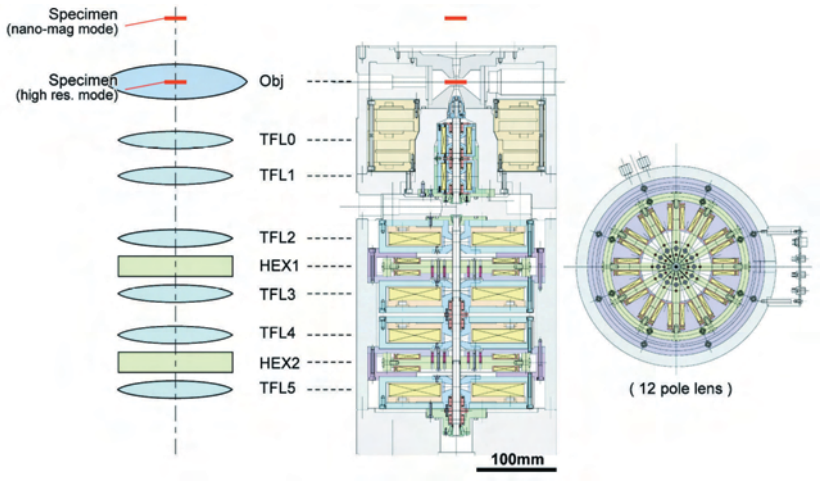


Fig. 4 Electron optical and mechanical design of the long focal aberration corrector
(Obj: Objective lens, TFL: Transfer lens, HEX: Hexapole lens, hexapole lens is formed by 12 poles to suppress the appearance of higher order aberrations.)

The aberration corrector satisfying both of (1) and (2) was designed to minimize not only spherical aberration but also the coma aberration using the electron optical simulation. [1]

The design of the aberration corrector is shown in Fig. 4. The aberration corrector is based on the H. Rose's hexapole corrector[2] . But the transfer lenses (TFL0~2) between the objective lens and the first hexapole (HEX1) were relocated to minimize the coma aberration in Nano-mag mode, and transfer lens triplet (TFL0~2) was employed instead of the doublet in Rose's corrector to satisfy the aberration correction both in Nano-mag and High-resolution modes.

The resolution improvement was also estimated using the electron optical simulation as shown in Fig. 5. The large improvements in resolution are expected both in Nano-mag and High-resolution mode owing to the aberration correction; in both modes, the spherical aberration is completely corrected, while coma and chromatic aberrations are well suppressed.

B. Manufacture and test of the prototype Nano-mag aberration corrector

According to the electron optical design stated above, we designed the mechanical

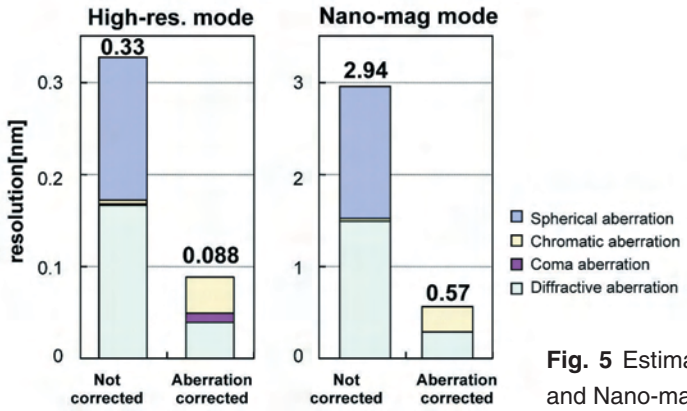


Fig. 5 Estimated spatial resolution in High resolution and Nano-mag mode.

structure of the prototype aberration corrector. The prototype corrector was precisely manufactured with the assembling errors less than 10 μm. The performances of each element in the corrector, transfer lenses and hexapole lenses (12 poles), checked individually; especially it was confirmed that the strength of each lenses coincided with the expectation by electron optical simulations.

After the fundamental tests, the prototype corrector was experimentally installed in a transmission electron microscope with 100kV accelerating voltage to confirm the aberration correction function using the electron beam. The optical axial adjustments procedure was also examined. These investigations are in progress.

1 H. Liu, E. Munro, J. Rouse, X. Zhu "Simulation methods for multipole imaging system and aberration corrector" ,Ultramicrocopy vol.93 (2002) 271

2 H. Rose "Outline of spherically corrected semiaplanatic medium-voltage transmission electron microscope", Optik vol.85 (1990) 19; M. Haider, G. Braunshausen, E. Schwan, "Correction of spherical aberration of a 200 kV TEM by mean of hexapole corrector", Optik vol.99 (1995) 167

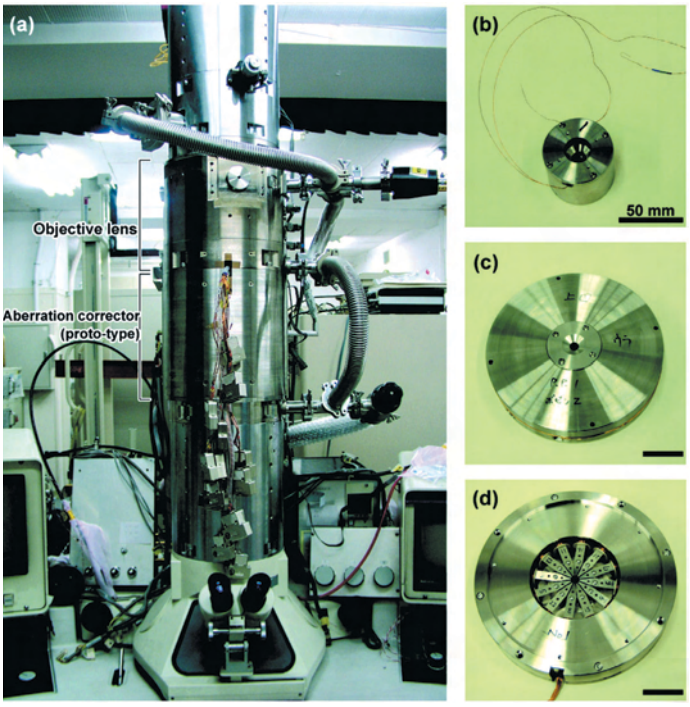


Fig. 6 Manufacture and test of prototype aberration corrector (a) Prototype aberration corrector installed in 100kV TEM, and lenses in the prototype corrector; (b) Transfer lens (TFL0),(c) Transfer lens (TFL 2), (d) 12 poles (used as hexapole lens)

3. Publications

3.1. Journals

A. Tonomura: "The Aharonov-Bohm effect and its applications to electron phase microscopy" Japan Academy, Ser. B82 No.2, (2006)45-58.

Kitsakorn Locharoenrat, Akira Sugawara, Saho Takasea, Haruyuki Sanoa, and Goro Mizutania, "Shadow Deposition of Copper Nanowires on the Faceted NaCl(110) Template" ,accepted for publication on Surf. Sci.

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

Nothing to be reported.

3.3. Oral Presentations and Posters

Oral Presentations:

- A. Tonomura: "The Quantum World Unveiled by Electron Waves", Innovative Nanoscale Approach to Dynamic Studies of Materials, Okinawa Japan, January 10, 2006
- A. Tonomura: "Single Quantum Dynamics Research Group- Observing, understanding, and manipulating quantum phenomena at extremely small (nano-scale) distances-", FRS Symposium, Saitama Japan, May 12, 2006
- A. Tonomura: "Dynamic Observation of Vortices in Superconductors by Lorentz Microscopy", Nanoscale Superconductivity and Magnetism 2006, Leuven Belgium,

July 6, 2006

- A. Tonomura: "Dynamic Observation of Vortices in Nb and Bi-2212 Thin Films using Lorentz Microscopy", M2S, Dresden Germany, July 10, 2006
- A. Tonomura: "Field-emission Electron Microscopes to Observe Microscsopic Phase Objects", Cornell Workshop in honor of Prof. John Silcox, Ithaca USA, July 19, 2006
- A. Tonomura: "Electron Waves Univeil the Microcosmos -Reenactment of Friday Evening Discourse-", International Conference on Physics Education 2006, Tokyo Japan, August 14, 2006
- A. Tonomura: "Observation of quantum objects and phenomena using electron waves ", The Brijuni Conference -Exploring Fundamental Problems in Science "Imaging in Space and Time", Brijuni Croatia, August 31, 2006
- A. Tonomura: "Electron Holography", IMC, Sapporo Japan, September 5, 2006
- A. Tonomura: "Applications of 1-MV Field Emission Electron Microscope to the Vortex Observation in High Tc Superconductors", IMC, Sapporo Japan, September 7, 2006
- A. Tonomura: "Quantum Phenomena Visualized Electron Waves", LEEM PEEM V, Himeji Japan, October 16, 2006
- A. Tonomura: "Electron Phase Microscopy to Observe Microscopic Objects", NANOMEC-06, Bari Italia, November 22, 2006
- A. Tonomura: "The Quantum World Observed Using Electron Waves", Catholic University of Leuven, Leuven Belgium, November 27, 2006
- A. Tonomura: "Observation of Vortices in Superconducting Films Using Lorentz Microscopy", Catholic University of Leuven (Discussion on Collaboration), Leuven Belgium, November 28, 2006
- A. Tonomura: "Quantum Phenomena Visualized by Electron Waves", Stockholm University Department of Chemistry, Stockholm Sweden, November 30, 2006
- A. Tonomura: "Coherent Electron Microscopy to Observe Vortices in Superconductors", Stockholm University Department of Physics, Stockholm Sweden, December 1, 2006
- A. Tonomura: "Observation of Plastic Flows of Vortices in Superconductors by Lorentz Microscopy", NVLS2006, Kyoto Japan, December 11, 2006

Posters:

(Nothing to be reported).

4. Intellectual Property Rights and Other Specific Products

(Nothing to be reported)

5. Meetings and Events

OIST Workshop "Future of Electron Microscopy" – New technologies and new applications –

Date: March 24-30, 2007

Place: The Naha Terrace and OIST Sea Side House

Speakers: S. Iijima : (Meijyo Univ., AIST, NEC), H. Rose : (Technical University of Darmstadt), M. Haider : (CEOS), H. Lichte : (Dresden University), Y. Takai : (Osaka University), K. Takayanagi: (Tokyo Institute of Technology), K. Suenaga : (AIST), U. Dahmen : (Lawrence Berkeley Laboratory), A. Howie : (Cambridge), H. Ichinose : (Riken), C. Colliex: (CNRS, Orsay), H. Mori : (Osaka University), R. Dunin-Borkowski : (Cambridge), M. McCartney : (Arizona State University), Y. Zhu: (Brookhaven National Laboratory), H. Lichte (Dresden University), T. Hirayama : (Fine Ceramics Center), J. Plitzko : (Max-Planck-Institut of Biochemistry), F. R. Chen : (National Tsing Hua University), K. Downing: (Berkeley Lab.), K. Namba : (Osaka University), W. Kühlbrandt : (Max-Planck-Institut für Biophysik) and A. Tonomura: (OIST, Riken and Hitachi)

IX. Molecular Genetics Unit

Principal Investigator: Sydney Brenner
Research Theme: Molecular Genetics of Salamander

Abstract:

Because very little nucleotide sequence of salamander has been known, we have initiated salamander cDNA project. We constructed a brain cDNA library of salamander (Ambystoma mexicanum). We have already carried out single-run sequencing of 80,000 clones from the brain library. We plan to make a salamander cDNA database for future studies on the neurons.

1. Participants:

1.1. Individuals:

-Technician (s): Saori Goda, Shinichi Yamasaki

1.2. Partner Organizations:

Okinawa Institute of Science and Technology PC

Type of study: Joint research

Principal researcher: Dr. Takayuki Naito

Theme: "Salamander project"

2. Activities and Findings:

2.1. Salamander project (See also Naito Unit)

Construction of cDNA database of Ambystoma mexicanum

Because very little nucleotide sequence of salamander has been known, we have started salamander cDNA project. We constructed a brain cDNA library of Ambystoma mexicanum by using vector-capping method developed by Dr. S. Kato. Eukaryotic mRNA has the cap structure (7-methyl-GpppG) at the 5' end. If cDNA is full-length, the first nucleotide of the cDNA should be G. As shown in Table 1, the population of G at 5' end of cDNA clones in the brain library was found to be 71 %, which shows the high content of full-length clones in this library.

Table 1 Clones having G at 5'-end in the salamander brain cDNA library

G	A	T	C	N	total
44441	5113	9296	3456	15	62301
71%	8%	15%	6%	0%	100%

We plan single-run sequencing of 5'-end of 100,000 clones from brain cDNA library, 50,000 clones from retina cDNA library, and 50,000 clones from spinal chord cDNA library. We classify clones which have the similar sequences into a cluster. Relationship between the number of sequenced clones and the number of clusters at present situation is shown in Fig. 1.

After clustering of single-run sequences, we proceed to full-length sequencing of the clones. We have already done single-run sequencing of 80,000 clones from brain library, and obtained about 14,000 clusters. We have carried out second-run sequencing of 8,000 clones from the clusters.

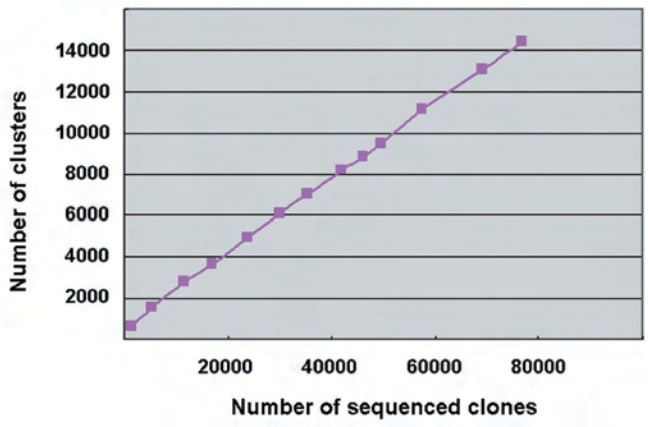


Fig. 1 The relationship between the number of sequenced clones and the number of clusters.
BLAST parameters are set to distinguish alternative form

X. Information Processing Biology Unit

Principal Investigator: Ichiro Maruyama
Research Title: Information Processing by Life

Abstract:

Our research objective is to understand how external information is perceived by sensory neurons and processed by the nervous system at cellular and molecular levels. Following the completion of laboratory settings in 2005, this year we have initiated several lines of experiments using cultured cells and the nematode *Caenorhabditis elegans* (*C. elegans*) as model systems. We have already made the following major findings in the field: (i) The epidermal growth factor receptor (EGFR) has a preformed homodimeric structure and a preformed heterodimeric structure with ErbB2, another member of the receptor family, as determined by fluorescence resonance energy transfer (FRET) and fluorescence cross-correlation spectroscopy (FCCS) at physiological expression levels in cultured cells. (ii) All the members of the EGFR family have preformed homo- and heterodimeric structures between the member receptors when analyzed by bimolecular fluorescence complementation (BiFC) assay. (iii) In *C. elegans*, alkaline pH (OH^-) is perceived by the ASE amphid neurons as well as the IL2 neurons, whose function was previously unknown. The two neuronal networks triggered by the two sensory neuron pairs seem to compete each other to regulate *C. elegans* behaviors so that the worm is attracted towards pH ranges from 7.0 to 10.0 but is repelled by pH ranges higher than 10.0. (iv) We have also found signaling components that play crucial roles in *C. elegans* synaptogenesis, an essential process for the establishment of neuronal networks. Apart from these major findings, we have also determined experimental conditions for measuring pharyngeal pumping to monitor *C. elegans* responses to external stimuli. These results give us insights into an understanding of a molecular mechanism underlying information transfer from the outside of cells to the inside as well as an understanding of neuronal networks that control animal behaviors in response to external stimuli. These findings are also vital steps towards the development of artificial intelligence as well as of pharmaceuticals for human diseases such as schizophrenia and cancers.

1. Participants

1.1. Individuals

Researcher:

Satoshi Hasegawa, Stephan Shuichi Haupt, Kazunobu Hirose, Ichiro Kawasaki, Takashi Murayama, Katsunori Nakata, and Rong-Hua Tao

Technical Staff:

Mayuki Fujiwara, Kanako Hisata, and Hiraku Miyagi

Research Assistant:
Yuko Toyama

1.2. Partner Organizations

Department of Chemistry, National University of Singapore
Type of Research: Collaborative
Principal Investigator: Thorsten Wohland
Research Title: Investigation of the dimerization of proteins from the epidermal growth factor receptor family by single wavelength fluorescence cross-correlation spectroscopy.

Department of MCD Biology, University of California, Santa Cruz
Type of Research: Collaborative
Principal Investigator: Yishi Jin
Research Title: Genetic and biochemical analyses of synaptogenesis in the nematode *C. elegans*.

Nara Medical University
Type of Research: Collaborative
Principal Investigator: Takashi Fujimoto
Research Title: Application of the lambda phage surface display system.

Centre for Molecular Medicine, Singapore
Type of Research: Collaborative
Principal Investigator: Sohail Armed
Research Title: Investigation of the dimerization of proteins from the epidermal growth factor receptor family by single wavelength fluorescence cross-correlation spectroscopy.

University of the Ryukyu School of Medicine
Type of Research: Collaborative
Principal Investigator: Ken-ichi Kariya
Research Title: Total internal reflectance fluorescence microscopic analysis of membrane proteins.

University of Tokyo
Type of Research: Collaborative
Principal Investigator: Yuichi Iino
Research Title: Germline development in the nematode *Caenorhabditis elegans*.

Keio University School of Medicine

Type of Research: Joint

Principal Investigator: Takanori Moriki

Research Title: Application of the lambda phage surface display system.

2. Activities and Findings

2.1. Project Aims

Life is separated from non-life by cell membranes, and all the cells have cell-surface receptor proteins that span the membranes in order to transfer external information, such as environmental changes and cell-cell communication, to the inside of the cell. Such information flow is fundamental for all living systems ranging from bacteria to humans. Dysregulation of the cell surface receptor molecules often causes a variety of impairments including mental and developmental diseases and cancers.

(i) We wish to understand at the molecular level how the external information is sensed and transmitted into the inside of the cell by cell-surface receptor molecules, and how the information is processed, transferred to other parts of the cells, and regulates other cellular activities. (ii) We wish also to understand information processing at higher levels through cell-cell communications; namely, how the external information sensed and transmitted through neuronal cells, processed by the nervous system, and how it controls animal behavior, learning and memory.

2.2. Approaches.

Approaches to be used in this project are based on the assumption that all the organisms from unicellular bacteria to multicellular humans are using common fundamental principles underlying all the biochemical processes. More specifically, principles underlying the processing of external information by cell surface sensors are identical in all the organisms ranging from bacteria to humans. Moreover, fundamental principles underlying information processing are conserved in the nervous system of worms and in the brain of humans.

(i) Processing of external information by cells. We will use animal tissue culture cells as a model system, and will try to find fundamental principles underlying the processing of external information by cell surface receptors. Using cultured cells, we will analyze how growth factor receptor proteins are activated by their ligand on the cell surface, how the receptor activation is transferred to the inside of the cell, and how the information is processed by other cellular components and regulates other cellular activities.

(ii) Processing of external information by the nervous system. In this section, we will

analyze (a) which organs sense what chemical and physical stimuli, (b) which neurons are responsible for the information processing, (c) how the information is transmitted through neuronal networks, (d) which groups of neurons are involved in learning and memory, and (e) how the nervous system controls animal behaviors. For this research, we will exclusively use *C. elegans* as a model system. Based upon the outcome of the research, we will try to find fundamental principles underlying the information processing, and principles to be found will be confirmed in higher organisms such as mice and rats by constructing transgenic animals and/or using disease models.

2.3. Progress report

2.3.1. Processing of external information by cells

In this section of the project, we have specifically focused on the epidermal growth factor receptor (EGFR) family of cell-surface receptor tyrosine kinases, also known as ErbB or HER. The epidermal growth factor (EGF) receptor, or ErbB, family of receptor tyrosine kinases are expressed highly in brain, and plays important roles not only in the development of the nervous system, but also in the modulation of synaptic plasticity. The receptors regulate proliferation, differentiation and migration of neurons. Furthermore, one of the receptor's ligands, Neuregulin-1 (NRG-1) has recently been identified genetically as a schizophrenia susceptibility gene. NRG-1 reverses long-term potentiation (LTP) at CA1 hippocampal synapses by depotentiation through a reduction in the number of surface AMPA receptor molecules. The enhanced activity of ErbB4, a member of the receptor family, is observed in schizophrenia, and mediates NMDA receptor hypofunction.

The receptor family consists of four members, EGFR/ErbB1, ErbB2/Neu/HER2, ErbB3/HER3 and ErbB4/HER4, and has a large (~620 amino acid residues) extracellular ligand-binding region, a single transmembrane α-helix, and an intracellular region containing the tyrosine kinase and its regulatory domains. They form a network of homo- and heterodimers. ErbB2 can only be regulated indirectly, and is thought to be the preferred heterodimerization partner for other ErbB receptors. ErbB3, on the other hand, must associate with an ErbB family member that has an active tyrosine kinase in order to respond to its own NRG binding.

Ligand-induced dimerization has been proposed to be a property common to the transmembrane signaling mechanism of all known growth factor receptors including the EGF/ErbB receptors (Dimerization Model). According to the model, receptor dimerization is responsible for autophosphorylation of the intrinsic kinase activity, which is mediated by an intermolecular process. Namely, ligand binds to the monomeric form of the receptor, and induces its dimeric form for the activation. Prior to ligand binding, however, it remains controversial whether the receptor has a monomeric or dimeric structure.

We have recently found by chemical cross-linking and sucrose density-gradient centrifugation that in the absence of bound ligand EGFR has an ability to form a dimer and the majority, >80%, of the receptor exists as a preformed dimer on the cell surface. We also analyzed the receptor dimerization by inserting cysteine residues at strategic positions about the α-helix axis of the extracellular juxtamembrane region. The mutant receptors spontaneously formed disulfide bridges and transformed NIH3T3 cells in the absence of ligand, depending upon the positions of the cysteine residues inserted. Kinetic analysis of the disulfide bonding indicates that ligand binding induces flexible rotation or twist of the juxtamembrane region of the receptor in the plane parallel with the lipid bilayer. The binding of an ATP competitor to the intracellular kinase domain also induced similar flexible rotation/twist of the juxtamembrane region. All the disulfide-bonded dimers had flexible ligand-binding domains with the same biphasic affinities for the ligand as the wild type. These results demonstrate that ligand binding to the flexible extracellular domains of the receptor dimer induces rotation/twist of the juxtamembrane regions, hence the transmembrane domains, and dissociates the dimeric, inactive form of the intracellular domains. The flexible rotation/twist of the intracellular kinase and regulatory domains may be necessary for the intrinsic catalytic kinase to become accessible to the multiple tyrosine residues present in the regulatory domain and various substrates such as PLCγ and PI3 kinase.

Indeed, this rotation/twist model (Fig. 1) is consistent with the homodimeric structure of the receptor kinase, transmembrane and unactivated extracellular domains that have recently been determined by others. Ligand-induced intermolecular interaction of the extracellular domains may induce the rotation/twist of their transmembrane domains of the receptor homo- and heterodimers, resulting in dissociation of the dimeric kinase domains for the receptor activation. The dissociated kinase is likely to phosphorylate tyrosine residues in the receptor's regulatory domain and other substrates.

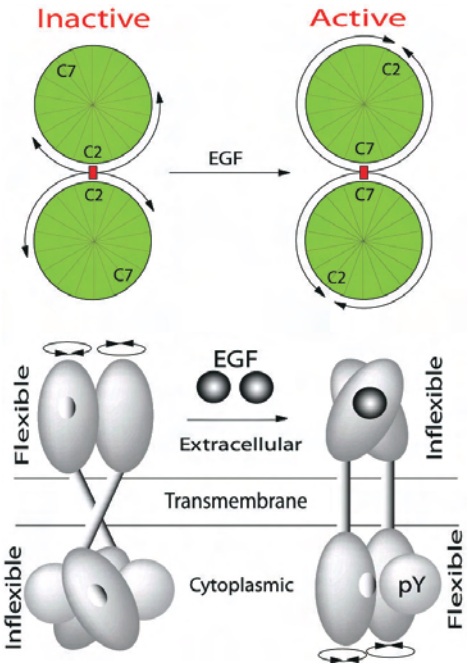


Fig. 1 "Rotation/twist" model for the receptor activation

2.3.1.1. Detection of preformed homo- and heterodimeric structures of EGFR and ErbB2 at physiological expression levels. We have detected preformed homo- and heterodimers of EGFR and ErbB2 at physiological expression levels (< 10^5 molecules per cell)

by fluorescence resonance energy transfer (FRET) and fluorescence cross-correlation spectroscopy (FCCS). When EGFR and ErbB2 fused with a fluorescent protein (FP) were expressed on the cell surface of Chinese hamster ovary (CHO) cells at physiological levels, FRET was detected between the donor and acceptor FPs. Furthermore, cross-correlation between FPs separately fused to EGFR or ErbB2 was also observed by FCCS, indicating that EGFR and ErbB2 molecules diffuse together as homo- or heterodimers in the cell membrane. These results demonstrate that the unactivated cell-surface receptors can spontaneously form homo- and heterodimers as major forms, irrespective of their expression levels ranging from ~2 x 10^4 to ~5 x 10^6 molecules per cell. This work has been carried out in collaboration with T. Wohland (National University of Singapore) and S. Ahmed (Centre for Molecular Medicine, Singapore).

2.3.1.2. Detection of preformed homo- and heterodimers between the EGFR family members. We have also analyzed preformed homo- and heterodimeric structures between all the members, EGFR, ErbB2, ErbB3, and ErbB4, of the receptor family by employing bimolecular fluorescence complementation (BiFC) assay, and have found that all the members display preformed homo- and heterodimeric structures in the absence of bound ligand. Furthermore, we have also found that ErbB3 was localized in the nucleus when expressed alone or together with ErbB4. When coexpressed with EGFR or ErbB2, however, ErbB3 was relocated to the plasma membrane (Fig. 2). Binding of ligands such as EGF and HRGβ1 had no effect on the efficiency of the dimer formation, indicating that there is no significant amount of monomers on the cell surface. The nuclear localization of the ErbB3 homodimer and ErbB3/ErbB4 heterodimer suggest that the receptors may also play roles in the nucleus. These provide new insights into an understanding of transmembrane signal transduction mediated by the EGFR family.

2.3.1.3. Mutagenesis analysis of EGFR domains required for homo- and heterodimerization. We have also initiated mutagenesis analysis of EGFR to find the receptor

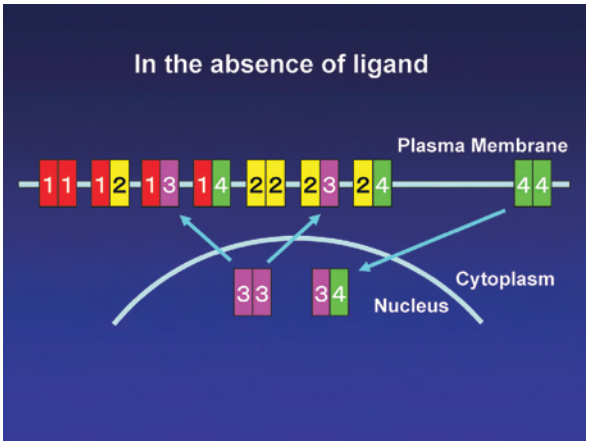


Fig. 2 Preformed homo- and heterodimeric structures of the ErbB receptor family and their subcellular localization

domain(s) involved in spontaneous homo- and heterodimerization. Three-dimentional structures of the extracellular domains of EGFR and ErbB2 as well as of the intracellular kinase domain of EGFR recently determined by others suggest that the extracellular juxtamembrane region and intracellular kinase domain may play vital roles in the formation of the dimers. By constructing point mutations of amino-acid residues, we will identify crucial ones for the dimer formation.

2.3.2. Processing of external information by the nervous system

In this section of the project, we have focused on the analysis of the nematode *C. elegans* chemotaxis towards alkaline pH (2.3.2.1.), the genetic analysis of signaling pathways involved in *C. elegans* synaptogenesis (2.3.2.2.), and the electrophysiological analysis of pharyngeal pumping for monitoring the perception of external stimuli in *C. elegans* (2.3.2.3.).

2.3.2.1. *C. elegans* chemotaxis towards alkaline pH. *C. elegans* responds to water-soluble chemicals such as ions and amino acids. *C. elegans* senses acid pH (H^+) as an aversive stimulus, and alkaline pH (OH^-) as an attractive stimulus. Some of the amphid chemosensory neurons have been shown by neuronal ablation to be responsible for acidic pH avoidance. In general, however, neural mechanisms underlying the OH^- response have not been elucidated in any organisms.

To investigate the OH^- chemosensation, we have developed an assay system for the worm's response to alkaline pH. On the assay plate with a pH gradient from pH 7.0 to pH 10.0, wild-type animals moved towards alkaline pH along the gradient (Fig. 3). *osm-6*, a sensory cilium-defective mutant, did not show any response to alkaline pH. In addition, some chemotaxis mutants with normal sensory cilia are found to be insensitive to the pH gradient.

In *C. elegans*, many attractive ions are sensed by ASE chemosensory neurons. Interestingly, an ASE defective *che-1* mutant avoided around pH 10.0 region as shown in Fig. 3. These suggest that while ASE neurons may be required for chemotaxis towards alkaline pH

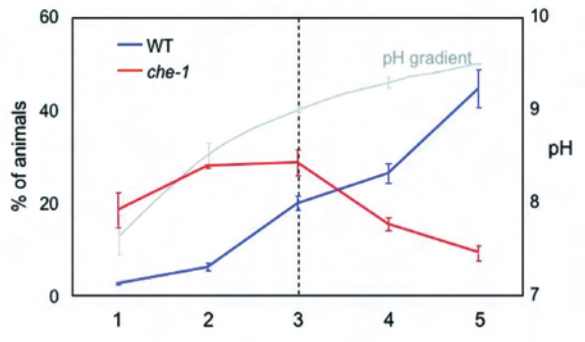


Fig. 3 Distribution patterns of wild-type (blue) and *che-1* mutant (red) animals along the alkaline pH gradient in square plates. Horizontal axis indicates five sections, 1.5-cm wide each, of the assay plate. Worms were put at the center, dotted line, of the plate. Data are the mean ± SEM of more than three separate assays.

ranges, some other neurons than ASE may be responsible for avoidance of higher alkaline pH ranges. Furthermore, ASE-specific rescue of the gustatory defective *dyf-3* mutant induced normal orientation towards alkaline pH, suggesting that ASE functions are sufficient for this type of chemotaxis (Fig. 4).

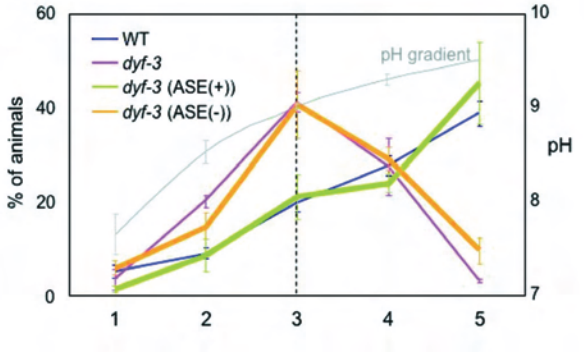


Fig. 4 ASE specific rescue in *dyf-3*. Note that *dyf-3* animals (pink) did not respond to the pH gradient. *dyf-3* rescued by the ASE(+) construct (green) moved toward higher pH, whereas *dyf-3* injected with a negative control construct (orange) did not. Data are the mean ± SEM of more than three separate assays.

Among known chemotaxis mutants, *daf-6* avoided pH 10.0 like *che-1*. Among three types of chemosensory organs in *C. elegans*, the *daf-6* mutant is defective in sensory pore formation in two types of chemosensory organs, and has normal structure in IL2 chemosensory endings. Our results imply that IL2 chemosensory neurons may be involved in alkaline pH avoidance.

2.3.2.2. Genetic analysis of synaptogenesis in *C. elegans*. Synapses display a stereotyped ultrastructural organization, commonly containing a single electron-dense presynaptic density surrounded by a cluster of synaptic vesicles (Fig. 5). The mechanism controlling subsynaptic proportion is not understood. Loss of function in the *C. elegans rpm-1* gene, a putative RING finger/E3 ubiquitin ligase, causes disorganized presynaptic cytoarchitecture. RPM-1 is localized to the presynaptic periactive zone and negatively regulates a novel p38 MAP kinase pathway, composed of the dual-leucine-zipper-bearing MAPKKK DLK-1, MAPKK MKK-4, and p38 MAP kinase PMK-3. To identify the additional signaling components of this pathway, we are characterizing a suppressor mutation of *rpm-1, ju587*. We identified that this gene functions downstream of MKK-4, and mapped *ju587* between +3.51 and +5.06 on chromosome I. This work has carried out in collaboration with Yishi Jin at University of California, Santa Cruz.

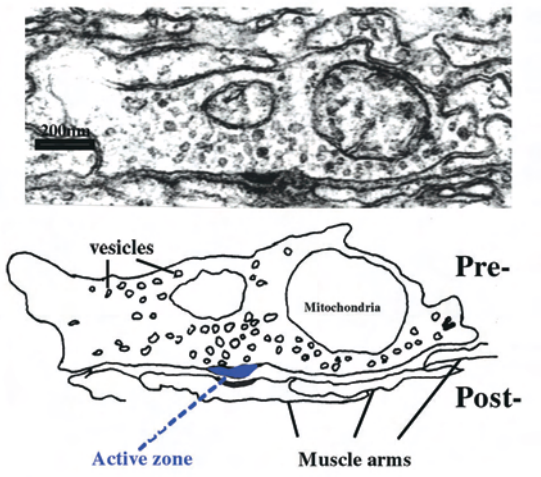


Fig. 5 Neuromuscular synapse

2.3.2.3. Electophysiological analysis of pharyngeal pumping in *C. elegans* for monitoring the perception of external stimuli and its plasticity. The perception of external stimuli in *C. elegans* is generally tested using locomotor responses, visually assessing whether worms are attracted or repelled by stimuli. Pharyngeal pumping is proposed as an alternative behavioural response for monitoring the perception of external stimuli and plasticity in *C. elegans*. Under normal conditions, spontaneous pumping measured using electrophary ngeograms is low or absent. So far, several stimuli were identified that could modulate pharyngeal pumping activity (Fig. 6). Whether stimulant ions or molecules act through sensory cells or via neurotransmitter or neuromodulator receptors of muscles or motor neurons or other pathways remains to be determined. Of particular interest is the fast activation of pumping by stimulation with bacterial suspensions. Denaturated bacteria and latex beads have no effect, thus current work focuses on identifying components of the bacterial cell wall that are effective stimulants. The short-term aim is to identify the sensory cells and possibly the receptor proteins involved in *C. elegans* food perception. At the moment, nothing is known about how *C. elegans* perceives bacteria, although unknown odorants are supposed to play a role in long-range detection. Beyond its general significance, the food stimulus is of particular interest because it represents the unconditioned stimulus for positive reinforcement learning in *C. elegans*. We are currently developing paradigms for the conditioning of pharyngeal pumping, that would have potential to become a model system. Unlike other preparations, this system would be tractable at all levels from molecules to cells, networks, and behaviour.

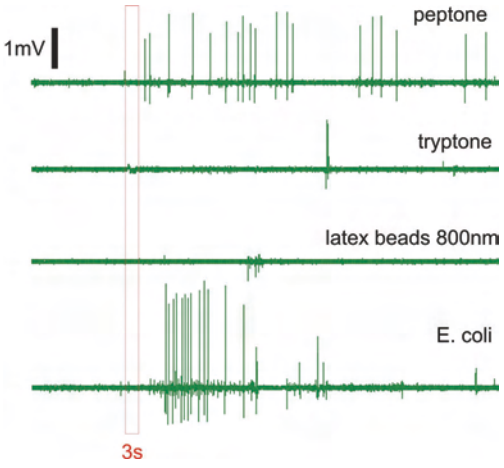


Fig. 6 Pharyngeal pumping activity upon presentation of various stimuli.

3. Publications

3.1. Journals

Kannan, B., Har, J. Y., Liu, P., Maruyama, I., Ding, J. L., and Wohland, T. (2006) Electron multiplying charge-coupled device camera based fluorescence correlation spectroscopy. *Anal. Chem*. 78, 3444-3451.

Hwang, L. C., Liu, P., Maruyama, I., Gosch, M., Lasser, T., and Wohland, T. (2006) Single wavelength fluorescence cross-correlation spectroscopy for multicolor detection of molecular interaction. *Biophys. J.* 90, 11.

Liu, P., Thankiah, S., Hwang, L. C., Koh, M. L., Maruyama, I., and Wohland, T. (2006) In vivo study of the epidermal growth factor receptor by fluorescence cross-correlation spectroscopy. *Biophys. J.* 90, 1865.

Sudhaharan, T., Liu, P., Koh, R., Hwang, L. C., Wohland, T., and Maruyama, I. (2006) Preformed homo- and heterodimers of EGF/ErbB2 receptors as major forms on the cell surface. *Mol. Biol. Cell* 17, 61.

Kawasaki, I., Hanazawa, M., Gengyo-Ando, K., Mitani, S., Maruyama, I., and Iino, Y. (2007) ASB-1, a germline-specific isoform of mitochondrial ATP synthase b subunit, is required to maintain the rate of germline development in *Caenorhabditis elegans*. *Mech. Dev.* 124, 237-251.

Liu, P., Thankiah, S., Koh, M.L., Hwang, L.C., Ahmed, S., Maruyama, I.N., and Wohland, T. (2007) Investigation of the dimerization of proteins from the epidermal growth factor receptor family by single wavelength fluorescence cross-correlation spectroscopy. *Biophys. J.* (in press)

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

None

4. Scientific Meeting Presentation

4.1. Oral

Kannan, B., Har, J.-H., Liu, P., Maruyama, I., Ding, J. L., and Wohland, T. Fluorescence correlation spectroscopy performed using EMCCD camera. Asia-Pacific Workshop on Biological Physics, Singapore (July 3-5, 2006).

Liu, P., Sudhaharan, T., Koh, M. L., Hwang, L. C., Maruyama, I., and Wohland, T.

Interaction and activation of ErbB family receptors investigated by single-wavelength fluorescence cross-correlation spectroscopy. Asia-Pacific Workshop on Biological Physics, Singapore (July 3-5, 2006).

Fujimoto, T., Miwa, M., Maruyama, I., and Nakamura, S. Rheumatoid arthritis autoantigens identified by using lambda phage surface display. 16th Annual Meeting of Japanese Society of Spondyloarthritis, Nara, Japan (September 9, 2006).

Murayama, T., and Maruyama, I. Chemotactic response toward basic pH in *C. elegans*. 2nd Biennial East Asia *C. elegans* Meeting, Seoul National University, Seoul, Korea (November 15-18, 2006).

Kawasaki, I., Hanazawa, M., Gengyo-Ando, K., Mitani, S., Maruyama, I., and Iino, Y. ASB-1, a germline-specific isoform of mitochondrial ATP synthase b sununit, is required to maintain the rate of germline development in *Caenorhabditis elegans*. 2nd Biennial East Asia *C. elegans* Meeting, Seoul National University, Seoul, Korea (November 15-18, 2006).

Tao, R.-H., and Maruyama, I. N. Cell-surface and nuclear localization of preformed ErbB receptor homo- and heterodimers in living cells. OIST-Korea Workshop "Neuroscience and Beyond", Okinawa, Japan (February 21-23, 2007).

4.2. Posters

Liu, P., Sudhaharan, T., Hwang, L. C., Koh, M. L., Maruyama, I., and Wohland, T. In vitro study of the epidermal growth factor receptor by fluorescence cross-correlation spectroscopy. 50th Annual Meeting of American Biophysical Society, Salt Lake City, Utah, USA (February 18-22, 2006).

Sudhaharan, T., Liu, P., Koh, M. L., Hwang, L. C., Wahland, T., and Maruyama, I. EGF/ErbB receptors exist as preformed homo- and heterodimers on the cell surface. Gordon Research Conferences "frontiers of science: Growth Factor Signalling", Connectcut College, New London, Connecticut, USA (July 16-21, 2006).

Thankiah, S., Liu, P., Koh, M. L., Hwang, L. C., Wahland, T., and Maruyama, I. Preformed EGF/ErbB receptor dimers on the cell surface. 46th Annual Meeting of The American Society for Cell Biology, San Diego, California, USA (December 4-8, 2006).

Kannan, B., Hong, Y., Thankiah, S., Liu, P., Maruyama, I., and Wohland, T. A system for

spatially-resolved total internal reflection-fluorescence correlation spectroscopy using an EMCCD camera. 51st Annual Meeting of Biopysical Society, Baltimore, Maryland, USA (March 3-7, 2007).

Wohland, T., Liu, P., Thankiah, S., Maruyama, I., and Ahmed, S. Single wavelength fluorescence cross-correlation spectroscopy (SW-FCCS) as a tool to determine biomolecular interactions in vivo. 51st Annual Meeting of Biopysical Society, Baltimore, Maryland, USA (March 3-7, 2007).

5. Invited Lecture and Seminar

Maruyama, I. Contemporary Life Sciences. Kaiho High School, Okinawa, Japan (August 23, 2006).

Maruyama, I. Epidermal growth factor receptor and targeted cancer therapy. Kanazawa University School of Medicine, Kanazawa, Japan (October 5, 2006).

Maruyama, I. Targeted cancer therapy and cancer diagnostics. Kanazawa University School of Health Sciences, Kanazawa, Japan (October 6, 2006).

Maruyama, I. Application of basic life sciences to Okinawa's natural resources. Annual Meeting of Okinawa Industrial Technology Center, Okinawa, Japan (October 18, 2006).

Maruyama, I. Application of FCS and FCCS to cell biology: Analysis of homo- and heterodimeric structures of the EGF receptor family. 5th East Asian Biophysics Symposium & 44th Annual Meeting of the Biophysical Society of Japan, Okinawa, Japan (November 12-16, 2006).

Maruyama, I. Environmental signal detection and processing by cells and the nervous system. Okinawa-Belgian Workshop, Okinawa, Japan
(November 20, 2006).

Maruyama, I. Information processing by life. 2nd Annual Meeting of Okinawa Institute of Science and Technology, Okinawa, Japan (February 2, 2007).

Maruyama, I. Rotation/twist Model for Activation of the EGF/ErbB receptors. OIST-Korea Workshop "Neuroscience and Beyond", Okinawa, Japan (February 21-23, 2007).

6. Intellectual Properties and Other Products

None

7. Meetings and Events

7.1. Workshop hosted:

Super Science High School Workshop (Kaiho High School, Naha, Okinawa, Japan)
Date: August 28, 2006

7.2. Seminars hosted:

Speaker: Takashi Murayama
Department of Biology, Kyushu University, Fukuoka, Japan
Title: The nematode C. elegans mutants defective in a food response behavior.
Date: February 2, 2006

Speaker: Rong-Hua Tao
Department of Medicine, Kyushu University, Fukuoka, Japan
Title: Testicular zinc finger (TZF) proteins affecting androgen receptor activity.
Date: March 20, 2006

Speaker: Satoshi Hasegawa
Riken Institute Center for Developmental Biology, Kobe, Japan
Title: Roles of histone deacetylases (HDACs) in endothelial cell development.
Date: April 28, 2006

Speaker: Masataka Kinjo
Research Institute for Electronic Science, Hokkaido University, Sapporo, Japan
Title: Study of protein dynamics in living cell using fluorescence correlation spectroscopy.
Date: May 16, 2006

Speaker: Klaus Weisshart
Carl Zeiss, Germany
Title: What is fluorescence correlation spectroscopy (FCS)?
Date: May 16, 2006

Speaker: Stephan Haupt
Institute of Ecology, Technical University Berlin, Germany
Title: The gustatory system and antennal learning in the honeybee.
Date: June 2, 2006

Speakers: Atsuhiko Toyama, and Taka-Aki Sato
Proteomics Research Center, Shimazu Corp., Tsukuba, Ibaraki, Japan.
Title: Comprehensive proteomic analysis of ovarian cancer for Molecular tumor typing.
Date: March 7, 2007

Speaker: Masaru Furuta
Proteomics Research Center, Shimazu Corp., Tsukuba, Ibaraki, Japan.
Title: MS Imaging.
Date: March 7, 2007

Speaker: Masaki Yamada
Proteomics Research Center, Shimazu Corp., Tsukuba, Ibaraki, Japan.
Title: MS analysis of phosphorylated proteins.
Date: March 7, 2007

XI. Molecular Neurobiology Unit

Principal Investigator: Takayuki Naito
Research Theme: Single-cell Biochemistry and Molecular Analysis of Brain Functions

Abstract:
Setting up the laboratory:
We spent most of our time this fiscal year setting up the laboratory and we started experimental work in the summer of 2006. Three researchers and 3 technicians were recruited in April, a further researcher and a secretary in September and most of the apparatus were set up.
Research activity:
The central theme of our research is the study of activity-dependent gene expression in the nervous system. Activity-dependent gene expression means modulation of gene expression occurring in individual cells as a result of various stimuli. It is thought to be correlated with self-reorganizing activities of the nervous system such as neuronal plasticity.
We have started the following major projects along with the central theme:

1. Imaging study of activity-dependent gene expression
2. Single-cell biochemistry
3. Neumap
4. Molecular profiling of the brain by mass spectrometry
5. Salamander project in collaboration with the Brenner Unit

Some initial findings are described in Section 2.

1. Participants:

1.1. Individuals:

-Researchers:

Setsuko Nakanishi, Kiyotaka Akiyama, Nozomu Nakamura, Michael Chandro Roy

-Technicians:

Seiko Kuraba, Sayaka Arai, Saori Ishida

-Research Assistant:

Kaori Yamashiro

1.2. Partner Organizations:

Okinawa Institute of Science and Technology, PC

Type of study: Joint research

Principal researcher: Dr. Sydney Brenner

Theme: "Salamander project"

University of Tokyo, Department of Metabolome, Graduate School of Medicine
Type of study: Joint research
Principal researcher: Prof. Ryo Taguchi
Theme: "Mass Imaging"

National Institute for Physiological Sciences, Okazaki Institute for Integrative Bioscience
Type of study: Joint research
Principal researcher: Associate Prof. Mitsutoshi Setou
Theme: "Mass Imaging"

Tokyo Women's Medical University
Type of study: Joint research
Principal researcher: Dr. Kazuhiko Tatemoto
Theme: "Peptidome in the brain"

Nagoya University, Department of chemistry
Type of study: Joint research
Principal researcher: Prof. Daisuke Uemura
Theme: "Marine microorganism project"

2. Activities and Findings:

2.1. Imaging study of activity-dependent gene expression

We plan to visualize dynamics of activity-dependent gene expression in the cells of the nervous system.

Immediate-early genes are our first targets in this project, It is known that some of the transcription factors and postsynaptic effectors, such as Egr1 (known as Zif268, NGFI-A, and Krox24), Arc (known as Arg3.1), and Homer1a (known as Vesl-1S), which are called immediate-early genes, are activity-dependently induced in hippocampal pyramidal neurons, and are identified as critical indicators of synaptic plasticity and memory processing. We will use primary cultures of neurons, and then brain slices and individual animals. We focus on the following cell-types at present: 1) pyramidal cells of the hippocampus, 2) Purkinje cells of the cerebellum, 3) granule cells of the cerebellum, and 4) striatal cells of the caudate putamen. Some of these primary cultures have been prepared.

2.2. Single-cell biochemistry

In this project, we are interested in the action of single cells in cellular (neural) networks, because it is believed that individual cells (neurons) are differentially activated in cellular (neural) networks depending on a variety of stimuli. We will develop single-cell

biochemistry and apply it to this study. At first, we will study what kind of mRNAs and how many copies of each mRNA are expressed in single cells.

To prepare RNA from single cells, some experiments using LMD (laser microdissection) have been carried out, as shown in Fig. 1.

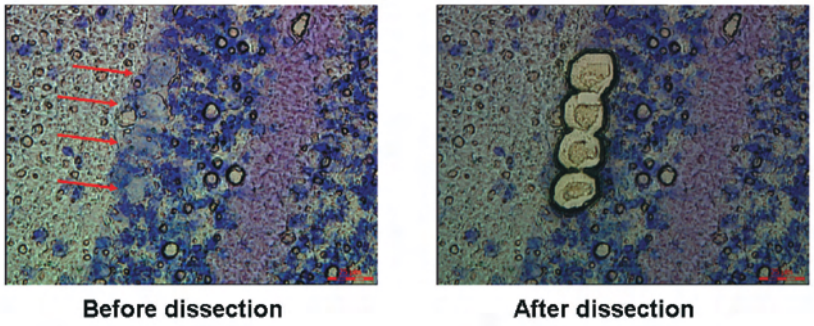


Fig. 2 LMD (laser microdissection) of mouse Purkinje cells

2.3. NeuMap

NeuMap refers to the identification of different cell types in the nervous system by the genes expressed within them. We want to study how many neuron types are present in the nervous system.

Neurons receive signals from the environment or other neurons in the network through membrane receptors and transfer signals to other neurons by releasing neurotransmitters. Neurons can be classified by these signaling molecules, such as receptors on the cell surface (Ion-channel receptors and G protein-coupled receptors (GPCRs) are especially important) and neurotransmitters. Most of these molecules can be detected through gene expression analysis.

For example, we analyzed the gene expression of cultured cerebellar granule cells using DNA microarray analysis (RNA material used in this study is a kind gift from Dr. Shigetada Nakanishi of Osaka Bioscience Institute). We identified about 30 candidate genes coding for ion-channel receptors and about 100 candidate genes coding for GPCRs. The expression of these candidates will be confirmed by Q-PCR.

We also applied MPSS (Massively parallel signature sequencing) to the analysis of the gene expression of cultured granule cells. A comparative study of DNA microarray data and MPSS data is underway.

2.4. Molecular profiling of the brain by mass analysis

Based on molecular biology, we intend to develop an interdisciplinary approach including optical imaging, electrophysiology, mass analysis, etc. Mass spectrometry is used to identify signal molecules in intercellular space and to discover novel neurotransmitters.

Set up of two Mass Spectrometers

1. LTQ Orbitrap MS^n (Thermo-Finnegan) hybrid mass spectrometer with ESI probe (Electron Spray Ionization), and NSI probe (Nano Spray Ionization) along with the

following interfaces:

a) HPLC (High Pressure Liquid Chromatography, Paradigm) equipped with an auto-sampler (CTC PALAnalysis) and UV-detector; b) CE (Capillary Electrophoresis, Beckman Coulter).

2. MALDI-MS (Matrix Assisted Laser Desorption Ionization Mass spectrometer, Applied Bio System). Both Mass spectrometers have been tested and their performance evaluated with known standard compounds.

Application of LC-Mass method for molecular profiling of pig cerebrospinal fluid (CSF):

Molecular profiling of the brain is in progress. For example, the Methanol-soluble fraction of freeze-dried CSF was analyzed by Nano-LC-MS (LTQ Orbitrap MS^n) and over one hundred molecules with lower molecular mass (200~1500 Da) were detected in this fraction.

2.5. Salamander project

General matter

We have started the salamander project in collaboration with Brenner Unit of OIST. In general, cell size is proportional to DNA content/cell. As many species of salamander have high DNA content per cell, salamander is thought to be a good material to study neuronal networks in vertebrate nervous systems and for single-cell biochemistry.

After screening 4 species of salamander (*Ambystoma mexicanum, Amphiuma tridactylum, Cynops ensicauda, and Cynops pyrrhogaster*), *Ambystoma mexicanum* has been selected as the experimental material, because it is easy to obtain, maintain, operate, and the cell size is large enough comparing with mammalian cells.

In addition, we extracted DNA and RNA, and mRNA from salamander (*Ambystoma mexicanum*) and mouse liver and estimated the cell number/gram of tissue, RNA content/cell, and mRNA content/cell for each animals, using C-value (haploid DNA content; pg) of 3 for mouse and 30 for salamander. As shown in Table 1, we found that mouse has about 30 times more cells in 1 g of the liver tissue than salamander, and RNA (and mRNA) content/cell is about 10 times higher in salamander than in mouse.

Table 1 Comparison of RNA content between ambystoma and mouse

	Tissue weight (g)	DNA weight (g)	Total RNA (mg)	PolyA RNA (mg)	Cell number	RNA content / Cell (pg/cell)	PolyA RNA content / Cell (pg/cell)
Ambystoma	1	3.83 (0.36)	1.58 (0.11)	1.29×10^{-2} (1.5×10^{-3})	6.38×10^{7} (6.1×10^{6})	24.8 (1.7)	0.202 (2.4×10^{-2})
Mouse	1	12.3 (1.0)	5.50 (0.50)	3.84×10^{-2} (9.7×10^{-3})	2.05×10^{9} (1.7×10^{8})	2.69 (0.25)	0.0188 (4.8×10^{-3})

SD Value is indicated by parenthesis
n=6

Morphological study

A morphological study of the salamander has been carried out. A brain atlas of Ambystoma mexicanum is under construction using coronal, horizontal and sagittal sections. Some preliminary results are presented in Figs.2 and 3.

Electron microscopic examination of the salamander's neuronal cells is on going.

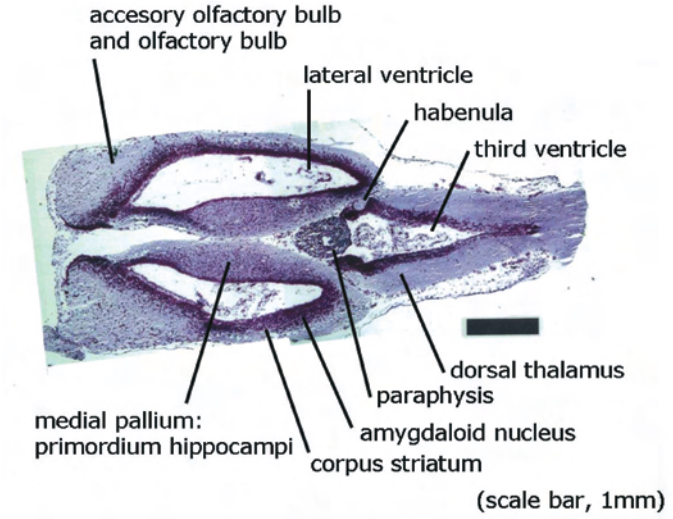


Fig. 2 Forebrain and midbrain of Ambystoma mexicanum, horizontal section (8 μm thick) stained by Gomori's method.

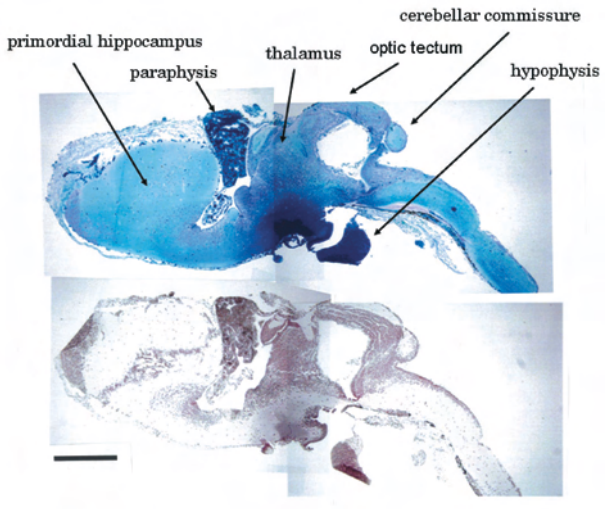


Fig. 3 Two sagittal sections of Ambystoma mexicanum stained by the Klüver-Barrera method (upper figure) and Bodian's method (lower figure). In the Bodian's specimen the cell bodies were also stained brown-purple. Some identified brain areas are indicated with arrows. Scale bar: 1 mm

Construction of a cDNA database of Ambystoma mexicanum (Details; see Brenner Unit)

Based on the cDNA database, we proceed to studies including gene expression analysis,

imaging analysis, and producing transgenic salamanders in the near future.

3. Publications

3.1. Journals

Tatemoto K., Nozaki Y., Tsuda R., Konno S., Tomura K., Furuno M., Ogasawara H., Edamura K., Takagi H., Iwamura H., Noguchi M., and Naito T. "Immunoglobulin E-independent activation of mast cell is mediated by Mrg Receptors" *Biochemical and Biophysical Research Communications,* ***349,*** 1322-1328 (2006).

Nakamura N. H., Akama K. T., Yuen G. S., and McEwen B. S. "Thinking outside the pyramidal cell: unexplored contributions of interneurons and neuropeptide Y to estrogen-induced synapse formation in the hippocampus" Reviews in the Neurosciences, in press.

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

Nothing to be reported.

3.3. Oral Presentations and Posters

Oral Presentations:

Roy M.C., Kondo S., Noma I., Takiguchi T., Koyama T., Yamada K., Kita M., Uemura D. "Durinskiol A, a High Molecular Weight Polyol from a Dinoflagellate, Durinskia sp"; *The 86th Japan Chemical Society Meeting*, Spring **2006**, Nihon Daigaku, Funabashi, Japan, March 27-30, 2006.

Naito T. "Introduction to Naito Unit and salamander project", *Okinawa-Belgian Workshop*,Okinawa, Japan, November 20, 2006

Naito T. "Brain and Genes", 2nd OIST Research Project Seminar, Okinawa, Japan, February 2, 2007

Naito T. "Single-cell biochemistry and molecular analysis of brain functions", *Neuroscience and Beyond (OIST-Korea Workshop)*, Okinawa, Japan, February 21-23, 2007

Posters:

Nothing to be reported

4. Intellectual Property Rights and Other Specific Products

Patent:

Title of the invention: Methods for screening substances inhibiting mast cell degranulation

through receptor #168 and the development of anti-inflammatory drugs containing these substances

Inventors: Kazuhiko Tatemoto, Takayuki Naito, Yuko Nozaki, Masahiro Huruno, and Keiko Tomura

Application Number: PCT/JP2006/323063

5. Meetings and Events

Nothing to be reported

XII. Mathematical Biology Unit

Principal Investigator: Robert Sinclair
Research Theme: Biologically Inspired Mathematics

Abstract:

Mathematics is not a natural science, and yet it has in the past grown as a result of interactions with the natural sciences, particularly physics. It is only reasonable to expect that mathematics will also benefit from the challenges biology is presenting it with. The long-term goal of this research unit is to develop new areas of mathematics which are inspired by, and should also be of use in, studying biological systems. This can only be done successfully as a result of both continuing to be active in pure mathematical research and also close interactions with experimental biologists. OIST provides a unique opportunity for such continued and close contact with experimental biologists.

1. Participants: This unit started operation July 1, 2006.

1.1. Individuals:

-Research Assistant: Shino Fibbs (started December 13, 2006).

-Visiting Researchers: Prof. Minoru Tanaka of Tokai University Department of Mathematics, November 18, 2006.

Dr. Hitomi Terajima, Kyoto University Research Institute for Mathematical Sciences (RIMS), January 18-31, 2007.

1.2. Partner Organizations:

Nothing to be reported

2. Activities and Findings:

2.1. Activities and Findings in Mathematical Biology

2.1.1. Putting evolution into reverse. The molecular basis of evolution is quite well understood, and mathematical equations which can capture (some aspects of) the process of evolution have been known for some time. What is not at all well understood is how to look back in time. If we find a plant with a certain genetic code, what can we say with certainty about its ancestors? We must expect that there will be questions which cannot be answered. Identifying those questions which have real meaning, and finding precise answers to these questions where possible, will both bring a new level of rigour into the study of evolution and also give new insights into its fundamental structures.

At this point in time, it would seem that it is indeed possible to compute reverse

transition probabilities within the framework of Mendelian genetics, despite the fact that the forward Markov process is absorbing. Current work is divided into a number of activities. These include (i) an extensive literature search, necessary because of the enormous amount of work that has been done in this area in the last 70 years, (ii) the development of software to use as experimental tools, and (iii) investigations into the appropriate structure to use as the basis of a mathematical formulation.

Clearly, Mendelian genetics is only a first step in this direction. The influence of non-coding regions in genomes would appear to present the greatest challenges, since their lengths can change dramatically over time. The development of appropriate mathematical models of even the forward evolutionary process including non-coding regions is an immediate goal, to be followed by an investigation of the reverse process.

2.1.2. A study of the nervous system of the worm C. elegans, which has only about 300 nerve cells. The entire wiring diagram is known, but we do not yet understand how it actually works. Using known biological data, we plan to construct first a computational and then a mathematical model of the worm's nervous system. This mathematical model will then form the basis for more theoretical studies concerning the relationships between the component parts, and, indeed, how to define concepts such as "part of a brain" at all.

Initial studies of the neural network associated with locomotion clearly indicate that the network is not composed of repeating segments, as has often been assumed in previous models. Rather, there appear to be a number of different networks (as one might guess, given that C. elegans can move in a number of different ways) which are interconnected in a non-trivial way. This complex structure must be optimal in some respect, since it is a product of evolution. Already these data present one with a number of challenges. How many "components" does the network have? How many independent functions does it perform? In what sense is it optimal?

From a study of the available literature, it has become clear that the way forward is to attempt to model (parts of) the neural network using more biological data than previous models have included. In particular, more recent data concerning the various synaptic connections and gap junctions seems to be necessary for one to be able to construct a functioning model. This is a long-term project being pursued in collaboration with Dr. Maruyama's Molecular Neuroscience group.

2.1.3. The relationship between neuronal form and function. This research is being undertaken in collaboration with Dr. Stiefel's Theoretical and Experimental Neurobiology Unit. Dr. Stiefel already has many results relating neuronal form or shape to function, but a robust mathematical framework which can accommodate these findings is desirable. This research involves not only the symmetries of three-dimensional Euclidean space, but also considerations of how best to define a distance between two given dendritic structures. Of particular interest is the question of how many different classes or types of dendritic trees can perform a given function in an optimal way.

2.1.4. Introns: The fact that so much sequence data is now becoming available means that this is a time of great opportunity for investigations into the structure of introns. While it is abundantly clear that a complete understanding of introns can only come from both sequence and spatial data, it appears that there are still a number of questions which can be posed on the basis of sequence data alone. Such questions relate to correlations between nucleotides at intron/exon boundaries and repeated sequences found within introns, for example. Software is currently being developed by Dr. Sinclair which will generate a database of experimentally verified intron sequences (based upon RefSeq).

Preliminary computations using experimental versions of this database are already suggesting that correlations previously reported in the literature are perhaps non-existent. Looking forward, there seems to be a real chance of moving beyond "consensus sequences" for the description of splice sites and branch points. It is already known that there are biological differences between the introns of yeast, plants (even between monocots and dicots) and animals. These differences should be visible in the experimental sequence data.

2.1.5. "Few-measurement statistics". Many biological experiments are too difficult to be performed more than a small number of times. As a result, a large number of current research papers (also in the best journals) contain cases of standard statistical machinery applied to sets of ten or less data points. It is however a fact that statistics is based upon the assumption that there are "many" data points, and there is here an apparent need for new mathematical approaches.

As an example, imagine two cages placed in a forest, one empty and one containing an odorant. One could count the number of times a certain type of animal enters each cage. A simple question might be "Is this animal attracted to this substance?" If the available experimental data are "three times in the empty cage and never in the other", what can be said? A naive application of standard statistical methods would lead one to the conclusion that there is absolutely no chance (0%) that the animal will ever enter the cage with the odorant. Is this supported by the data? No! Having said that, what can be said about the probability that the animal might enter this cage?

The mathematical techniques to be used here are all elementary, and yet do not seem to have been applied to such problems before. At this point, it is clear that knowledge of the space of possible outcomes of an experiment (the two cages, in the case above) is vital.

2.2. Activities and Findings in Pure Mathematical Research

2.2.1. Dr. Sinclair continues to perform mathematical work of an experimental nature in collaboration with Prof. Minoru Tanaka of Tokai University and others. The current focus of this work is an application of pure mathematical understanding in global differential geometry to (satellite) orbital transfer problems. A loose collaboration has begun with Prof. Jean-Baptiste Caillau of the Institut de Recherche en Informatique de Toulouse and Prof. Bernard Bonnard of Bourgogne University, France.

2.2.2. The front cover of the December 22 issue of Science Magazine was produced by

Cameron Slayden of Cosmocyte, Inc. (www.cosmocyte.com) using data provided by Dr. Sinclair. This cover illustration accompanied a news article describing the "Breakthrough of the Year", the proof of the Poincaré Conjecture by Grigori Perelman. The illustration depicts the flow of a two-dimensional surface under what is known as the Ricci Flow. Dr. Sinclair developed an algorithm and wrote software to implement this while still at Melbourne University. The software was then rewritten at OIST to allow export of the shape data to a standard graphics format. This data became the basis of the illustration.

3. Publications

3.1. Journals

Sinclair R. and Tanaka M., "The cut locus of a two-sphere of revolution and Toponogov's comparison theorem", to appear in the Tohoku Mathematical Journal.

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

Nothing to be reported.

3.3. Oral Presentations:

Robert Sinclair, "Mathematical Biology", Neuroscience and Beyond: OIST-Korea Workshop, Okinawa, Feb. 21-23 2007

4. Intellectual Property Rights and Other Specific Products

Nothing to be reported

5. Meetings and Events

5.1 OIST IRP Seminars

Date: November 24, 2006.

Title: "The transfer operator method: An introduction and an application to quantum chaos"

Speaker: Dr. Tobias Mühlenbruch

Institute for Theoretical Physics, Clausthal University of Technology, Germany.

Date: November 29, 2006.

Title: "Global existence results for semilinear evolution equations under generalized dissipativity conditions"

Speaker: Asst.Prof. Paul Georgescu, Gheorghe Asachi Technical University of Iasi, Romania.

Date: December 4, 2006.

Title: "Lasers in the Biosciences"

Speaker: Prof. Dr. Hans Schuessler, Department of Physics, Texas A&M University.

Date: December 18, 2006.

Title: "Motions of Curves and their Discretization"

Speaker: Prof. Atsushi Fujioka, Hitotsubashi University, Tokyo.

Date: February 7, 2007.

Title: "From the nematode C. elegans to macaque monkeys: General characteristics of neural network organisation"

Speaker: Dr. Marcus Kaiser, School of Computing Science, Newcastle University, UK.

5.2 Public Relations

5.2.1. A visit to Kaiho High School, November 11, 2006. Dr. Sinclair gave a talk titled "The Soul of Mathematics" to school students of first to senior grades, using the book "A Mathematician's Apology" by G.H. Hardy (first published in 1940) as a basic text.

5.2.2. The 2nd OIST Research Project Seminar, February 2, 2007. Organized by the Science & Technology Promotion Office, Okinawa Prefectural Government. Dr. Sinclair gave a talk titled "Mathematical Biology", followed by a presentation of mathematical animations. This event, which included talks by Dr. Maruyama and Dr. Naito of OIST, was covered by the Japanese Press.

XIII. Developmental Neurobiology Unit

Principal Investigator: Dr. Ichiro Masai
Research Theme: Mechanism Underlying Retinal Neurogenesis in Zebrafish

Abstract:

In vertebrate animals, the retinal region is initially specified in the anterior neural plate and evaginates from the ventral forebrain to form the optic cup, where six major classes of neurons and one class of glial cells differentiate to form a neural circuit responsible for phototransduction and visual processing. Because the retina is derived from the brain, it provides a good model for studying the mechanisms underlying neuronal differentiation and neural circuit formation. To elucidate these mechanisms, we will focus on retinal development in the zebrafish (*Danio rerio*). The zebrafish is an animal model that has been recently developed and that is suitable for genetic and cell biological studies. Previously, we found that the generation of retinal neurons is regulated by five signaling molecules, namely, Hedgehog, Fibroblast growth factor, Wnt, Notch and Histone deacetylase 1. One of the aims of our research is to elucidate the molecular mechanism that regulates the spatial and temporal patterns of retinal neurogenesis in zebrafish. Furthermore, we have identified zebrafish mutants that show various defects in neuronal differentiation in the retina. By analyzing phenotypes of these mutants and cloning these mutant genes, we will elucidate the mechanisms that regulate neuronal differentiation and neural circuit formation.

1. Participants:

1.1. Individuals:

-Researchers: Yuko Nishiwaki, Masahiro Yamaguchi, Toshiaki Mochizuki

-Technicians: Keiko Iriyama, Ayako Nagashima

1.2. Partner Organizations:

RIKEN Brain Science Institute

Collaborative research

Principal researcher: Dr. Hitoshi Okamoto

Theme: "Molecular cloning of zebrafish genes that regulate neuronal differentiation in the brain"

2. Activities and Findings:

On October 1, 2006, a new group, Developmental Neurobiology Unit, was established. The laboratory space of this unit including zebrafish facility is being constructed in an annex

building of the Okinawa Industrial Technology Center, Uruma, Okinawa. Since them, active recruitment of researchers and technicians has been carried out, and purchase of major equipment has also been completed. Until the construction of laboratory facility is completed, we conduct research in the Institute of Physical and Chemical Research (RIKEN), Wako, Saitama. The main results of our research carried out in this fiscal year are described below.

2.1. Mechanism that regulates the spatial and temporal patterns of retinal neurogenesis in zebarfish

In the vertebrate developing retina, almost retinal cells initially proliferate, but exit from the cell cycle and differentiate into post-mitotic neurons after the stage when retinal neurogenesis occurs. Although the generation of postmitotic neurons is the first step of neuronal differentiation, it is not fully understood how mitotic retinoblasts determine when they should generate postmitotic progenies. Previously, we identified a zebrafish mutant, *ascending and descending (add)*, in which retinal progenitor cells fail to exit from the cell cycle but continue to proliferate (Fig.1). Our cloning of the *add* mutant gene revealed that the *add* gene encodes Histone deacetylase 1 (HDAC1). HDAC1 is associated with the transcription repressor and co-repressor complexes, and inhibits the transcription of their target genes by modulating the acetylation state of chromatin. We found that Wnt and Notch signaling pathways promote cell proliferation and inhibit neurogenesis in the zebrafish retina, respectively, and that both signaling pathways fail to be suppressed in the *add* mutant retina. Taken together, these data suggest that HDAC1 promotes retinal neurogenesis in zebrafish by suppressing both Wnt and Notch signaling pathways.

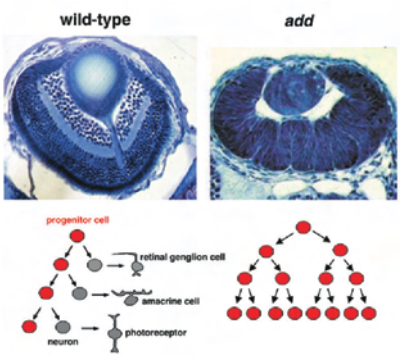


Fig. 1 *add* mutant retina at 2dpf. In the add mutant, retinal progenitor cells (red) fail to exit from the cell cycle and continue to proliferate.

It is generally accepted that HDAC1 is recruited to several transcription repressor and co-repressor complexes, suggesting that HDAC1 interacts with signaling pathways other than Wnt and Notch pathways. To elucidate the molecular network of an HDAC1-mediated switch from proliferation to differentiation, we will identify factors interacting with HDAC1 and its downstream targets. For this purpose, we are currently screening for zebrafish mutations that modify *add* mutant phenotypes (Fig. 2). Using chemical mutagens, new mutations (Mut) were introduced in male genomes and these mutagenized F0 males were mated with *add* mutant carrier females (*add*+/-) to produce F1 generation, which included fish of the Mut+/-; *add*+/- genotype. The mating of individual F1 Mut+/-; *add*+/- fish with *add* mutant carriers produced F2 Mut+/-; *add*+/- embryos. If mutations occur in proteins that interact with the HDAC1 pathway in retinal neurogenesis, it is expected that they modify *add*-induce hyperproliferation of

retinal progenitor cells. To date, we screened 72 mutagenized genomes and identified two mutations that modified *add*-mediated hyperproliferation of retinal progenitor cells. We are now establishing these mutant strains and will start to characterize phenotypes of homozygous mutant embryos.

Fig. 2 Procedure of screening of mutants modifying *add*-mediated retinal phenotypes. WIK wild-type strain, which is highly polymorphic to the *add* mutant strain, is used for mutagenesis. We will find mutations modifying *add* mutant phenotypes in F2 generation. Mutational loci can be mapped to chromosomes using F2 embryos Mut+/+; *add*-/-.

2.2. Mechanism that regulates neuronal differentiation and neural circuit formation in the zebrafish

After exiting the cell cycle, postmitotic retinal cells differentiate into six major classes of retinal neurons and form the neural network responsible for phototransduction. To elucidate the mechanisms underlying these late stages in neuronal differentiation, we identified zebrafish mutants that show various defects in neuronal differentiation and neural circuit formation in the retina (Fig. 3). These mutants were classified into the following five groups.

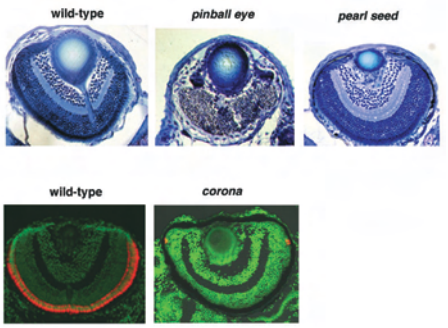


Fig. 3 (upper) plastic sections of wild-type and two zebrafish mutants, *pinball eye* and pearl seed. Severe apoptosis occurs in differentiating retinal neurons in the *pinball eye* mutant. Lens vesicle is smaller in the *pearl seed* mutant eye.

Four mutants showing severe apoptosis of differentiating retinal neurons (*pinball eye*^{rw255}, *helmet*^{rw329}, *sun glasses*^{rw337}, *rw564*)

Three mutants showing disorganization of retinal layers (*fractal lamina*^{rw147}, *rw698, amorphous*^{rw708})

Two mutants showing abnormal axonogenesis of retinal ganglion cells (*rw393, rw440*)

One mutant showing defects in photoreceptor specification (*coronarw*^{76b})

Three mutants showing defects in lens differentiation (*pearl seed*^{rw259}, *rw341, volvox*^{rw619})

Among them, to elucidate the mechanism that underlies severe apoptosis of retinal neurons, we mapped mutational loci of four apoptotic mutants, namely *pinball eye (piy), helmet, sun glasses and rw564,* to zebrafish chromosomes. Furthermore, we cloned the *piy* gene and found that it encodes DNA primase small subunit (Prim1) (Fig. 4, upper-left panel). Prim1 is an enzyme that synthesizes RNA primers in the lagging strands of DNA replication forks, which are required for subsequent DNA synthesis by DNA polymerase α. It was reported that the RNA synthesis by Prim1 is required for the activation of DNA replication checkpoint (Fig. 4, upper-right panel). This raises the possibility that DNA checkpoint-mediated activation of a tumor suppressor protein, p53, is involved in severe apoptosis of retinal neurons in the *piy* mutant. We found that the blockade of DNA checkpoint pathway or p53 significantly rescued the *piy*-mediated defects (Fig. 4, lower photo panels). These data suggest that DNA checkpoint-mediated p53 activation induces severe apoptosis of retinal cells in the *piy* mutant retina. Taken together, these data suggest that the surveillance system of genomic integrity strongly influences the choice between differentiation and apoptosis during retinal neurogenesis in zebrafish.

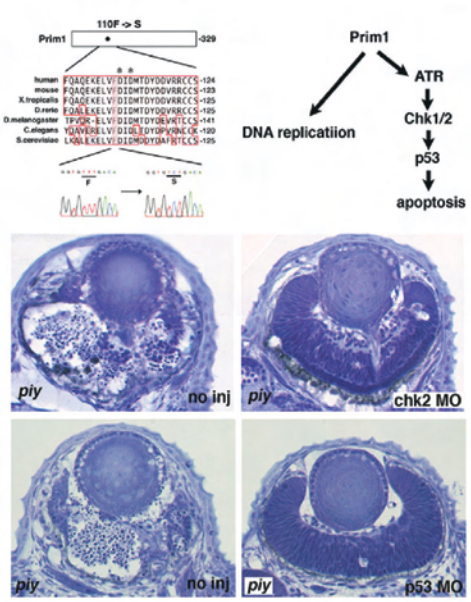


Fig. 4 (upper) plastic sections of wild-type and two zebrafish mutants, *pinball eye* and *pearl seed*. Severe apoptosis occurs in differentiating retinal neurons in the *pinball eye* mutant. Lens vesicle is smaller in the *pearl seed* mutant eye.
(lower) The blockade of p53 and Chk2 rescues *piy*-mediated defects. Severe apoptosis is inhibited in the *piy* mutant retina injected with anti-sense oligos of Chk2 and p53.

2.3. Mechanism that regulates the structural and functional integrities of photoreceptors

There are hereditary retinal diseases in human, for example, retinitis pigmentosa, in which photoreceptor cells degenerate. To date, about 185 genetic loci associated with retinal diseases have been mapped to human chromosomes. The human genome project accelerated the cloning of mutant genes, and more than 100 genes have been identified (see RetNet http://www.sph.uth.tmc.edu/Retnet). These genes function in signaling pathways involved in various biological aspects of photoreceptors: phototransduction, retinoid metabolism, and protein transport within photoreceptors. However, there are still many molecules whose functions are unclarified. To elucidate the mechanisms underlying the structural and functional integrities of photoreceptors, we previously screened zebrafish mutants with disrupted visual behaviors such

as optokinetic response, and identified two mutants, *twilight*^{rw18} (*tli*) and *eclipse*^{rw76a} (*els*).

In the *tli* mutant, photoreceptor cells differentiate, but degenerate until 8 day-post-fertilization. Electron microscopy revealed that the regular stacking of photoreceptive membranes in the outer segment is disrupted in the *tli* mutant, suggesting that Tli is required for the structural integrity of the outer segment (Fig. 5). To elucidate the mechanism underlying the structural integrity of photoreceptor cells, we are currently cloning the *tli* mutant gene. Last year, we isolated a series of BAC clones that cover the genomic region where the *tli* mutation locus is located. Using the zebrafish genomic database released from the Wellcome Trust Sanger Institute, we found that there are several candidate genes mapped within this genomic region. To identify the tli mutant gene among them, we currently determine cDNA sequences of these candidate genes prepared from the *tli* homozygous embryos.

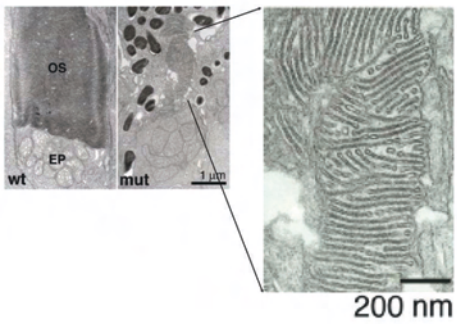


Fig. 5 EM observation of wild-type and *tli* mutant photoreceptor cells. The stacked membranes in the outer segment (OS) is irregular in tli mutant photoreceptors (middle and right panels). EP, ellipsoids

In the *els* mutant embryos, photoreceptors do not degenerate but are retained in the late stages of development (Fig. 6, upper panels). We examined whether the *els* mutant shows a normal response in electroretinogram (ERG) and found that there is no ERG response in the *els* mutant, suggesting that phototransduction pathway is severely compromised in the *els* mutant (Fig.6, middle panels). We cloned the *els* mutant gene and found that it encodes cGMP-

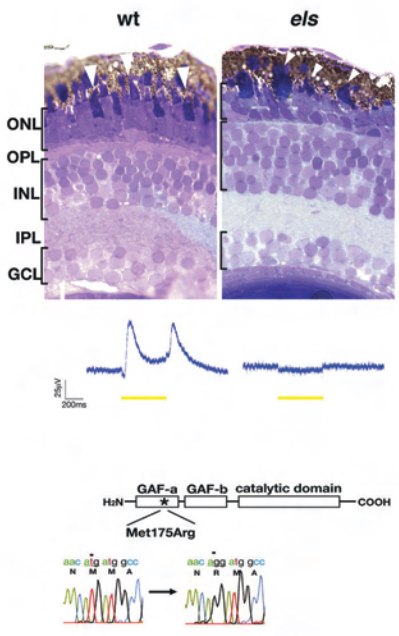


Fig. 6 (upper) Plastic sections of wild-type and zebrafish *els* mutant. Although the shapes of photoreceptor cells is irregular, they are maintained in the late stages.

(middle) ERG response in wild-type and *els* mutant. There is no ERG response in the mutant.

(lower) Mis-sense mutation occurs in the PDE6a' gene in the *els* mutant.

phosphodiesterase 6 α'-subunit (PDE6α') (Fig. 6, lower panel). PDE6 is an enzyme that mediates phototransduction in vertebrate photoreceptors, rods and cones. Rod PDE6 is composed of two catalytic subunits, α and β, and two inhibitory subunits γ, while cone PDE6 consists of two identical catalytic subunits α' complexed with two inhibitory γ subunits. We found that only cone-mediated phototransduction pathway is disrupted in the *els* mutant, suggesting that zebrafish PDE6α' is required for phototransduction in cones. Furthermore, to examine whether visual functions are normal in the *els* heterozygous mutant larvae, we carried out quantitative measurement of the optokinetic response (OKR) to the *els* heterozygous mutant larvae. We found that contrast sensitivity is compromised in the *els* heterozygous mutant larvae. These data suggest that the dose of PDE6α' activity influences the threshold of the contrast sensitivity.

3. Publications

3.1. Journals

Nothing to be reported

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

Nothing to be reported.

3.3. Oral Presentations and Posters

Presentations:

Ichiro Masai, "Signaling pathways regulating neuronal differentiation and maintenance in the zebrafish retina", Gordon Research Conferences – visual system development, IL Ciocco, Italy, May 14-19, 2006

Masahiro Yamaguchi, Noriko Tonou-Fujimori, Hitoshi Okamoto, Ichiro Masai, "Role of DNA primase in p53-dependent apoptosis of retinal cells (in Japanese)", 2006 Annual conference of the Japanese society of Developmetal Biologists, Hiroshima, Japan, May 31-June 3, 2006

Masahiro Yamaguchi, Noriko Tonou-Fujimori, Hitoshi Okamoto, Ichiro Masai, "Role of DNA primase in p53-dependent apoptosis of retinal cells", 7th International conference on zebrafish development & genetics, Madison, USA, June 14-18, 2006

Yuko Nishiwaki, Atsuko Komori, Tomonori Manabe, Toshihiko Hosoya, Hiroshi Sagara, Emiko Suzuki, Hitoshi Okamoto, Ichiro Masai, "The els mutation affects visual performance in both mutant and heterozygous larvae", 7th International conference on zebrafish development & genetics, Madison, USA, June 14-18, 2006

Ichiro Masai, "Genetic analysis of zebrafish retinal mutants shedding light on human genetic eye diseases", 2006 Annual meeting of the Japanese Neuroscience Society, Kyoto, Japan, July 19-21 , 2006

Ichiro Masai, "Signaling pathways regulating neuronal differentiation and maintenance in the zebrafish retina", 2nd Asia-Oceania zebrafish meeting, Palau Tioman, Malaysia, October 9-11, 2006

Ichiro Masai, "Mechanisms underlying retinal neurogenesis in zebrafish", OIST-Korea workshop "Neuroscience and beyoud", Okinawa, Japan, February 21-23, 2007.

Posters:

Masahiro Yamaguchi, Noriko Fujimori, Hitoshi Okamoto, Ichiro Masai, "Role of DNA primase in p53-dependent apoptosis of retinal cells", 2nd strategic conference of zebrafish investigators, Asilomar, USA, February 2-6, 2007

04. Intellectual Property Rights and Other Specific Products

Nothing to be reported

5. Meetings and Events

Nothing to be reported

XIV. Theoretical and Experimental Neurobiology Unit

Principal Investigator: Klaus Stiefel

The Theoretical and Experimental Neurobiology Unit was established in mid-November 2006 with my arrival in Okinawa.

In the three months since then, I, together with the invaluable assistance of the OIST staff, have ordered the electrophysiology lab equipment and the computational cluster. The lab equipment consists of one *in-vitro* patch clamp setup, including the necessary accessory equipment such as a scale and pipettes and was ordered via the scientific supply company Tomy. The cluster, consisting of 20 dual core AMD processor nodes was ordered from SUN microsystems. In addition, the lab setup was agreed on and the office furniture for the post-doc/graduate student room was ordered and delivered.

We have hired Ms. Ryoko Uchida as a secretary. So far, we have two visitors in the Theoretical and Experimental Neurobiology Unit: Dr. Arthur Flexer, from the University of Vienna, working on the application of data mining techniques on multi-compartmental neuron simulation data, and Ben Torben-Nielsen, M.Sc. from the University of Maastricht, working on structure-function relationships in neurons. We are in the process of hiring Mr. Maxence LeVasseur, M.Sc., as an experimentalist.

We are also organizing the OIST workshop on inverse problems (April 2007), together with Dr. Robert Sinclair, the Okinawa Computational Neurobiology Course 2007, together with Drs. Doya, De Schutter and Wilkins, and the OIST-Salk joint Neuroscience Meeting (November 2007 in San Diego, CA).

XV. Neurobiology Research Unit

Principal Investigator: Jeffery Wickens
Research Theme: Cellular Mechanisms of Learning and Adaptive Behaviour in the Brain

Abstract:

The overall aim of this research is to advance understanding of the brain mechanisms underlying learning and adaptive behaviour. The focus is on the basal ganglia, a set of forebrain nuclei thought to play a key role in adaptive behaviour through the selection of actions, goals and strategies on the basis of previous reward-related learning. The basal ganglia are also involved in major neurological and behavioural disorders, such as Parkinson's disease and attention-deficit hyperactivity disorder. Central issues in basal ganglia research include the manner in which the cortical input to the basal ganglia is processed and how neuromodulators such as dopamine and adenosine modify and influence the operations performed on the cortical inputs. The core strengths of the unit are in cellular electrophysiology and computational neuroscience, with strong collaborative relationships in behavioural analysis, neuroanatomy, systems physiology and clinical psychology. The research has the forward goal of informing the development of better treatments for the debilitating neurological and behavioural disorders of the basal ganglia, which are of such importance to both children and adults.

1. Participants:

1.1. Individuals:

Researchers: Dr Tomomi Shindou (Started 29 January 2007), Dr Cathy Vickers (Started 29 January 2007)

1.2. Partner Organizations:

University of Otago, New Zealand
Joint Research
Principal Researcher: Assoc Prof. Brian Hyland
Theme: Role of dopamine in reward-related learning

2. Activities and Findings:

The Neurobiology Research Unit was established January 29, 2007. Currently, the laboratory is being completed and the equipment is being commissioned.

3. Publications

3.1. Journals

Arbuthnott GW, Wickens JR. Space, time and dopamine. Trends in Neuroscience 30, 62 - 69 (2007)

Pitcher TL, Wickens JR, Reynolds JNJ. Differences in striatal action potentials between two behaviorally distinct rat strains. Neuroscience (in press)

Wickens J, Arbuthnott GW, Shindou T. Stimulation of GABA function in the basal ganglia: Computational models of GABAergic mechanisms in basal ganglia function Progress in Brain Research (in press)

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

Bolam, J.P,. Bergman, H., Graybiel, A., Kimura, M., Plenz, D., Seung, H.S., Surmeier, D.J. and Wickens, J.R. Molecules, microcircuits and motivated behaviour: Microcircuits in the striatum; in: S. Grillner and A.M. Graybiel (eds) Microcircuits: the interface between neurons and global brain function. Dahlem Workshop Report 93. The MIT Press, Cambridge, MA: 2006, p 165-190.

Bergman, H., Kimura, M. and Wickens, J.R. Modulation of striatal circuits by dopamine and acetylcholine; in: S. Grillner and A.M. Graybiel (eds) Microcircuits: the interface between neurons and global brain function. Dahlem Workshop Report 93. The MIT Press, Cambridge, MA: 2006, p 149-164.

Wickens, J. R. Hyland, B. I., and Tripp, G. Frontostriatal mechanisms in reinforcement: implications for ADHD, in Recent breakthroughs in basal ganglia research. E. Bezard (ed), Nova Science Publishers, Inc., NY: 2006, p 65-80.

3.3. Oral Presentations and Posters

(List authors (underline presenter), title, conference, Country (or City) and date)

Presentations:

Wickens, J. Frontostriatal mechanisms in positive reinforcement. Reward and decision-making, Los Angeles, June 2006

Wickens, J. Computational aspects of the dopamine signal, Winter Conference on Brain Research, Colorado, January 2006

Posters:

Pitcher TL, Wickens JR, Reynolds JND (2006) Cellular properties of striatal spiny neurons in the SHR. 17th European Network on Hyperkinetic Disorders (EUNETHYDIS) Brugge, Belgium.

4. Intellectual Property Rights and Other Specific Products

Nothing to be reported

5. Meetings and Events

Nothing to be reported

XVI. Human Developmental Neurobiology Unit

Principal Investigator: Gail Tripp

1. Participants:

1.1. Individuals:

Unit staff have yet to be appointed.

1.2. Partner Organizations:

Active collaborations with

Dr Elizabeth Schaughency, Department of Psychology, University of Otago, New Zealand.
Dr Brent Alsop, Department of Psychology, University of Otago, New Zealand.
Dr Barbara Galland and Professor Barry Taylor, Medical School, University of Otago.
Dr Jeffery Wickens, Okinawa Institute of Science and Technology.

2. Activities and Findings:

Laboratory under construction.

3. Publications

3.1. Journals

Tripp, G., Schaughency, E. A., & Clarke B. (2006). Parent and teacher rating scales in the evaluation of ADHD: Contribution to diagnosis and differential diagnosis in clinically referred children. *Developmental and Behavioral Pediatrics*, *27*, 209-218.

Bird, A., Reese, E., & Tripp, G. (2006). Parent and child talk about past emotional events: Associations with child temperament and goodness-of-fit. *Journal of Cognition and Development*, *7*, 189-210.

Galland, B. C., Dawes, P. J., Tripp, G., & Taylor, B. J. (2006). Changes in behavior and attentional capacity after adenotonsillectomy. *Pediatric Research, 59,* 711-716.

Tripp, G., Schaughency, E. A., Langlands, R., & Mouat, K. (in press). Family interactions in children with and without ADHD. *Journal of Child and Family Studies*.

Reese, E., Bird, A., & Tripp, G. (in press). Children's self esteem and moral self: Links to parent-child conversations about emotion. *Social Development*.

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

Wickens, J. R., Hyland, B. I., & Tripp, G. (2006). Frontostriatal mechanisms in reinforcement: Implications for ADHD. In E. Bezard(Ed), *Recent Breakthroughs in Basal Ganglia Research* (pp 65-80). Nova Science Publishers: New York.

Tripp, G. (2006). Abnormal and clinical psychology. In A. Weatherall, M. Wilson, D. Harper, and J. McDowall (Eds). *Psychology in Aotearoa New Zealand.* Pearson Education: New Zealand.

3.3. Oral Presentations and Posters

Presentations:

Smith, A., Schaughency, E., Tripp, G., & Fahey, S. Predicting internalizing symptoms in children with ADHD in US and New Zealand clinical samples. *Joint conference of the Australian and New Zealand Psychological Societies,* Auckland, New Zealand, September, 2006.

Tripp, G. Acquisition of response bias in children with and without ADHD. *17th European Network on Hyperkinetic Disorders (EUNETHYDIS,)* Brugge, Belgium, October 5-8, 2006.

Galland, B., Tripp, G., Phillips, A., Taylor, B. Sleep and breathing difficulties in children diagnosed with DSM-IV ADHD. *New Zealand Paediatric Society Conference,* Nelson, New Zealand, November, 2006.

Posters:

Nothing to be reported

4. Intellectual Property Rights and Other Specific Products

Nothing to be reported

5. Meetings and Events

Nothing to be reported

XVII. Brain Mechanisms for Behaviour Unit

Principal Investigator: Gordon W. Arbuthnott

Research Theme: An exploration of the electrophysiological mechanisms in the basal ganglia related to behaviour with particular emphasis on network properties in striatum in slices and in cultured neuronal circuits.

Abstract:

The unit was formed only in late Jan. 2007. Equipment is ordered and being delivered at present.

1. Participants:

1.1. Individuals:

-Researcher(s): Marianela Garcia-Munoz Ph.D.

-Technician(s): One to be appointed

1.2. Partner Organizations:

University of Otago, Dunedin, New Zealand

Joint research

Principal researcher: Dr. Brian Hyland

Theme: The brain mechanisms underlying the effectiveness of deep brain stimulation of the subthalamic nucleus in Parkinsonism. Research Project supported by the Neurological Foundation of New Zealand. Post Doctoral Researcher Dr Cyril DeJean.

2. Activities and Findings:

Nothing to be reported

3. Publications

3.1. Journals

Michelle Day, Zhongfeng Wang, Jun Ding, Xinhai An, Cali A. Ingham, Andrew F. Shering, David Wokosin, Ema Ilijic, Zhuoxin Sun, Allan R. Sampson, Enrico Mugnaini, Ariel Y. Deutch, Susan R. Sesack, Gordon W. Arbuthnott, and D. James Surmeier Selective elimination of glutamatergic synapses on striatopallidal neurons in Parkinson disease models. *Nat Neurosci* (2006) 9 251-259.

C. A. Vickers, B. Stephens, J. Bowen, G.W. Arbuthnott, S.G.N Grant,C.A. Ingham. Neurone specific regulation of dendritic spines in vivo by Post Synaptic Density 95 protein (PSD-95). *Brain Research* (2006) 1090 89-98

Gillingwater, T.H., Ingham, C.A., Parry, P.E., Wright, A.K. Haley, J.E., Wishart, T.M., Arbuthnott, G.W., Ribchester, R.R. Delayed Synaptic Degeneration In The CNS of Wlds Mice After Cortical Lesion. *Brain* (2006) 129 1546-1556

Gordon W. Arbuthnott & Jeff Wickens Space, time and dopamine. T.I.N.S. (2007) 30 62-69

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

Nothing to be reported.

3.3. Oral Presentations and Posters

Nothing to be reported.

Presentations:

Nothing to be reported.

Posters: **G. W. Arbuthnott**, Su, Li, Jaeger, D. Cortical effects of subthalamic nucleus stimulation: An intracellular study in anesthetized rat. (2006) *FENS Abstracts Vienna July 2006*

4. Intellectual Property Rights and Other Specific Products

Nothing to be reported

5. Meetings and Events

Nothing to be reported

XVIII. Education and Training Activities

The Seaside House renovation has provided an excellent facility for workshops and educational activities. During fiscal 2006, there were seven (7) workshops held:

International Workshop "Single Molecule Analysis"

Outline: The aim of workshop "Single Molecule Analysis" was to provide opportunities for young researchers with backgrounds of not only biological sciences but also material sciences and engineering to learn about the frontier of single molecule science carried out by the most advanced techniques of optical, scanning probe, and electron microscopy.

Date: April 17 - 21, 2006
Organizer: Keiichi Namba (Osaka University)
Akihiko Ishijima (Tohoku University)
Yasushi Sako (RIKEN)
Venue: Bankoku Shinryo-kan (Nago-shi)
Participants: 14 Speakers, 32 Participants


Lecture


Poster session

Group photo

OIST Workshop "Future Directions in Primate Brain Research" (Domestic Workshop)

Outline: The aim of workshop "Future Directions in Primate Brian Research" was to exchange ideas about the important research topics, new technology, lab facility, breeding and ethics in primate brain research, and to discuss possible contributions of a new primate brain research center at OIST and what could be the major challenges.

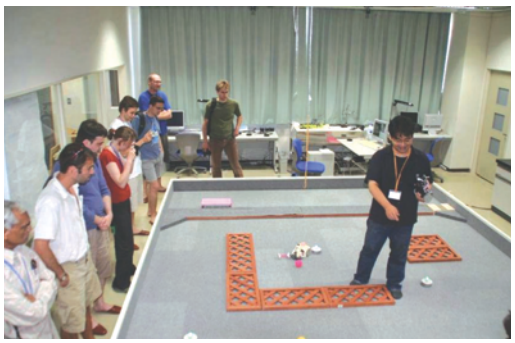
Date: May 14 - 16, 2006
Organizer: Kenji Doya (OIST)
Venue: Okinawa Marriott Resort and Spa (Nago-shi)
Participants: 34

Group photo

Okinawa Computational Neuroscience Course (OCNC) 2006

Outline: The aim of OCNC 2006 was to provide opportunities for young researchers with theoretical backgrounds to learn up-to-date neurobiological findings, and those with experimental backgrounds to have hands-on experience in computational modeling.

Date: June 26 - July 6, 2006
Organizer: Kenji Doya (OIST)
Upinder Bhalla (National Center for Biological Sciences, India)
Shinya Kuroda (University of Tokyo)
Nicolas Le Novère (European Bioinformatics Institute)
Venue: OIST Seaside House
Participants: 17 Speakers, 10 Tutors, 38 Students



IRP lab tour

Group photo

OIST-Korea Workshop "Neuroscience and Beyond"

Outline: The aim of OIST-Korea workshop "Neuroscience and Beyond" was to establish a strong bond among OIST and other institutes in the region having participation from OIST, University of the Ryukyus, Kyungpook National University (Korea) and Seoul National University (Korea).

Date: February 21- 23, 2007
Organizer: Shogo Endo (OIST)
Hee Kyung Jin (Kyungpook National University)
Venue: OIST Seaside House
Participants: 8 Speakers, 27 Participants

Opening session

Session

Group photo

OIST Workshop on Systems Biology of Yeast Signaling

Outline: The aim of OIST Workshop on Systems Biology of Yeast Signaling was to discuss and share the broad range of experimental techniques and future of Yeast MAPK System Biology. Furthermore, this workshop was aimed to obtain integrated systems perspectives and approach for understanding MAPK system using budding yeast, thereby augment such understanding and approach to mammalian MAPK systems.

Date: February 24 - 27, 2007
Organizer: Hiroaki Kitano (OIST)
Venue: OIST Seaside House
Participants: 21

Group photo

OIST Workshop on Cognitive Neurobiology

Outline: The aim of OIST Workshop on Cognitive Neurobiology was to bring together scientists who are trying to understand the neurobiological origins of cognitive functions in order to exchange recent experimental findings and ideas about future research directions.

Date: March 11 - 15, 2007
Organizer: Kenji Doya (OIST)
Naotaka Fujii (RIKEN Brain Science Institute)
Tadashi Isa (National Institute for Physiological Sciences)
Daeyeol Lee (Yale University)
Nikos Logothetis (Max Planck Institute)
Barry Richmond (National Institutes of Health)
Wolfram Schults (University of Cambridge)

Keiji Tanaka (RIKEN Brain Science Institute)
Venue: OIST Seaside House
Participants: 21 Invited speakers, 1 Discussant, 25 Poster presenters

The Second International Workshop on Cell Regulations in Division and Arrest

Outline: Subsequent to the first workshop held last March, to study 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.

Date: March 25 - 29, 2007
Organizer: Mitsuhiro Yanagida (OIST)
Venue: OIST Seaside House
Participants: 72 (31 invited speakers)

OIST Workshop "Future of Electron Microscopy"

Outline: The aim of OIST Workshop "Future of Electron Microscopy" is to discuss the elementary technologies of electron microscopy and new applications to nano-bio-technology.

Date: March 27 - 31, 2007
Organizer: Akira Tonomura (OIST)
Venue: Naha Terrace and OIST Seaside House
Participants: 23