OIST

Annual Report 2007

Okinawa Institute of Science and Technology Promotion Corporation

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Okinawa Institute of Science and Technology Promotion Corporation

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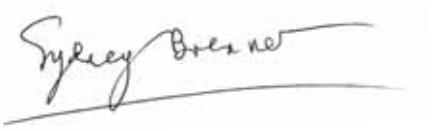
Preface

I am pleased to report continued progress in the development of our research activities. Two of our research units established at the beginning of FY2004 have been reviewed by independent international committees, chaired by distinguished scientists: Dr. T. Wiesel (Nobel Laureate in 1981) for the review of the K. Doya Unit, and Dr. T. Hunt (Nobel Laureate in 2001) for the review of the M. Yanagida Unit. Both PIs were recommended for renewal for a further 5 years from 2009 to 2014, and this was officially confirmed by me. We have appointed two additional PIs and have preserved a majority of non-Japanese scientists consistent with our plan to become an international center of research.

We have also progressed with the construction of the new facility and major tenders for the construction of the first phase have been awarded. The landscape at the campus site will soon undergo major changes.

I wish to thank all members of OIST P.C. for their contributions to creating what we have today. It will provide a secure platform for future development, as we enter the long and hard road to becoming the "best in the world".



President
Okinawa Institute of Science and Technology
Promotion Corporation

General Report

I. Events

The Okinawa Institute of Science and Technology Promotion Corporation (OIST P.C.) was established on September 1st of 2005. The Corporation was established in order to conduct outstanding research and development in Okinawa and to prepare for the opening of the world-class graduate university of science and technology.

The main events in FY2007 were as follows:

April 6, 2007
Groundbreaking ceremony was held at the main campus site in Onna Village

July 9, 2007
The fourth Board of Governors meeting was held in Okinawa.

October 23, 2007
Construction of faculty housing at the Seaside Campus commenced.

January 25, 2008
The fifth 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 OIST P.C. and advise the President. In addition to the existing 8 Board members, 4 Board members, Dr. Hunt, Dr. Kanazawa, Dr. Lee, and Dr. Rees, were appointed by then Prime Minister Shinzo Abe in 2007.

Since December 2006, Dr. Arima and Dr. Wiesel have been 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. Timothy Hunt	Cancer Research UK Nobel Laureate (Physiology or Medicine, 2001)
Dr. Ichiro Kanazawa	President, Science Council of Japan
Dr.Yuan-Tseh Lee	Former President, Academia Sinica Nobel Laureate (Chemistry, 1986)
Dr. Jean-Marie Lehn	Professor, University Louis Pasteur-Strasbourg Nobel Laureate (Chemistry, 1987)
Sir Martin Rees	President, The Royal Society
Dr. Hiroko Sho	Professor Emeritus, University of the Ryukyus
Dr. Susumu Tonegawa	Professor, MIT Nobel Laureate (Physiology or Medicine, 1987)
Dr. Torsten Wiesel*	Secretary General, Human Frontier Science Program Organization Nobel Laureate (Physiology or Medicine, 1981)
Dr. Kiyoshi Kurokawa (ex officio)	Special Cabinet Adviser in charge of science, technology and innovation Former President, Science Council of Japan

* Co-chairs

The 4th BOG meeting

The 4th BOG meeting was held on July 9, 2007 in Okinawa. Attending Board members were: Dr. Akito Arima, Dr. Jerome Friedman, Dr. Ichiro Kanazawa, Dr. Yuan-Tseh Lee, Dr. Hiroko Sho, Dr. Susumu Tonegawa, and Dr. Torsten Wiesel. Invited guests included Director-General Mr. Osamu Shimizu of the Okinawa Development and Promotion Bureau, Cabinet Office.

The following items were covered in the meeting agenda.

- Mr. Shimizu conveyed a message from Minister Takaichi.
- President, Dr. Brenner reported on the scientific program, recruitment, dissemination of research results, education and training activities, preparation work for the foundation of the university, construction of facilities, administrative and financial matters, and Board matters.
- Status of campus development was reported including: (a) final site development plan, (b) summary of the schedule, (c) current priorities, (d) land acquisition, (e)contracting progress, and other issues.
- Reports about academic activities and others were given that included (a) workshops and seminars, (b) statement of business principles and medium-term plan of OIST P.C. revisions.
- Cabinet Office reported on financial issues of the campus development plan and the budget request for fiscal year 2008.
- Okinawa Prefectural Government reported on the basic plan for development of the OIST P.C. peripheral area.

The 5th BOG meeting

The 5th BOG meeting was held on January 25, 2008 in Tokyo. Attending Board members were: Dr. Akito Arima, Dr. Jerome Friedman, Dr. Ichiro Kanazawa, Dr. Yuan-Tseh Lee, Dr. Hiroko Sho, Dr. Susumu Tonegawa, and Dr. Torsten Wiesel. Invited guests included Minister of State for Okinawa and Northern Territories Affairs, Science and Technology Policy Mr. Fumio Kishida; Director-General Mr. Osamu Shimizu.

The following items were covered in the meeting agenda.

- Minister Kishida delivered a statement.
- President Dr. Brenner reported on future plans for the development of new research areas.
- Executive Director Dr. Baughman reported on research and educational activities for operations and planning of OIST.
- Progress report was presented on campus development and plans for next fiscal year.
- Cabinet Office reported on budget proposal for fiscal year 2008 and on the Evaluation Committee on Independent Administrative Institutions.

III. Campus Development and Facility Status

Civil works

The site preparation work continued to make good progress during of the fiscal year. The dramatic progress is evident in the cut-and-fill work completed so far transforming the site from “forested rolling hills” to the “spectacular campus site”. The site preparation for the first key cluster, “Laboratory 1 and Center Building”, was completed and is ready for the next phase of building work. The site preparation for “Village Zone”, adjacent to the main campus entrance, and a new Bypass were also completed by the end of the fiscal year 2007. The site development work for the second cluster, “Laboratory 2 and Laboratory 3”, located on the hill opposite the first cluster site, was commenced on July 24, 2007.

One of the most characteristic features of the campus plan, the tunnel and vertical shaft, is the principal connection between the “Village Zone” and the “Laboratory Zone”. The earth works began on June 19, 2007,

ahead of the commencement of Center Building construction over the shaft, and immediately following issuing of the Building Permit on June 18, 2007. For the first two months, activities concentrated on the reinforcement work around the mouth at the top of the vertical shaft and at the tunnel exit, after which excavation was started from both entrances. Commencing from the top, excavation of the shaft was stopped at the top level of the tunnel and the tunnel excavation broken through to the shaft on October 5, 2007. The structural work for the second lining has been continued.

Five of the seven bridges connecting the various hills were ordered in FY2007 to be completed in the first half of FY2008.

Environment impact assessment

Throughout the construction, the environmental impact was monitored carefully. This campus project was subjected to an Environmental Impact Assessment (EIA) process. The contractors were required to follow the environment-management instructions, which were established in the course of EIA process. Additionally a third party consultant was hired to monitor and investigate the environmental impact. The construction progressed in compliance with Okinawa Prefectural Ordinance for Prevention of Red Clay Outflow, and precise coordination skills and construction planning were demanded to prevent the discharge of turbid stormwater runoff to the rivers. This was achieved by implementing turbid water treatment facilities and inspection patrols on rainy days.

Facility construction

Laboratory 1 and Center Building are connected by a corridor to form a single structure. Based on the construction drawing prepared in FY2006, the first package of the structure and exterior for this building was contracted on March 4, 2008. The mechanical, electrical and plumbing work is scheduled to commence in May 2008 with the aim of completing this phase of the construction in June 2009 and starting the research works in this campus after moving from the Uruma site.

At the seaside campus, construction of the first 4 faculty housing units commenced in October 2007 and the other 4 faculty housing units commenced in February 2008 to be completed in FY2008. These faculty housing units will be utilized as the accommodation for invited lecturer at academic workshops in the Seaside House, or for temporary accommodation for new principal investigators.

IV. Administration and Finance

Overall matters

The organizational structure has been changed to align more closely with the objectives of the Promotion Corporation which are (I) to conduct research in Okinawa and (II) to prepare for the graduate university. The Promotion Corporation now has two divisions, Operations, and University Planning, to cover the two activities.

The meetings of the Committee of PIs (COPI) continue to be held regularly and we have established subcommittees to deal with specific issues, such as the workshop program and computing.

The Management Committee (MACO) has been replaced by an Executive Committee which deals with major corporation issues. The Executive Committee members include the President, Executive Director, Executive Assistants to the President and a PI representative.

Dr. Robert Baughman was appointed as Executive Director on September 31, 2007 and was designated Vice President.

Finance

The total budget for FY2007 was 8,702 million yen, which was approximately 13% increase from the previous year, 7,678 million yen. Major constructions were carried out in 2007.

<Budget comparison between FY2006 and FY2007>

Million Yen

Breakdown \ Year	2006 Budget	2007 Budget
Subsidy for Facilities	3,530	4,419
1) Land Development	903	725
2) Construction	1,323	2,864
3) Infrastructure Improvement	650	650
4) Land Acquisition	654	180
5) Hakuun-so Renovation	-	-
6) Hakuun-so Purchase	-	-
Subsidy for Operation	4,148	4,283
1) Research Expenses, Workshops etc.	3,461	3,558
2) General Administrative Expenses	687	725
Grand Total	7,678	8,702

Operation

The payroll outsourcing system has been introduced. Contrary to the previous payroll system manually carried out by OIST P.C. staff, the new system allows the payroll process to be taken care by highly reliable computer mainframe. It is safer, more effective and more secure because it has back-up mainframe systems at different locations on mainland Japan. The new system enables the payroll process to continue even in case of emergency including absence of payroll staff, computer crashes and natural disasters such as earthquakes or fire.

Rules and regulations

With the advice from legal consultants, some of the rules and regulations etc. have been revised. Review work has been continuing to improve the current rules and regulations so that they are flexible enough for conducting research while securing compliance.

In order to increase the number of general competitive biddings, OIST P.C. has carefully revised the criteria used for contracts without tender by lowering the cap on the amount of contract effective August 1, 2007.

Furthermore, as for the contracts to be concluded after April 1, 2008, OIST P.C. revised necessary regulations on March 31, 2008 to conform the standards set forth by the central government uses.

Personnel affairs

The total number of full-time employees was 146 as of March 1, 2008. Of these, 115 were in research units.

Staff training

To improve administrative capabilities, staff were provided with appropriate training opportunities. We have established cross-cultural communication classes for Japanese administrative staff to develop English communication skills required for dealing with foreign researchers. We have also provided Japanese classes to meet the needs of an increasing number of foreign researchers and technicians.

<Training Programs in FY2007>

Dates	Program Theme	Organized by	Partici-pants
April 25, 2007	Explanatory meeting of Technological Standard	MEXT	1
July 5, 2007-September 27,2007	Self Study Class	OIST P.C.	147
July 10, 2007	Grants-in-Aid for Scientific Research	MEXT	2
August 30 & 31, 2007	Evaluation and Audit	Ministry of Internal Affairs and Communications	1
September 11, 2007	Sexual Harassment	21Century Foundation	4
September 13, 2007	Lotus Notes Ver. 8 Seminar	IBM	3
October 10, 2007-February 26, 2008	Accounting work	OIST P.C.	56
October 17, 2007-March 27, 2008	Cross Cultural Communication	OIST P.C.	68
November 30, 2007	Pubic Building Construction Quantity Survey Criteria	Research Institute on Building Cost	1
December 7, 2007	Collusive Bidding Problem Seminars	Fair Trade Commission	2
January 18, 2008	Promotion of Elderly Employment	Associate of Employment Development,Okinawa	2
January 25, 2008	Information Disclosure and Personal Information Protection	Ministry of Internal Affairs and Communications	1
March 26, 2008	Safe Control of Nuclear Fuel Materials	Nuclear Safety Technology Center	1
All year- round	Japanese (language)	OIST P.C.	12
As needed	Information Disclosure and Personal Information Protection	OIST P.C.	4
As needed	Training seminars for new recruits	OIST P.C.	9

IT Infrastructure

IT implemented the following systems to strengthen the infrastructure.

- 12 new servers (including back-up servers) were installed.
- IP phones replaced normal digital phone to support multi-language display and functions such as conference call and voice mail.
- Groupware (Lotus Notes) was installed as the standard mail system, facility reservation management, and schedule sharing.
- BlackBerrry was implemented in addition to groupware.
- IT call tracking system was enhanced to analyze incidents.
- Official and internal websites were upgraded.
- E-mail filtering system was installed to filter out spam messages.

Public Relations/Community Relations Activities

OIST P.C. PIs conducted two scientific lectures for local senior high school students, sponsored by the Okinawa Prefectural Government Council for Promotion of the Okinawa Institute of Science and Technology. Another two scientific lectures for local junior high school students were conducted as well. These were sponsored by the Onna Village Government.

Three issues of the newsletter were published and distributed to the central and local governments, local schools, research institutions at home and abroad, as well as to visitors to OIST P.C..

Scientific Report

During fiscal year 2007, the number of research units increased from 13 to 17. The new research units are:

1. Computational Neuroscience Unit
   Principal Investigator: Dr. Erik De Schutter
   Establishment date: April 2007

2. Trans-Membrane Trafficking Unit
   Principal Investigator: Dr. Fadel Samatey
   Establishment date: April 2007

3. Cellular and Molecular Synaptic Function Unit
   Principal Investigator: Dr. Tomoyuki Takahashi
   Establishment date: April 2007

4. Developmental Signalling Unit
   Principal Investigator: Dr. Mary Ann Price
   Establishment date: May 2007

V. Neural Computation Unit

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

Abstract

The goal of Neural Computation Unit is to elucidate the mechanisms of animal behaviors and human minds at multiple levels; from actions and emotions via the networks and neurons to the molecules and genes. Our specific focus is on how the brain realizes reinforcement learning, in which an agent, biological or artificial, learns novel behaviors by exploration and reward feedback. We combine top-down, computational modeling and bottom-up, neurobiological experiments to achieve this goal. The major achievements of the three subgroups in this fiscal year 2007 are the following.

a) The Dynamical Systems Group developed novel Bayesian and reinforcement learning algorithms and applied the methods to variety of issues in neuroscience. We applied the Bayesian inference framework to the problems of parameter fitting and model selection in complex models in systems biology and developed a software tool, called “LetItB,” which can automatically estimate parameters of models defined in SBML (systems biology markup language) to fit experimental data. The software tool is being tested on a model of the molecular cascade for dopamine-dependent synaptic plasticity in cortico-striatal synapses, which we constructed to understand the molecular basis of reinforcement learning.

b) The Systems Neurobiology Group examined the hypotheses from theoretical models by rodent experiments and human brain imaging. We recorded neuronal activities of the basal ganglia while rats performed a decision making task and found neurons that evaluate the goodness of action candidates at different stages of the basal ganglia circuit. We also tested the role of serotonin by chemical and electric recordings from the dorsal raphe nucleus, the major source of ascending serotonergic projection, and found elevated activities of serotonin while rats are working for delayed rewards.

c) The Adaptive Systems Group worked on the meta-level control mechanisms for reinforcement learning algorithms using a colony of robots called “Cyber Rodents,” which have the distinctive capabilities for self-preservation by recharging and self-reproduction in software by infrared communication. We implemented distributed evolution and learning frameworks in Cyber Rodents and investigated how reward functions and meta- parameters for reinforcement learning algorithms can evolve by selection under different environments.

1. Staff

Dynamical Systems Group
Researchers: Junichiro Yoshimoto
Technical staff: Tomofumi Inoue
Research assistant/graduate student: Shinji Kimura, Takashi Nakano, Makoto Otsuka

Systems Neurobiology Group
Researchers: Masato Hoshino, Makoto Ito, Katsuhiko Miyazaki, Kayoko Miyazaki
Research assistant/graduate student: Alan Rodrigues, Takehiko Yoshida

Adaptive Systems Group
Researchers: Stefan Elfwing, Takashi Sato, Eiji Uchibe
Research assistant/graduate student: Takumi Kamioka, Mikihiro Kobayashi, Tetsuro Morimura

2. Partner Organizations

ATR Computational Neuroscience Laboratories
Type of partnership: Joint Research
Name of principal researcher: Dr. Mitsuo Kawato
Name of researcher: Kenji Doya
Research theme: Functional brain imaging study of molecular basis of mind

Nara Institute of Science and Technology Graduate School of Information Science
Type of partnership: Joint Research
Name of principal researcher: Prof. Shin Ishii
Name of researchers: Kenji Doya, Junichiro Yoshimoto
Research theme: Application of Bayesian method to identification of biological reaction system

Honda Research Institute Japan Co., Ltd.
Type of partnership: Joint Research
Name of principal researcher: Dr. Hiroshi Tsujino,
Name of researchers: Kenji Doya, Makoto Ito, Katsuhiko Miyazaki, Kayoko Miyazaki, Osamu Shono, Eiji Uchibe
Research theme: Biological modeling of basal ganglia in behavioral learning

NEC Corporation
Type of partnership: Contract Research
Name of principal researcher: Nobuki Kajihara
Name of researchers: Kenji Doya, Junichiro Yoshimoto, Shinji Kimura
Research theme: A feasibility study of IP core implementation of reinforcement learning

3. Activities and Findings

3.1 Dynamical Systems Group

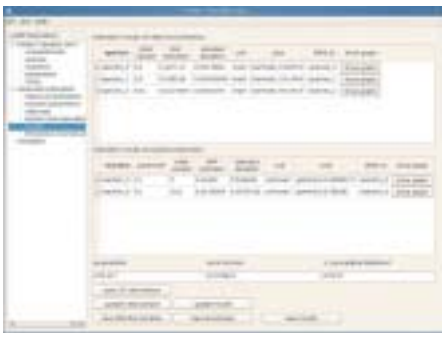
Bayesian system identification of biological networks [Yoshimoto, Inoue]

Bayesian system identification paradigm has a potential to 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 are made explicit in a form of prior distribution.

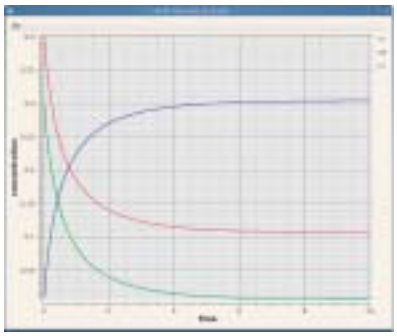
In this fiscal year, we focused on a model selection problem in molecular cascades, wherein the object is to infer the most likely structure of molecular cascades among multiple options with different model structures. We developed a feasible Monte Carlo method to approximate the marginal likelihood that is a criterion for evaluating the fitness of a model structure to a given dataset. We tested the validity of the criterion in benchmark problems including the model selection problem in HIV proteinase cascades. The results demonstrated that our method can determine whether a complex structure of a molecular cascade can be further simplified or not.

We further developed a general-purpose parameter estimation tool for models described by SBML (system biology markup language) and combined the parameter estimation engine with a graphical user interface for easy operation by biologists (Figure 1).

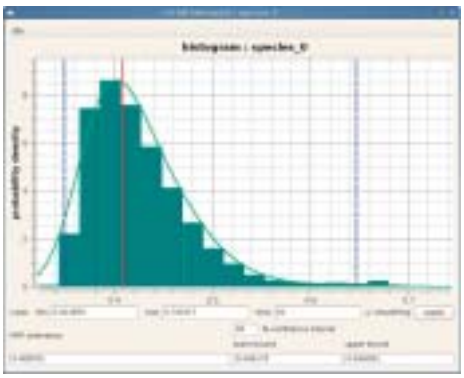
Biochemical model of cortico-striatal synaptic plasticity [Nakano, Yoshimoto]



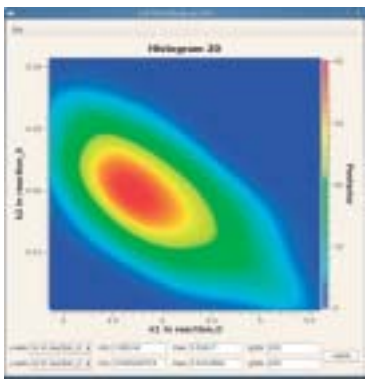
(a) graphical user interface



(b) simulated time course

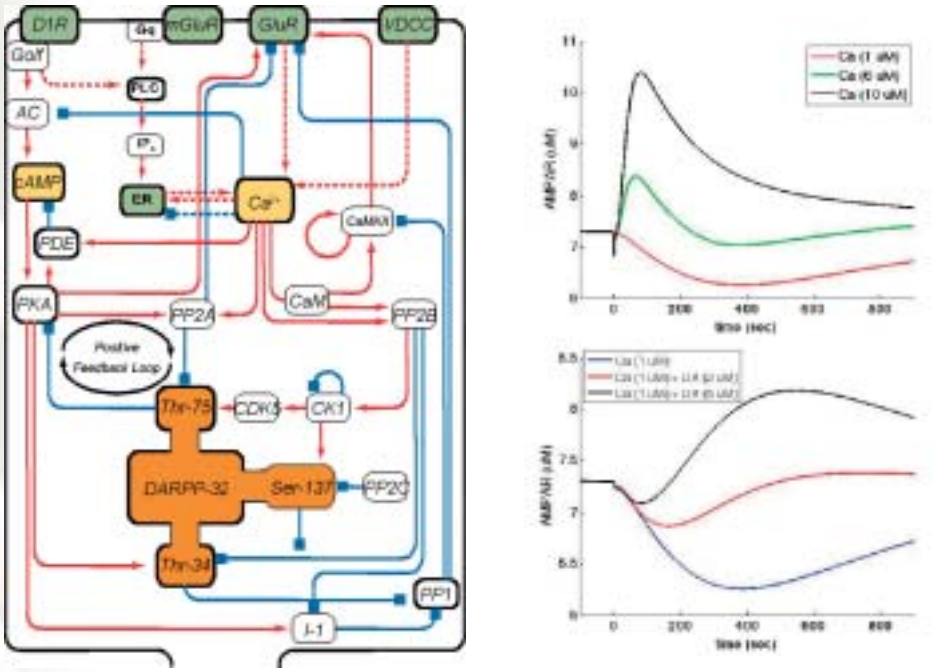


(c) posterior probability of one parameter



(d) joint posterior probability

Figure 1: The parameter estimation tool “LetItB”. The user will provide the structure of the model in the SBML format, and specify which parameters to be estimated and the measurement time course as the inputs. Using a Monte Carlo sampling method, the tool returns the posterior probability distribution of the parameters, which can be shown graphically.



(a) molecular cascade model (b) calcium- and dopamine-dependent plasticity

Figure 2: (a) Biochemical model of dopamine-dependent synaptic plasticity. The medial spiny neurons in the striatum receive two major inputs to the spines: the glutamatergic input from the cortex and the dopaminergic input from the substantia nigra, which increases calcium and c-AMP concentrations, respectively. The multi-site phosphoprotein DARPP-32 integrates these input pathways and regulates phosphorylation of AMPA receptors, which results in the change in synaptic efficacy. The model (a) was constructed using GENESIS/Kinetikit based on the literature and similar models in the depositories (e.g., DOQCS). It reproduced both calcium- and dopamine-dependent plasticity, and was used to clarify the dynamic function of the pathways around DARPP-32.

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 had build a computer model of intracellular signaling cascades in D1-type cortico-striatal neurons and confirmed that the model could replicate the bidirectional synaptic plasticity depending on dopaminergic input magnitude. In this fiscal year, we further refined our model and investigated its dynamic properties more comprehensively. As a result, we have obtained new findings as follows:

- Our model can also replicate the bidirectional synaptic plasticity depending on the intracellular calcium concentration, which is regulated by the intensity of the glutamate input.
- The domains of synaptic potentiation and depression were clarified by visualizing the two-dimensional parameter space of dopamine and calcium concentrations.
- DARPP-32 (dopamine- and cAMP-regulated phosphoprotein of Mr 32kDA) is crucial for not only dopamine- but also calcium-dependent plasticity.
- PKA activation is crucial for bidirectional synaptic plasticity depending on dopaminergic input.

- PKA-PP2A-DARPP-32 positive feedback loop plays a role to enhance and sustain PKA activation, leading to long-term potentiation of synaptic efficacy depending on dopaminergic input.
- Bidirectional synaptic plasticity depending on calcium- input intensity is induced by either PKA activation or CaMKII activation, but becomes more prominent in the presence of both activations.

Efficient state representation in reinforcement learning tasks with high-dimensional sensor inputs [Otsuka, Yoshimoto]

Reinforcement learning (RL) is a way to build decision-making function into autonomous agents. Many methods for solving RL problems (e.g. Q-learning, SARSA, Actor-Critic, policy gradient, etc.) have been proposed to solve a Markov decision process (MDP), but many real problems do not fall into the class of MDP due to the presence of hidden states and limitation of computational resource in the agents. The key for enhancing the performance of the RL methods in such problems is to extract critical spatiotemporal features in the environment. In this fiscal year, we focused on spatial feature extraction and explored a feasible method to realize it during reinforcement learning episodes.

We adopted the restricted Boltzmann machine (RBM) as a basic architecture of the learning system, and investigated how the energy-based SARSA algorithm for the RBM worked in a decision-making task with high-dimensional and noisy sensor inputs. The simulation results showed:

- The learning algorithm was able to obtain nearly optimal decision-making policy even if the sensory inputs were disturbed by large noises.
- Reward-dependent and reward-invariant information codings were both found in the distributed activation patterns of the hidden units of the RBM.
- The distributed information coding persisted even though the number of hidden nodes is varied.

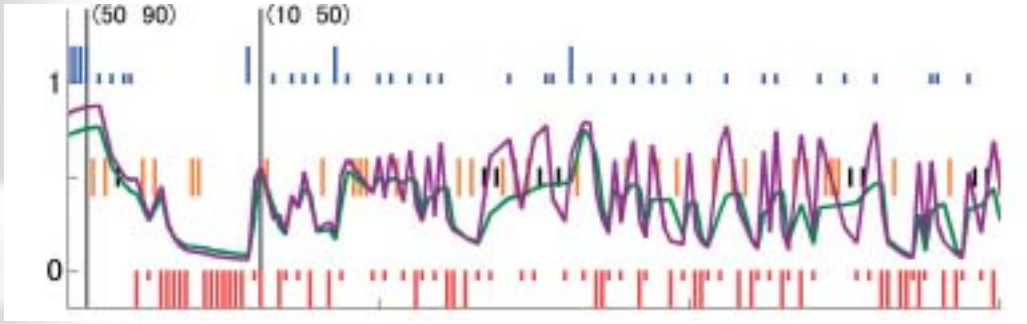
3.2 Systems Neurobiology Group

Action and action value coding in the basal ganglia network [Ito, Yoshida]

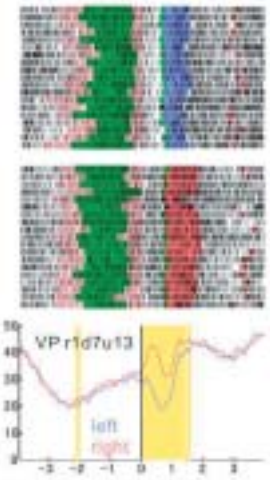
In order to understand the mechanisms of action selection by prediction of reward, we analyzed the rats' choice strategy and neural coding in the basal ganglia during a probabilistic reward-based free choice task.

By model-based prediction of rats' choice from the preceding sequence of actions and rewards, we showed that the generalized Q-learning model with the forgetting rate and no-reward aversion parameters can predict the rats' choice sequence as good as the best multi-step Markov model does. The generalized Q-learning model, which includes the local matching law (Sugrue et al., 2004), the standard Q-learning (Sutton and Barto, 1998; Samejima et al., 2005), and a modified Q-learning (Barraclough et al., 2004) as its subset, does not only serve as the best descriptive model of rats' choice strategies, but also serves as a normative model that explains 'why' rats should take such strategies (Corrado and Doya, 2007).

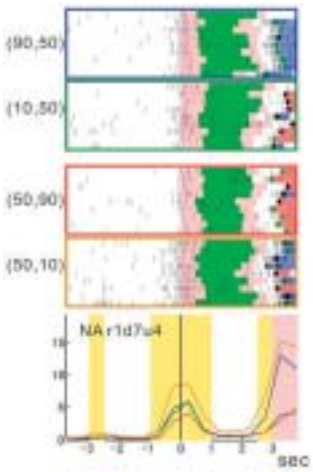
Information analysis revealed that neurons in the striatum and the pallidum coded the trial type, action values (reward expected for a candidate action), executed action, and the reward availability. Coding of action values was less dominant, but was seen throughout the trial. We found contralateral-side dominance of action value coding in the pallidum (Ito and Doya, 2007d). From this result, we are considering a new model of action selection in the cortico-basal ganglia network.



(a) One-step prediction of rats' choices based on reinforcement learning models



(b) Action coding neuron in the pallidum

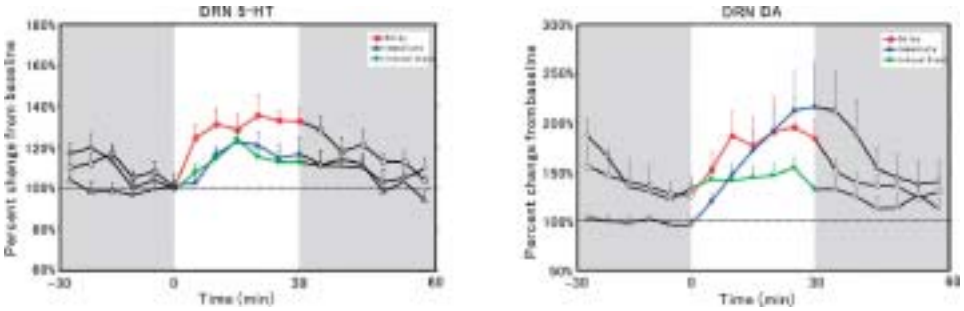


(c) Action value coding neuron in the striatum

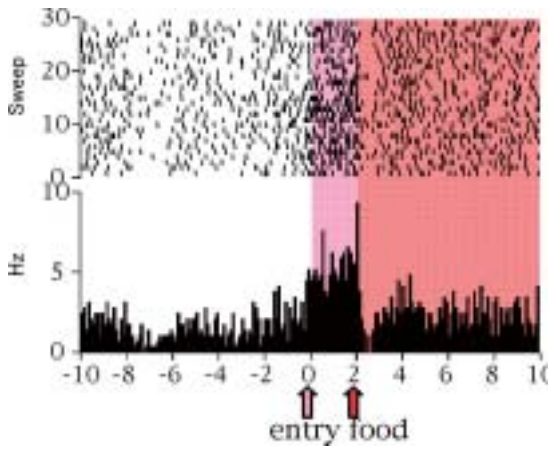
Figure 3: (a) One-step prediction of rats' choices by generalized Q-learning (purple) and original Q-learning (green). Blue bars and red bars indicate left and right choices of rats, respectively. (b) An example of action coding neuron in the pallidum. (c) An example of neuron in the striatum coding action value for the right choice.

Role of serotonin in actions for delayed rewards [Katsuhiko Miyazaki, Kayoko Miyazaki]

While serotonin is well known to be involved in a variety of psychiatric disorders including depression, schizophrenia, autism, and impulsivity, its role in the normal brain is far from clear despite abundant pharmacology and genetic studies. From the viewpoint of reinforcement learning, we earlier proposed that an important role of serotonin is to regulate the temporal discounting parameter that controls how far future outcome an animal should take into account in making a decision (Doya, 2002).



(a) time courses of serotonin and dopamine efflux during three task periods



(b) increased serotonin neuron firing during reward delay period

Figure 4: Chemical and electric recording of ascending serotonin in behaving rats.

We collaborated with Prof. Shigeto Yamawaki's group at Hiroshima University on human brain imaging experiments under dietary manipulation of the amino acid tryptophan, the precursor of serotonin (Tanaka et al., 2007). Through reinforcement learning model-based analysis of the fMRI data, verified our previous finding (Tanaka et al., 2004) that the there is a ventral-dorsal differentiation in the time scale of future reward prediction. In the trypotophan depletion and loading experiments, we found out that the ventral striatal activities correlated with short-term reward prediction was enhanced under lower serotonin levels, and that the dorsal striatal activities correlated with long-term reward prediction was enhanced under higher serotonin levels. The result supports our hypothesis that higher serotonin level promotes long-term prediction of action outcome.

In order to further clarify the role of serotonin in natural behaviors, we performed rat 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 food-water navigation task (Miyazaki et al., 2007d). 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 (Miyazaki et al., 2007b). 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.

Learning of complex action sequences [Hoshino, Rodrigues]

Humans and animals can learn highly complex sequence of actions. Movements are initially exploratory and require much attention, but they gradually become stereotyped and require little consciousness, which enables the sequence of actions as a building block for a more complex behaviors. Our aim is to clarify the computational principle of chunking of action sequences and the neural mechanisms behind it. We are building rat experimental models and preparing human brain imaging experimental paradigm.

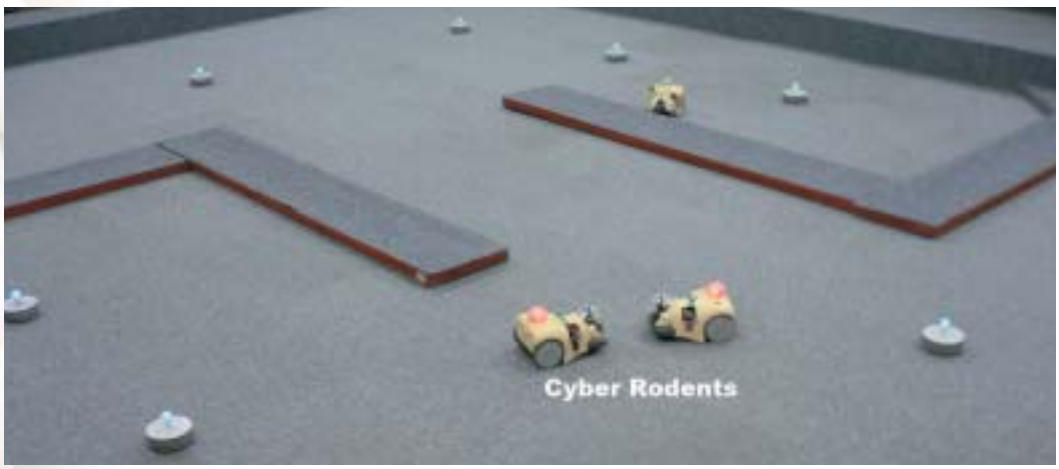


Figure 5: Cyber Rodents and battery packs

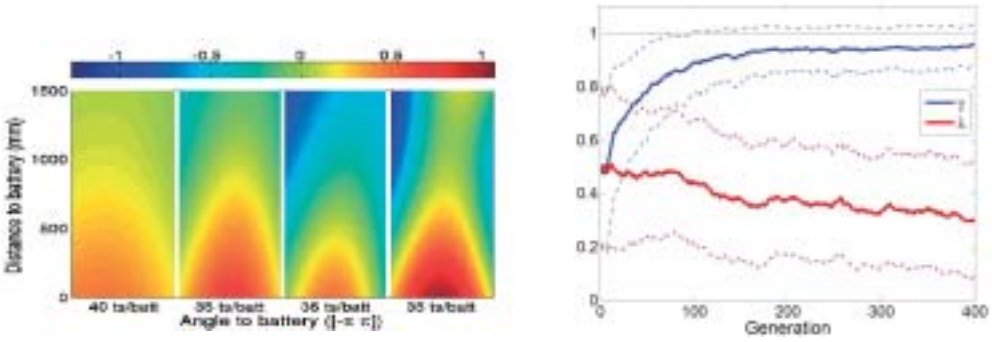
3.3 Adaptive Systems Group

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, or learning and evolution. 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.

Embodied evolution of reward functions and meta-parameters [Elfwing, Uchibe]

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.



(a) evolution of reward function (b) evolution of meta-parameters

Figure 6: Evolution of rewards and meta-parameters in Cyber Rodents.

Learning to communication and cooperate [Sato]

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 can learn 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.

Robust and efficient learning algorithms [Uchibe, Morimura, Kamioka, Otsuka]

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.

4. Publications

4.1 Journals

Corrado, G., Doya, K. (2007) Understanding neural coding through the model-based analysis of decision making, Journal of Neuroscience, 27, 8178-8180.

Doya, K. (2007) Reinforcement learning: Computational theory and biological mechanisms, HFSP Journal, 1, 30-40, DOI: 10.2976/1.2732246.

Doya, K. (2008) What are the objective functions of the brain?, Journal of the Society of Instrument and Control Engineers, 47(1), (in Japanese).

Doya, K. (2008) Computers in the brain: The circuit and molecular mechanisms of the cerebellum, the basal ganglia, the hippocampus, and the cerebral cortex, Seitai No Kagaku, 59(1), 10-19, (in Japanese).

Elfwing, S., Uchibe, E., Doya, K., Christensen, H. I. (2007) Evolutionary development of hierarchical learning structures, IEEE Transactions on Evolutionary Computations, 11, 249-264.

Kamioka, T., Uchibe, E., Doya, K. (2007) Max-min actor-critic for multiple reward reinforcement learning, IEICE TRANSACTIONS on Information and Systems (Japanese Edition), J90-D(9), 2510-2521, (in Japanese).

Samejima, K., Doya, K. (2007) Multiple representations of belief states and action values in corticobasal ganglia loops, Annals of New York Academy of Sciences, 1104, 213-228.

Sato, T., Uchibe, E., Doya, K. (2008) Emergence of communication and cooperative behavior by reinforcement learning agents, IPSJ Transactions on Mathematical Modeling and its Applications, 48, 55-67, (in Japanese).

Schweighofer, N., Tanaka, S. C., Doya, K. (2007) Serotonin and the evaluation of future rewards: Theory, experiments, and possible neural mechanisms, Annals of New York Academy of Sciences, 1104, 289-300.

Tanaka, Saori C., Schweighofer, N., Asahi, S., Shishida, K., Okamoto, Y., Yamawaki, S., Doya, K. (2007) Serotonin differentially regulates short- and long-term prediction of rewards in the ventral and dorsal striatum, PLoS ONE, 2(12), e1333.

Uchibe, E., Doya, K. (2007) Cyber rodent project, Journal of Japanese Neural Network Society, 14(4), (in Japanese).

Bissmarck, F., Nakahara, H., Doya, K., Hikosaka, O. (in press) Combining modalities with different latencies for optimal motor control, Journal of Cognitive Neuroscience.

Elfwing, S., Uchibe, E., Doya, K., Christensen, H. I. (in press) Co-evolution of shaping rewards and meta-parameters in reinforcement learning, Adaptive Behavior.

Morimura, T., Uchibe, E., Yoshimoto, J., Doya, K. (in press) A new natural gradient of average reward for policy search, IEICE Transactions on Information and Systems, (in Japanese).

4.2 Book(s) and other one-time publications

Doya, K. (2007) Introduction to Computational Neuroscience: Toward Understanding of the Mechanisms of Learning in the Brain, Science Sha/SGC Library, (in Japanese).

Elfwing, S., Uchibe, E., Doya, K., Christensen, H. I. (in press) Co-Evolution of Rewards and Meta-Parameters in Embodied Evolution, Elsevier/Creating Brain-Like Intelligence.

Elfwing, S. (2007) Embodied evolution of learning ability, Ph.D. Thesis/KTH School of computer science and communication, Stockholm, Sweden.

Morimura, T. (2008) Efficient task-independent reinforcement learning based on policy Gradient, Ph.D. Thesis/ Graduate school of Information science, Nara Institute of Science and Technology.

Morimura, T., Uchibe, E., Yoshimoto, J., Doya, K. (2007) Policy gradient reinforcement learning with log stationary distribution gradients, Information Science Technical Report/Graduate school of Information science, Nara Institute of Science and Technology, NAIST-TR-2007013.

Yoshida, T. (2008) A study on behavior and brain function for delayed and probabilistic reward, Master's Thesis/ Graduate school of Information science, Nara Institute of Science and Technology.

4.3 Oral presentations

Doya, K. Designing the reward system: Computational and biological principles, The first IEEE Symposium on Foundations of Computational Intelligence (FOCI 07) The first IEEE Symposium on Foundations of Computational Intelligence (FOCI 07), April 1-5, 2007.

Doya, K. Dopamine, serotonin and learning from delayed rewards, Brain reward systems: From molecular signaling pathways to neuronal networks, Fondation des Treilles, Provence, May 10-11, 2007.

Doya, K. Reinforcement learning model-based analysis of behavioral data and its application, Human Information Processing Workshop, May 24-25, 2007.

Doya, K. Reinforcement learning theory and neurobiology of decision making, The 2nd APCTP Summer School for Brain Dynamics: Complex Decision-making Processes, July 7, 2007.

Doya, K. In pursuit of the brain mechanism of reinforcement learning, Neuro2007: The 30th Annual Meeting of the Japanese Neuroscience Society, Yokohama, Japan, September 10, 2007.

Doya, K. Multiple pathways and algorithms for reinforcement learning, Neuro2007: The 30th Annual Meeting of the Japanese Neuroscience Society, Yokohama, Japan, September 12, 2007.

Doya, K. Reinforcement learning and decision making, Seminar at College de France, Paris, September 25, 2007.

Doya, K., Miyazaki, KW., Miyazaki, K. Metalearning and neuromodulation; raphe recordings in rats, Theoretical and Experimental Perspectives on Serotonin Function, Ericeira, Portugal, October 6, 2007.

Doya, K., Kimura, M. The basal ganglia and the encoding of value, Neuroeconomics: Decision Making and the Brain, New York University, New York, USA, Jnauary 11-13, 2008.

Doya, K. Roles of serotonin and dopamine in actions for delayed rewards, Center for Neural Science, New York University, New York, USA, Jnauary 14, 2008.

Doya, K. Roles of serotonin and dopamine in actions for delayed rewards, Center for Neuroeconomics, Schwartz Initiative in Theoretical Neuroscience, Yale University, New Haven, USA, Jnauary 15, 2008.

Morimura, T., Uchibe, E., Doya, K. Natural policy gradient with baseline adjustment function for variance reduction, Artificial Life and Robotics (AROB 08), Oita, Japan, January 31-February 2, 2008.

Nakano, T., Doi, T., Yoshimoto, J., Doya, K. A simulation study of the molecular mechianisms of striatal synaptic plasticity, The 9th Regular Meeting of Special Interest Groups of Bioinformatics and Genomics, Information Processing Society of Japan, 2007-BIO-9, 55-62, Onna, Japan, June 14, 2007.

Nakano, T., Yoshimoto, J., Doi, T., Doya, K. A kinetic model of molecular cascade for striatal synaptic potentiation and depression, Les Treilles meeting, Fondation des Treilles, Provence, May 10-11, 2007.

Nakano, T., Yoshimoto, J., Doi, T., Doya, K. A kinetic model for molecular mechanisms of cortico-striatal synaptic plasticity, Neuro2007: The 30th Annual Meeting of the Japanese Neuroscience Society, Yokohama, Japan, September 10-12, 2007.

Otsuka, M., Yoshimoto, J., Doya, K. Robust population coding for a free energy based reinforcement learning agent, The 18th International Conference on Artificial Neural Networks (ICANN 2008), Prague, Czech Republic, September 3-6, 2008 in press.

Uchibe, E., Doya, K. Constrained reinforcement learning from intrinsic and extrinsic rewards, The IEEE International Conference on Development and Learning, London, July 11-13, 2007.

Uchibe, E. Frontiers in Robotics, Japan-Germany Frontier of Science Symposium, Hayama, Japan, November 2-4, 2007.

Uchibe, E., Doya, K. Finding exploratory rewards by embodied evolution and constrained reinforcement learning in the cyber rodents, The 14th International Conference on Neural Information Processing, Kitakyushu, Japan, November 13-16, 2007.

Yoshimoto, J., Doya, K. Bayesian system identification of molecular cascades, The 14th International Conference on Neural Information Processing (ICONIP 2007), Kitakyushu, Japan, November 13-16, 2007.

4.4 Posters

Elfwing, S., Uchibe, E., Doya, K. Embodied evolution of the learning ability, The 8th Winter Workshop on Mechanism of Brain and Mind, Rusutsu, Japan, January 10, 2008.

Ito, M., Doya, K. Action-selection model for decision making task and information analysis of neural activity in basal ganglia, The 8th Summer workshop on mechanism of Brain and Mind, Sapporo, Japan, August 23-24, 2007.

Ito, M., Doya, K. Learning model-based analysis of information coding in the nucleus accumbens and the globus pallidus in a decision making task, The 37th Annual Meeting of the Society for Neuroscience, San Diego, California, November 3-7, 2007.

Ito, M., Doya, K. Neural activity of the nucleus accumbens and the globus pallidus in a decision making task, Neuro2007: The 30th Annual Meeting of the Japan Neuroscience Society, Yokohama, Japan, September 10-12, 2007.

Miyazaki, K., Miyazaki, KW., Doya, K. Activity of serotonergic neurons in the dorsal raphe nucleus of freely moving rats during reward and non-reward delay period, The 37th Annual Meeting of the Society for Neuroscience, San Diego, California, November 5, 2007.

Miyazaki, K., Miyazaki, KW., Doya, K. Activity of serotonergic neurons in the dorsal raphe nucleus of freely moving rats during reward and non-reward delay period, Neuro2007: The 30th Annual Meeting of the Japanese Neuroscience Society, Yokohama, Japan, September 10-12, 2007.

Miyazaki, KW., Miyazaki, K., Doya, K. Increased serotonin efflux in the dorsal raphe nucleus of rats working for delayed reward, The 37th Annual Meeting of the Society for Neuroscience, San Diego, California, November 5, 2007.

Miyazaki, KW., Miyazaki, K., Doya, K. Increased serotonin efflux in the dorsal raphe nucleus of rats working for delayed rewards, Neuro2007: The 30th Annual Meeting of the Japanese Neuroscience Society, Yokohama, Japan, September 10-12, 2007, in Japanese.

Nakano, T., Doi, T., Yoshimoto, J., Doya, K. Cortico-striatal synamtic plasticity and its signal transduction model, Symposium on Synapse, National Institute form Physiological Science, Okazaki, Japan, December 6-7, 2007, in Japanese.

Nakano, T., Doi, T., Yoshimoto, J., Doya, K. A kinetic model of the molecular cascade for cortico-striatal synaptic potentiation and depression, The 37th Annual Meeting of the Society for Neuroscience, San Diego, California, November 3-7, 2007.

Yoshida, T., Ito, M., Yoshimoto, J., Morimura, T., Doya, K. A study of mathematical models and experiments of decision-making under probabilistic and delayed rewards, The 8th Summer Workshop on Mechanism of Brain and Mind, Sapporo, Japan, August 23-24, 2007.

5. Intellectual Property Rights and Other Specific Achievements

5.1 Patent

Morimura, T., Uchibe, E., Yoshimoto, J., Doya, K. : A policy-gradient reinforcement learning algorithm that does not require a temporal discounting parameter (applied)

5.2 Awards

The 21st Tsukahara Award, Brain Science Foundation, to Kenji Doya
Best Paper Award, ICONIP 2007, to Eiji Uchibe and Kenji Doya
Yamashita Research Award, Information Processing Society of Japan, to Junichiro Yoshimoto

6. Meetings and Events

6.1 Okinawa Computational Neuroscience Course 2007

Date: June 26 - July 12, 2007
Venue: OIST Seaside House
Co-organizers: Erik De Schutter, Klaus Stiefel, Jeff Wickens (OIST)
Co-sponsors: Nara Institute of Science and Technology,
Japanese Neural Network Society
Speakers: Ad Aertsen (Universität Freiburg)
Gordon Arbuthnott (OIST)
Tom Bartol (Salk Institute)
Hagai Bergman (Hebrew University)
Nathaniel Daw (New York University)
Sophie Deneve (ENS Paris)
Erik De Schutter (OIST)
Markus Diesmann (RIKEN)
Kenji Doya (OIST)
Jerome Friedman (MIT)
Michael Häusser (University College London)
Shin Ishii (NAIST)
Dieter Jaeger (Emory University)
Mitsuo Kawato (ATR)
Eve Marder (Brandeis University)
Klaus Stiefel (OIST)
David Terman (Ohio State University)
Jeff Wickens (OIST)
Torsten Wiesel (Rockefeller University)

Centrally supported event.

6.2 Human Information Processing Workshop

Date: May 24 - 25, 2007
Venue: OIST Seaside House
Co-organizers: Shin-ichi Kita (Kobe U), Satoru Odo (Ryukyu U)
Co-sponsors: Institute of Electronics, Information and Communication Engineers
Human Interface Society
Social Psychology Society of Japan
Speakers: 32 domestic speakers.
http://www.ieice.or.jp/~hip/document/200705.htm

6.3 Neurocomputing and Bioinformatics Joint Workshops

Date: June 14 - 15, 2007
Venue: OIST Seaside House
Co-organizers: Hiroaki Gomi (NTT), Tatsuya Akutsu (NAIST)
Co-sponsors: Institute of Electronics, Information and Communication Engineers
Information Processing Society of Japan
Speakers: 26 domestic speakers.
http://www.ipsj.or.jp/katsudou/sig/sighp/bio/programs/2007_06.html

6.4 Summer Workshop on the Mechanisms of the Brain and Mind

Date: August 23 - 24, 2007
Venue: Sapporo
Co-sponsors: Integrative Brain Research Project
Speakers: Ann Graybiel (MIT)
A. David Redish (University of Minnesota)
Hajime Mushiake (Tohoku University)
Soichi Nagao (RIKEN BSI)

6.5 OIST Special Seminar

Date: August 27, 2007
Venue: OIST IRP seminar room
Speakers: A. David Redish, University of Minnesota

6.6 Winter Workshop on the Mechanisms of the Brain and Mind

Date: January 9-11, 2008
Venue: Rusutsu Resort
Co-sponsors: Integrative Brain Research Project
Speakers: Andreas Meyer-Lindenberg (University of Heidelberg)
Christian Keysers (University of Groningen)
Yoh Iwasa (Kyushu U)
Kumi Kuroda (RIKEN)
Naotaka Fujii (RIKEN)
Takaya Arita (Nagoya U)
Keiko Tanaka (Duke U)
Takanori Uka (Juntendo U)
Hirokazu Tanaka (NiCT)

VI. G0 Cell Unit

Principal Investigator: Mitsuhiro Yanagida

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

Abstract

Cell growth and multiplication are central problems in biology. Our research focuses on a cellular adaptation strategy for cell cycle arrest via nutrient starvation. We aim to investigate the molecular-switch problem; namely, how cells are able to control and execute an arrest at the G0-like differentiated state, and how cell growth and division resumes when the nutritional environment improves. The goal is to gain a molecular-level understanding of the transition from G0-arrest to the re-initiation of growth. We employ post-genomic methods that produce quantitative information regarding individual genes using fission yeast as a model organism.

This year we were successful in identifying the genes involved in the maintenance of, and exit from the G0 state, and conducted functional analyses on such genes. We found that a ubiquitin/proteasome system is required for maintenance of the G0 state, most likely by regulation of oxidative stress and mitochondrial function. On the other hand, we found a temperature-sensitive (ts) mutant of the *tor2* gene, encoding a phosphatidyl-inositol kinase (PIK), that shows both cell cycle and growth arrests in a nutrient-rich medium at the restrictive temparature. We compared the proteomes of both wild type G0 cells and *tor2* mutation-induced, arrested cells. New components of the TOR complexes were also detected.

On the basis of transcriptomic studies, we have thus far found 12 genes that are required for G0 maintenance. From proteomic analyses, proteins designated ‘ Degs’, whose levels are reduced immediately after the addition of a nitrogen source, were also analyzed by gene disruption experiments. Through the screening of 611 ts mutants, we found that 23% lose cell viability not only in the vegetative state, but also in the G0 state, and identified the responsible genes. Functional analyses of these genes required for the G0 function are ongoing. We have made substantial progress in metabolomic analysis, quantitatively detecting ~200 compounds and comparing their levels in several cases, including mutants defective in G0 maintenance .

Finally, we have started screenings for mutants showing resistance or hypersensitivity to the drugs tamoxifen and damnacanthal, which we have found disrupt G0 maintenance.

1. Staff

Okinawa

Group leader: Mizuki Shimanuki

Researchers: Koji Nagao, Kojiro Takeda

Technical staff: Sakura Kikuchi, Ayaka Mori, Aya Kokubu, Risa Uehara, Tomas Pluskal, Bryan Mathis, Alejandro Villar Briones

Research assistant/graduate student: Kenichi Sajiki

Research administrator/secretary: Tomomi Teruya

Kyoto
Researchers: Takeshi Hayashi, Mitsuko Hatanaka
Research administrator/secretary: Yukari Matsushita

2. Partner Organizations

Kansai Advanced Research Center, National Institute of Information and Communications Technology
Type of partnership: Collaboration
Name of principal researcher: Yasushi Hiraoka
Name of researcher: Yuji Chikashige
Research 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
Type of partnership: Technical Help
Name of principal researcher: Masayuki Yamamoto
Name of researcher: Kayoko Tanaka (Present: Department of Biochemistry, Univesity of Leicester)
Research theme: Gene disruption analysis of *S. pombe*

Chemical Genetics Laboratory, Discovery Research Institute, Wako Institute, Riken
Type of partnership: Collaboration
Name of principal researcher: Minoru Yoshida
Name of researcher: Shinichi Nishimura
Research 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
Type of partnership: Collaboration
Name of principal researcher: Chikashi Obuse
Research theme: Proteomic analysis of *S. pombe* G0 cells

Laboratory of Molecular Inheritance, Graduate School of Biostudies, Kyoto University
Type of partnership: Collaboration
Name of researcher: Takahiro Nakamura
Research theme: Metabolomic analysis of *S. pombe* cells using LC-MS mass spectrometer

Laboratory of Molecular Inheritance, Graduate School of Biostudies, Kyoto University
Type of partnership: Collaboration
Name of researcher: Yohta Fujita
Research theme: Identification of novel kinetochore components using LC-MS mass spectrometer

Unit for Molecular Neurobiology of Learning & Memory, OIST
Type of partnership: Collaboration
Name of principal researcher: Shogo Endo
Name of researcher: Nobuhiko Kojima (Present: Gunma University Medical School, Japan)

Bioneer Corporation (BIONEER) and Korea Research Institute of Bioscience and Biotechnology (KRIBB)
Type of partnership: Collaboration
Name of researchers: K.-L. Hoe, D.U. Kim, and H. Park
Research theme: Analysis of systematic genome wide haploid deletion mutants in *Schizosaccharomyces pombe* for the drug sensitivity of the G0 cells

The Department of Gene Mechanisms, Graduate School of Biostudies, Kyoto University
Type of partnership: Collaboration
Name of researcher: Junko Kanoh
Research theme: Analysis of TOR complexes by specific and common subunits

Laboratory of Molecular Inheritance, Graduate School of Biostudies, Kyoto University
Type of partnership: Collaboration
Name of researcher: Norihiko Nakazawa
Research theme: Identification of condensin accumulation components using LC-MS mass spectrometer

3. Activities and Findings

3.1 The Ubiquitin/proteasome system is required to maintain the G0 state

We found previously that the ubiquitin/proteasome system is essential for the survival of G0 cells. The 26S proteasome consists of a 19S regulatory and 20S core complex in vegetatively growing cells. It is unknown, however, whether the proteasome forms on identical 26S complex in G0. Purified proteasome was analyzed using immunoprecipitation and mass spectrometry (LC/MS/MS). We found that the 36 distinct subunits of the 26S proteasome were detected in both G0 and vegetative cells, clearly demonstrating that the composition of the G0 proteasome is identical to that of vegetative proteasome.

Intracellular localization of the proteasome in G0 cells, however, was different from that in vegetative cells. In vegetative dividing cells, the proteasome is mainly localized to the nucleus, thereby requiring a specific protein termed Cut8. The level of this protein was greatly reduced in the nitrogen-starved G0 cells (indicated by the arrow in Figure 1A), whereas the level of the proteasome subunit a4 was unchanged. Cut8 has a short half-life in both the vegetative and G0 conditions (Figure 1B). The proteasome in G0 cells is typically localized in the cytoplasm (the nuclear envelope and ER), but the addition of leptomycin B (LMB) an inhibitor of Crm1 caused nuclear accumulation of the proteasome (Figure 1C). These results indicate that proteasome localization was dynamically controlled through Cut8 and Crm1.

Electron micrographs (taken using the freeze substitution method) revealed the nuclear deposit of electron-dense materials and occasional membranous structures inside the nucleus (Figure 1D,

left panel). The origin of the amorphous-shaped nuclear deposits is unknown. The nuclear membrane appeared to become fragile if cells were treated with the $KMnO_4$ fixation procedure rather than the freeze substitution method. We analyzed the *mts3* (a gene for a 19S proteasome component) mutant extracts using mass spectrometry to gain additional information regarding the molecular defects in the G0 cells of the *mts3* mutant; approximately 2,000 proteins were detected in a semi-quantitative manner. In comparison with control extracts prepared under restrictive conditions, we found increased levels of many chaperone proteins, transcription factors, and proteins implicated in ubiquitin/proteolysis. Indeed, the level of cellular oxidative stress increased considerably in parallel with a reduction in cell viability. Interestingly, a number of mitochondrial proteins were greatly decreased in the *mts3* mutant cells at about the same time (Figure 1E). Among the 100 proteins that exhibited the greatest decreases, gene ontology suggested that 39 proteins were mitochondrial-specific. Consistent with this finding, the amounts of mitochondria stained by MitoTracker dye significantly decreased (Figure 1D, right panel). These results indicate that mitochondrial dysfunction occurred in the proteasome mutant.

Polyubiquitination of proteins requires three steps: ubiquitin activation by E1 enzyme, conjugation by E2, and ligation by E3. *S. pombe* has one E1 enzyme, eleven E2, and many E3. As expected, the E1 *ptr3* mutant lost cell viability under G0 condition. We then examined whether any of the eleven E2 mutants lost their viability under the G0 condition. Two E2 mutants (*ubc3* and *ubc8*) showed significant loss of viability in G0 (Figure 1F). The deletion-defective phenotypes of *ubc3* and *ubc8* resembled to some degree the phenotype of *mts3* proteasome mutant cells.
We speculate that the loss of proteasome function caused an increase in oxidative stress and mitochondrial dysfunction (Figure 1G). In short, we demonstrated that the proteasome system is essential in maintaining the G0 state, although many of the observed cellular phenotypes require further study to be fully understood.

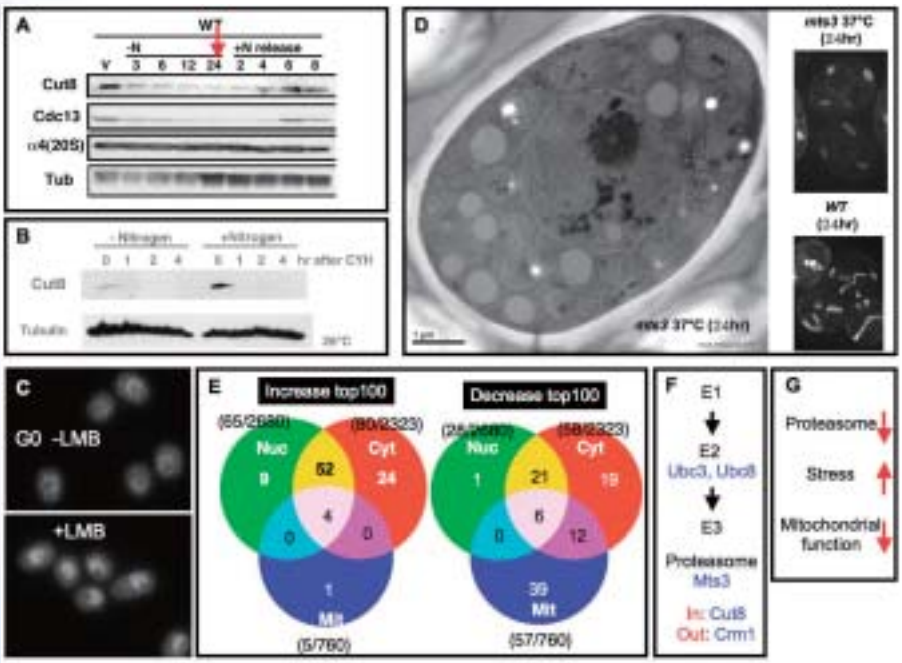


Figure 1. Proteasome is essential in G0 cells.

3.2.1 Rapamycin-sensitive *tor2* mutant shows G0-like phenotype and defects in metabolism

We found thet a ts mutant of *tor2* gene, encoding a phosphatidyl-inositol kinase (PIK), which played an important role in the nutrient-signaling pathway, arrested in the cell cycle progression with 1C DNA content at the restrictive temperature in a nutrient-rich culture medium as shown in Figure 2 (these results are published in Hayashi et al., 2007). The mutant revealed a G0-like phenotype in the presence of rapamycin at 26°C. This mutation is unique, as previously isolated *tor2* mutants are insensitive to rapamycin. Intracellular localization of *tor2* was speckled in the vegetative cytoplasm, and both speckled and membranous in the G0 cells.

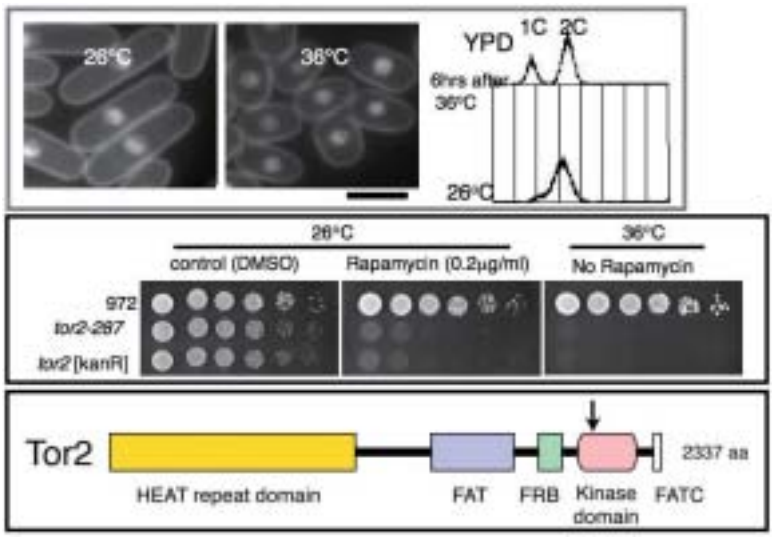


Figure 2. Rapamycin-sensitive *tor2-287* mutant was isolated by screening ts strains for the G0-like phenotype produced in the N-rich medium at 36°C.

We performed semi-quantitative LC/MS/MS proteome analysis on the wild type G0 and mutant *tor2* cells in the presence of a nitrogen source (Figure 3). Thirty-three proteins were significantly increased in both WT G0 and *tor2*, suggesting that WT G0 and *tor2* mutants at 36°C were similar. Most of these proteins were enzymes implicated in the metabolism of amino and sugar compounds, peptidases (protease), or transporters. Eight other proteins (including permease, glucosidase, and G-protein activator) were increased in WT G0 but not in the *tor2* mutant, and are therefore possibly specifically defective in *tor2* mutant cells. Four proteins showed a marked increase in *tor2* mutant cells but not in WT G0; three of these proteins are involved in amino acid metabolism and are closely related to each other. These results may provide a clue to the role of *tor2* in growing cells.

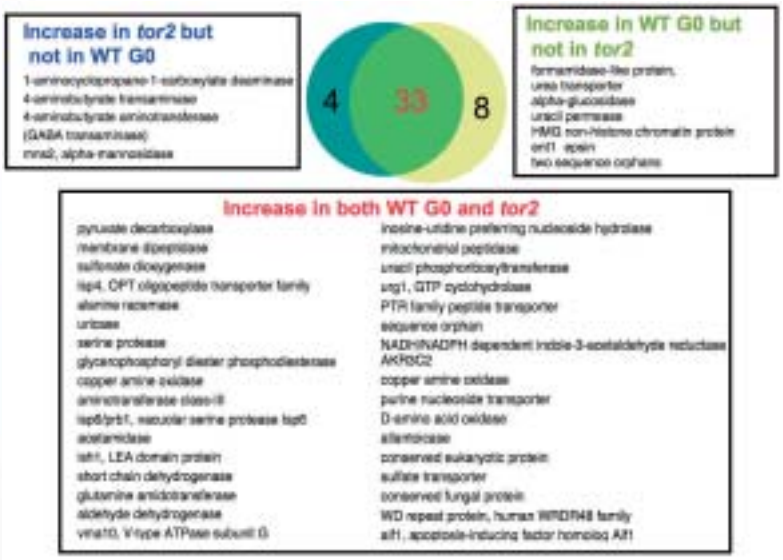


Figure 3. Mass-spectrometric analyses of proteomes in the cell extracts of wild type G0 and mutant *tor2*.

3.2.2 Organization of TOR complexes in *S. pombe*

To understand the role of *S. pombe* Tor2, it is essential to gather information on the molecular organization of TOR complexes (TORCs). Interpretation of the *tor2* mutant phenotypes requires knowledge on TORCs. To this end, a series of mass spectrometry analyses were taken and reported in Hayashi et al (2007). We identified six new proteins (Figure 4, top; Tco89, Bit61, Toc1, Tel2, Tti1, and Cka1) in addition to the six previously identified subunits (Tor1, Tor2, Mip1/Raptor, Ste20/Rictor, Sin1/Avo1, and Wat1/Lst8). All of the examined subunits exhibited multiple phosphorylation. Cka1 (casein kinase II), which binds to both Tor1 and Tor2, was found to be essential in maintaining the G0 state (described below).

To our surprise, Tel2, which is bound to Tti1, interacts with all six of the phosphatidyl inositol kinase (PIK)-related kinases (Tra1, Tra2, Rad3/ATR, Tel1/ATM, Tor1, Tor2; Figure 4, bottom). Tel2 is therefore a common partner of PIK-related kinases, and may act as a link between DNA checkpoint, nutritional response, and transcriptional regulation. The implications of this finding are discussed in Kanoh and Yanagida (2007).

S. pombe Tel2, a member of the Tel2/Rad-5/Clk-2 family, is known to be essential for viability, and interacts with Mrc1, a replication checkpoint regulator. The hypothesis that Tel2 is a common partner or regulator of PIK-related kinases may explain why this family is involved in a variety of cellular functions, including cell proliferation, regulation of the biological clock, telomere maintenance, and DNA damage checkpoint.

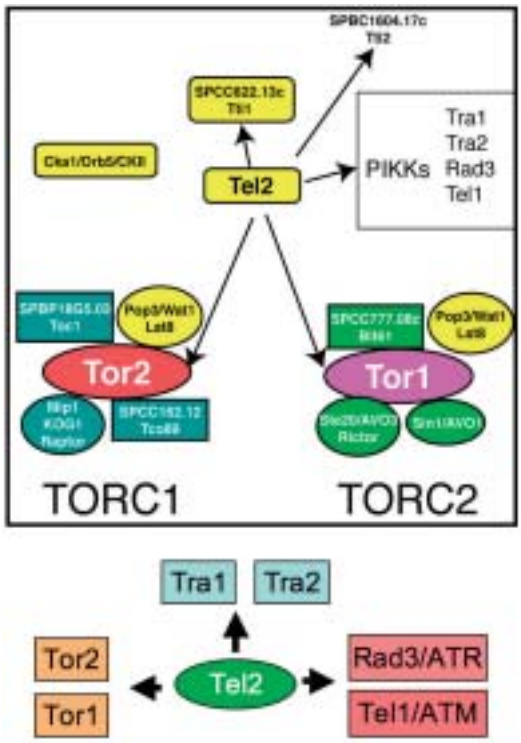


Figure 4. TOR complexes (TORC1 and TORC2) are functionally overlapping, as four of the eight subunits are common to both complexes.

3.3 Viability analysis of candidate gene deletions

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 examined 43 of these genes using deletion studies. We also studied 10 other genes without regard to their transcriptional regulation. Twelve strains lost cell viability faster than wild type during long-term incubation at 26°C in the G0 state (Figure 5, left).

We employed both electron microscopy and light microscopy in examining the phenotype of these deletion strains. The deletion Δ*klf1* mutant that was viable under the vegetative condition showed abnormal organella localization in vegetatively growing cells. In contrast, a large amount of unknown material was deposited between the plasma membrane and the cell wall in the G0 Δ*klf1* cells that lost cell viability (Figure 5, bottom).

Investigations into the functions of these gene products will shed light on the genetic control of the G0 state in *S. pombe*. We are currently conducting experiments to identify those genes regulated by Klf1. This study is described in part by Shimanuki et al. (2007). Figure 5 (top right) shows the genes required to maintain G0 cells, as discovered by genetic and high-throughput transcriptome approaches.

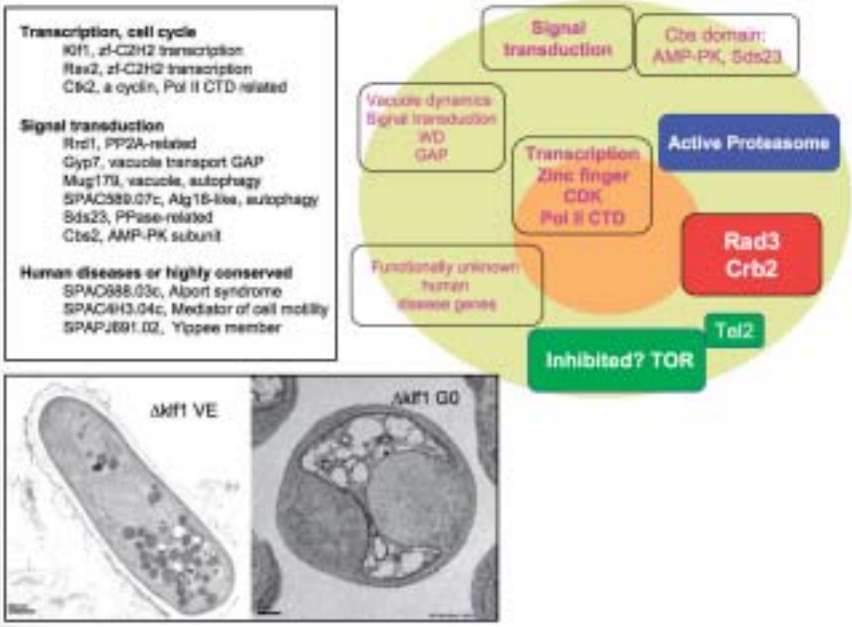


Figure 5. Genes required for G0 maintenance, as identified based on genetics and high-throughput transcriptome analysis.

3.4 Proteome analysis to identify candidates required for the maintenance of or exit from G0

We performed proteomic analysis of the G0 cells and cells shifting towards the proliferating state upon nutritional replenishment using a semi-quantitative liquid chromatography-mass spectrometry (nanoLC-MS) method. Significant progress was made by improving the sensitivity and reproducibility of detection and using our own program to quantify cellular proteins based on the emPAI (exponentially modified protein abundance index) parameter (Figure 6, left panel). As a result, more than 2000 proteins were identified and quantified using total cell extracts of G0, vegetative cells, and cells at times of 1, 2, 4, and 6 h after nutritional replenishment.

The levels of ~450 proteins were classified into five clusters: immediate, delayed, and late decrease, and immediate and delayed increase (Figure 6, right panel). Ontology is shown in Figure 15 for each of the five clustered genes. The figures in parentheses are the numbers of genes belonging to each cluster. For example, the group of 52 proteins classified as the immediate decrease cluster after release from the G0 state contained genes that are involved in either unknown biological processes or the transport of small molecules. For the delayed decrease cluster, proteins were abundant in signal transduction and adaptation to nutrient. In contrast, proteins of the immediate increase cluster contained ribosome biogenesis, ribosome proteins, amino acid biosynthesis, and proteins implicated in translation. The delayed increase cluster contained abundant proteins related to tRNA aminoacylation and amino acid metabolic processes, as with the immediate increase cluster.

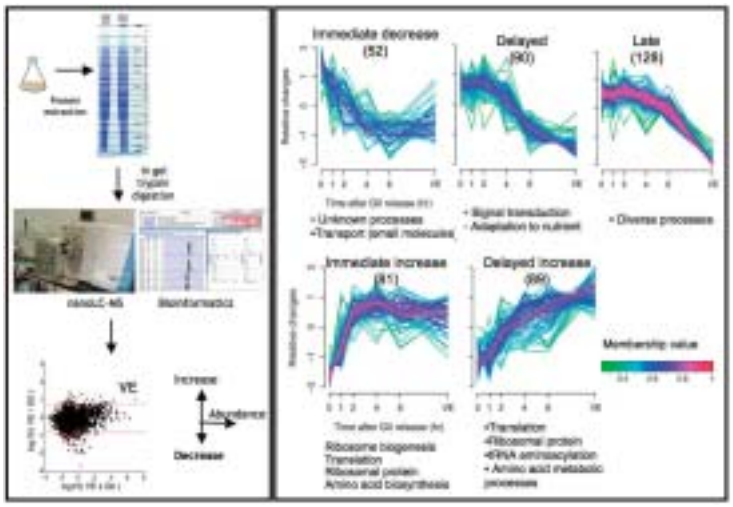


Figure 6. LC/MS/MS proteomic analysis of G0 cells and those showing proliferation after the addition of the nitrogen source.

The abundance profile for three representative proteins (urea transporter, formamidase-like protein, and Gly1:threonine aldolase) in the immediate decrease, delayed decrease, and immediate increase clusters, respectively, are shown in Figure 7 (left panel). The level of urea transporter decreased ~100-fold within 2 h, while Gly1 increased more than 10-fold within 2 h. Protein such as formamidase-like protein took a long time to decrease to a negligible level from the high level in G0 cells. Some of the representative proteins that belong to the immediate decrease cluster are shown in Figure 7 (right panel). This cluster contains many transporters, permeases, and proteolysis-related proteins, as well as nine functionally unknown proteins (Deg1–9). We are currently investigating the functionally unknown Deg1–9 proteins, Maf1 (a repressor of RNA polymerase III), and several transporter–permeases.

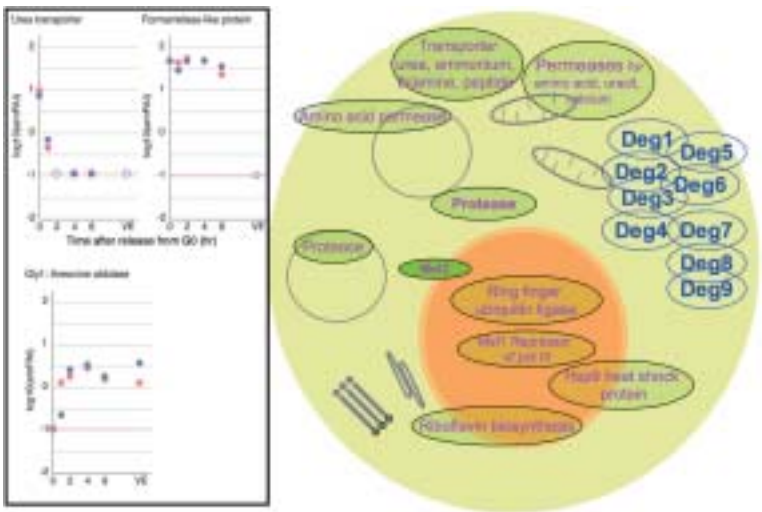


Figure 7. Proteomic analysis revealed individual proteins whose abundance changed markedly upon replenishment of the nitrogen source.

3.5 Comparative analysis of G0 and proliferating cells by metabolome

To quantitatively assay the compositions of metabolites in G0 versus vegetative cells, we employed a high performance liquid chromatography electro-spray ionization mass spectrometry (HPLC-ESI-MS) with ZIC-pHILIC columns. We developed a method for extracting the intracellular metabolites of *S. pombe* using cold methanol for quenching (rapid cessation of cellular metabolism), bead shock for breaking the cell wall, and centrifugation and filtration through a 5 KDa cut-off filter. Using this method, we obtained several thousand metabolite peaks and identified ~200 compounds based on their mass and retention time (Figure 8). Approximately 100 compounds showed significant differences in their concentrations. In the table of Figure 8, the quantified peak levels marked in orange and blue indicate a greater than 3-times increase or decrease, respectively, in G0 cells compared with vegetative cells. For further comparison, data obtained from *tor2* mutant cells cultured in the rich medium at 36°C are also shown.

In G0 cells, the concentration of the intracellular pool of amino acids required for the urea cycle shows a sharp decrease, whilst a number of methyl compounds such as methyl-adenosine and methyl-guanosine greatly increase. The methyl donor SAM (S-adenosyl methionine) also increases. The capping activity of RNA transcripts is possibly enhanced in G0 cells. One unknown compound possibly an amino acid derivative greatly increases in G0. Its identity is of interest.

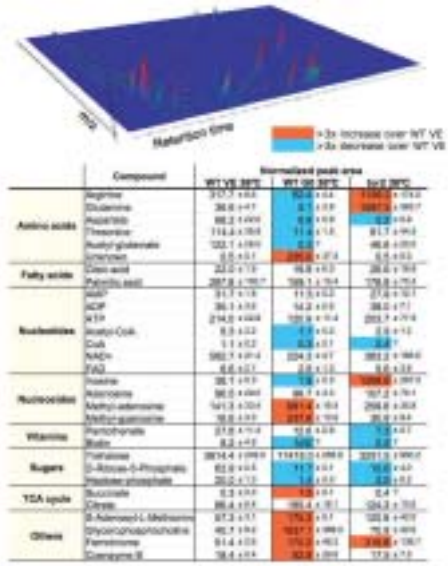


Figure 8. Metabolomic compound peaks with significant differences between G0 and VE cells.

3.6 Use of 611 ts strains to screen essential 'housekeeping' genes in G0

We screened the Hayashi ts mutant library to identify those genes essential in maintaining the G0 state in *S. pombe*. The ts mutations originally identified in the vegetative phase but that were also required for G0 maintenance are thought to be essential 'housekeeping' genes in both the G0 and vegetative phases. The cell viabilities of each strain were measured under three different conditions: 24 h after nitrogen deprivation, 3-day nitrogen-free culture at 26°C, and 37°C (Figure 9, top). Examination of the G0 viabilities of 610 ts strains revealed that 23% of the genes essential to vegetative growth are also required to maintain the G0 cell state. Forty nine strains lost viability at both 26 and 37°C, while 90 strains lost viability only at 37°C (Figure 9, bottom left).

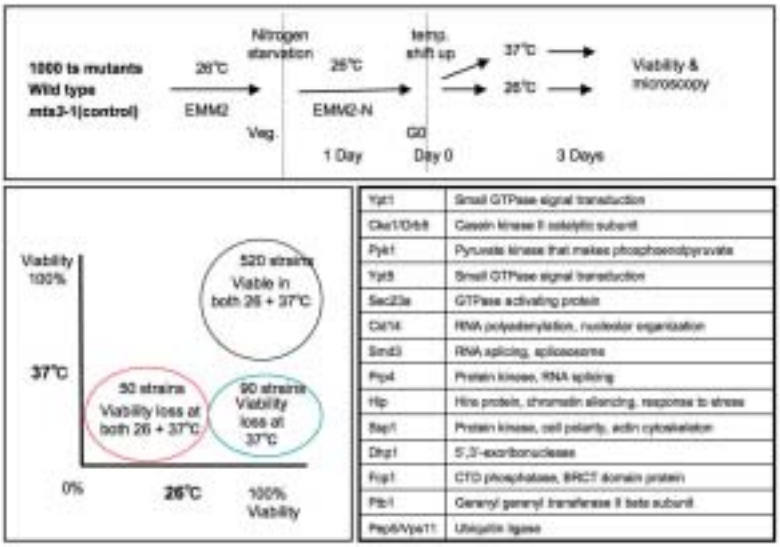


Figure 9. Genes required for both the vegetative and G0 conditions.

We identified the genes responsible for these mutants by subcloning, gene mapping by tetrad dissection, and nucleotide sequencing of the putative mutant genes; about twenty genes were identified (Figure 9, bottom right). A cartoon of Figure 10 illustrates the current status of research using the ts mutants. A variety of gene functions are essential in maintaining both the G0 and vegetative phases. GTPases (Ypt1, Ypt5, and Sec23a) are implicated in intracellular protein transport and endocytosis, and Ptb1 is involved in protein transport through the action of geranyl geranyl transferase. Protein kinases (Cka1/Orb5, Ssp1, and Prp4) are related to cell polarity, actin cytoskeleton, and RNA splicing. Cid14, Smd3, Dhp1, and Fcp1 are involved in transcription and RNA metabolism. The enzyme Pyk1 catalyzes phosphoenolpyruvate to produce ATP in the glycolytic pathway, while two other genes are implicated in chromatin assembly and proteolysis. We were also able to identify a *hcs1* mutation (the gene of HMG-CoA synthetase), which was involved in ergosterol synthesis.

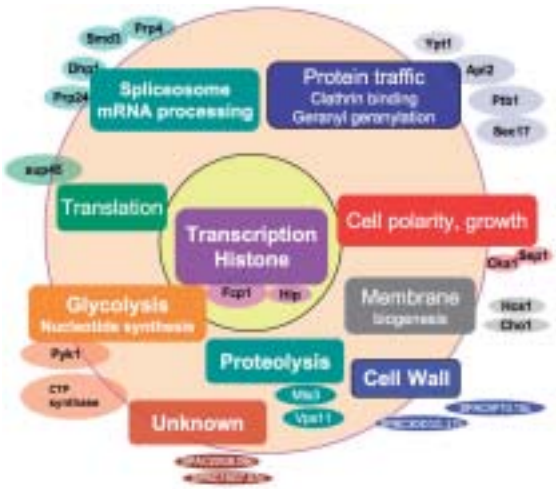


Figure 10. Gene functions identified to be essential for both G0 and VE.

We applied the metabolomic approach in studying *pyk1* mutant cells defective in pyruvate kinase. As expected, the level of phosphoenol pyruvate increased in *pyk1* mutant cells; however, an unexpected and interesting result was that the intracellular levels of nucleotide monophosphates (NMP) and diphosphates (NDP) were greatly diminished while the intracellular level of NTP is relatively normal. The reason for this decrease is unknown. We can speculate that the loss of viability of *pyk1* mutant cells is due to a severe depletion of the NMP and NDP nucleotide pool. Another example is *hcs1* mutant defective in HMG CoA synthase, in which the expected precursor compound is accumulated due to the inactivation of the enzyme, but the levels of various acetyl compounds were unexpectedly altered.

Transcriptome analysis was performed for the *fcp1* mutant, defective in the CTD (C-terminal domain) dephosphorylation of RNA polymerase II. Indeed, certain groups of transcripts were greatly altered in *fcp1* mutant cells in both the vegetative and G0 phases. Furthermore, the patterns of transcripts affected in vegetative and G0 cells were distinct. Given that Ctk2, the cyclin subunit of CTD kinase, has been shown to be essential in G0 by the transcriptome approach, we will focus on the role of Fcp1 in G0 maintenance.

3.7 *S. pombe* G0 cells are sensitive to certain drugs

Differentiated (non-dividing G0) somatic cells are generally thought to be resistant to drugs; this view forms the basis of cancer chemical therapy. We tested 95 other drugs in the SCADS inhibitor kit (a gift from the Screening Committee of Anticancer Drugs supported by a Grant-in-Aid for Scientific Research on Priority Area "Cancer" from the Ministry of Education, Culture, Sports, Science, and Technology, Japan), to identify those chemicals and drugs that lead to reduced cell viability in G0. Cell viability in G0 cells was significantly reduced by 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. In particular, damnacanthal and tamoxifen were more toxic to cells in the G0 state than those in the vegetative state. We are currently using the haploid deletion sets made by Bioneer Co. of Korea (in collaboration with Drs K.-L. Hoe, D.U. Kim, and H. Park) to search for genes that may affect sensitivity to specific drugs. If any deletion mutant is found to be resistant or hypersensitive to the drugs, we will investigate the relationship between drug effects and the function of the gene product.

4. Publications

4.1 Journals

Shimanuki, M., Chung, S. Y., Chikashige, Y., Kawasaki, Y., Uehara, L., Tsutsumi, C., Hatanaka, M., Hiraoka, Y., Nagao, K., Yanagida, M. (2007) Two-step, extensive alterations in the transcriptome from G0 arrest to cell division in *Schizosaccharomyces pombe*, Genes Cells, 12(5), 677-692 .

Hayashi, T., Hatanaka, M., Nagao, K., Nakaseko, Y., Kanoh, J., Kokubu, A., Ebe, M., Yanagida, M. (2007) Rapamycin sensitivity of the *Schizosaccharomyces pombe tor2* mutant and organization of two highly phosphorylated TOR complexes by specific and common subunits, Genes Cells, 12(12), 1357-1370.

Kanoh, J., Yanagida, M. (2007) Tel2: a common partner of PIK-related kinases and a link between DNA checkpoint and nutritional response?, Genes Cells, 12(12), 1301-1304.

Adachi, Y., Kokubu, A., Ebe, M., Nagao, K., Yanagida, M. (2008) Cut1/separase-dependent roles of multiple phosphorylation of fission yeast cohesin subunit Rad21 in post-replicative damage repair and mitosis, Cell Cycle, 7(6), 765-776.

Nakazawa, N., Nakamura, T., Kokubu, A., Ebe, M., Nagao, K., Yanagida, M. (2008) Dissection of the essential steps for condensin accumulation at kinetochores and rDNAs during fission yeast mitosis, J. Cell. Biol, 180(6), 1115-1131

4.2 Book(s) and other one-time publications

Shimanuki, M., Yanagida, M. (2008) Cellular strategy for G0 Arrest and Vegetative Proliferation in Fission Yeast, Cell Technology, 27, 260-265, (In japanease).

4.3 Oral presentations

Nagao, K. Strategy for Semi-quantitative Analysis in Mass Spectrometry-based Proteomics, Thermo Fisher Scientific Life Science Seminar 2007, Tokyo, Japan, May 18, 2007.

Yanagida, M. Non-dividing pombe enlightened in a tropical island, "Fourth International Fission Yeast, Meeting, Copenhagen, Fourth International Fission Yeast, Meeting, Copenhagen", Jun. 11-16, 2007.

Yanagida, M. Centromere/kinetochore mechanism for Cenp-A recruitment and spindle checkpoint protein association, 7th Chromosomen Segregation and Aneuploidy Workshop, Naantali, Finland, Jun. 16-20, 2007.

Shimanuki, M. A search for candidate regulators of G0 phase maintenance in fission yeast, Seminor at Inst. Life Sci, Kurume Univ, Kurume, Japan, July 26, 2007.

Shimanuki, M., Chung, S-Y., Chikashige, Y., Kawasaki, Y., Uehara, L., Tsutsumi, C., Hatanaka, M., Hiraoka, Y., Nagao, K, Yanagida, M. A search for candidate regulators of G0 phase maintenance, The 40th Forum on Yeast Genetics and Molecular Biology, Osaka, Japan, Sep. 11-13, 2007.

Yanagida, M. Pursuing Gene Networking Required for Entering, Maintaining and Exiting from the G0 Cell State of *S. pombe*, Cellular responses to DNA damage in *Schizosaccharomyces pombe*, Oslo, Oct. 1-4, 2007.

Yanagida, M. Essential centromere/kinetochore architecture for CENP-A loading and mitotic checkpoint, EMBO Workshop, Molecular Mechanisms of Cell Cycle Control in Nomal and Malignant Cells, Spetses Island, Greece, Oct. 5-8, 2007.

Nagao, K., Kokubu, A., Yanagida, M. Proteomic Analysis of Fission Yeast Cells at G0-Vegetative Phase Transition, The 30th Annual Meeting of the Molecular Biology Society of Japan, Yokohama, Dec. 11-15, 2007.

Shimanuki, M., Chung, S-Y., Chikashige, Y., Kawasaki, Y., Uehara, L., Tsutsumi, C., Hatanaka, M., Hiraoka, Y., Nagao, K, Yanagida, M. From transcriptomic analysis to isolation of mutans defective in maintenance the G0 state in fission yeast, The 3rd International Workshop on Cell Regulations in Division and Arrest under Stress, Okinawa, Japan, April 6-10, 2008.

Yanagida, M. Proliferating and non-proliferating yeast, National bioresource project symposium, Tokyo, Japan, March 10, 2008.

Yanagida, M. Comprehensive approach for understanding the network of genes required for G0 cell maintenance, The Workshop on transcriptions/signal networks of lifestyle-diseases, Kamakura, Japan, March 22, 2008.

Yanagida, M. The role of TOR complexes in the fission yeast *S. pombe* vegetative and G0 phase, The 3rd International Workshop on Cell Regulations in Division and Arrest under Stress, Okinawa, Japan, April 6-10, 2008.

4.4 Posters

Nagao, K.Obuse, C., Kokubu, A., Yanagida, M. Proteomic Analysis of Fission Yeast Cells at G0-Vegetative Phase Transition, Forth International Fission Yeast Meeting, Copenhagen, Jun. 11-16, 2007.

Mori, A., Nagao, K., Yoshida, T., Yanagida, M. A screening for drugs that inhibit the maintenance of *S. pombe* G0 state, Forth International Fission Yeast Meeting, Copenhagen, Jun. 11-16, 2007.

Sajiki, K., Yanagida, M. A search for essential genes in G0 by examining a collection of temperature sensitive mutants under the vegetative condition, Forth International Fission Yeast Meeting, Copenhagen, Jun. 11-16, 2007.

Shimanuki, M., Chung, S-Y., Chikashige, Y., Kawasaki, Y., Uehara, L., Tsutsumi, C., Hatanaka, M., Hiraoka, Y., Yanagida, M. A search for candidate regulators of Fission Yeast G0 phase: From transcriptome analysi, Forth International Fission Yeast Meeting, Copenhagen, Jun. 11-16, 2007.

Hayashi, T., Hatanaka, M., Nagao, K., Nakaseko, Y., Kokubu, A.Ebe, M., Yanagida, M. Proteomic Comparison between *S. pombe* G0 Cell and Rapamycin-Sensitive TORC1 Mutant Reveal Aimilar but Distinct Nitrogen Metabolic Regulations, Forth International Fission Yeast Meeting, Copenhagen, Jun. 11-16, 2007.

Sajiki, K. Yanagida, M. A search for genes regulating G0 phase: screening of a temperature sensitive mutant library, The 40th Forum on Yeast Genetics and Molecular Biology, Osaka, Japan, Sep. 11-13, 2007, (in Japanese).

Takeda, K., Kikuchi, S., Nagao, K., Kokubu, A., Yoshida, T., Yanagida, M. The essential role of the fission yeast ubiquitin/proteasome pathway on sustaining G0 cells, The 40th Forum on Yeast Genetics and Molecular Biology, Osaka, Japan, Sep. 11-13, 2007, (in Japanese).

Shimanuki, M., Chung, S-Y., Chikashige, Y., Kawasaki, Y., Uehara, L., Tsutsumi, C., Hatanaka, M., Hiraoka, Y., Yanagida, M. A search for essential genes in G0 by examining a collection of temperature sensitive mutants under the vegetative condition, The 30th Annual Meeting of the Molecular Biology Society of Japan, Yokohama, Dec. 11-15, 2007, (in Japanese).

Takeda, K., Kikuchi, S., Nagao, K., Kokubu, A., Yoshida, T., Yanagida, M., Yanagida, M. The ubiquitin/proteasome pathway is essential for maintainance of the fission yeast G0 phase, The 30th Annual Meeting of the Molecular Biology Society of Japan, Yokohama, Dec. 11-15, 2007, (in Japanese).

Nagao, K.Kokubu, A., Yanagida, M. Quantitative proteomic comparison between proliferating and non-proliferating cells in fission yeast, The 3rd International Workshop on Cell Regulation in Division and Arrest under Stress, Okinawa, Japan, April 6-10, 2008.

Nakamura, T., Nakaseko, Y., Pluskal, T., Nagao, K., Yanagida, M. Enzymes for CoA biogenesis are required for accurate chromosome segregation and histone acetylation in *S. pombe*, The 3rd International Workshop on Cell Regulation in Division and Arrest under Stress, Okinawa, Japan, April 6-10, 2008.

Pluskal, T., Nagao, K., Nakamura, T., Yanagida, M. Metabolomic comparison of vegetative and G0 cells of *S. pombe*, The 3rd International Workshop on Cell Regulation in Division and Arrest under Stress, Okinawa, Japan, April 6-10, 2008.

Sajiki, K., Hatanaka, M., Nakamura, T., Nakaseko, Y., Hayashi, T., Yanagida, M. Identification of mutants defective in G0 maintenance and entry, The 3rd International Workshop on Cell Regulation in Division and Arrest under Stress, Okinawa, Japan, April 6-10, 2008.

Takeda, K., Kikuchi, S., Nagao, K., Kokubu, A., Yanagida, M. Inactivation of ubiquitin/proteasome system causes mitochondrial dysfunction and cell death in fission yeast G0 phase, The 3rd International Workshop on Cell Regulation in Division and Arrest under Stress, Okinawa, Japan, April 6-10, 2008.

5. Intellectual Property Rights and Other Specific Achievements

Nothing to be reported.

6. Meetings and Events

6.1 OIST WorkshopInternational Workshop on Cell Regulations in Division and Arrest

Date: March 25th-29th, 2007

Venue: OIST Seaside House, Onna, Okinawa.

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)
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)

6.2 OIST seminar

Cell cycle regulation of organelle biogenesis in fission yeast: the role of the cytoskeleton and dynamin-related proteins

Date: November 14, 2007

Venue: IRP, OIST

Speakers: Jeremy Hyams (Institute of Molecular Biosciences, Massey University)

VII. Unit for Molecular Neurobiology of Learning and Memory

Principal Investigator: Shogo Endo
Research Theme: Molecular and genetic dissection of learning and memory

Abstract

The brain, organ of memory and cognition, performs as a result of the fine-tuned molecular mechanisms in neuronal cells. These 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 memory. Memory provides the basis for other higher brain functions such as thought, language and emotion. We have studied the molecular and cellular aspects of memory using the techniques of biochemistry, molecular biology and gene targeting. We continue to investigate the biochemical and cellular aspects of neuronal plasticity and memory as a model of brain function to reveal the intimate machinery of the brain.

1. Staff

Researchers: Gilyana Borlikova, Ph.D.
Jayne Nicole Rafferty, Ph.D.
Toshiro Sakamoto, Ph.D.
Réjan Vigot, Ph.D.
Technical staff: Masako Suzuki, M.Sc. (Laboratory Manager)
Michiko Arai, B.Sc.
Tomoko Arasaki, M.Sc.
Ms Nana Noumi
Mika Takiguchi, M.Sc.
Graduate student: Yukiko Uechi, M.Sc.
Research administrator/secretary: Ms Shoko Yamakawa

2. Partner Organizations

National Defense Medical College
Type of partnership: Collaborative Research
Name of principal researcher: Dr. Kunio Takishima
Name of researcher: Dr. Yasushi Satoh
Research theme: Generation of the mice with modified ERK2 gene.

RIKEN Brain Science Institute
Type of partnership: Collaborative Research
Name of principal researcher: Dr. Masao Ito
Research theme: Electrophysiological examination of genetically modified mice.

RIKEN Brain Science Institute
Type of partnership: Collaborative Research
Name of principal researcher: Dr. Soichi Nagao
Research theme: Behavioral examination of genetically modified mice.

University of the Ryukyus Graduate School of Medicine
Type of partnership: Collaborative Research
Name of principal researcher: Dr. Ken-ichi Kariya
Research theme: Genetic analysis of cancer-related genes.

Shinshu University Graduate School of Medicine
Type of partnership: Collaborative Research
Name of principal researcher: Dr. Tatsuo Suzuki
Research theme: Comprehensive characterization of mRNAs localized in synapse.

Harvard Medical School
Type of partnership: Collaborative Research
Name of principal researcher: Dr. Ole Isacson
Research theme: Roles of cGMP-PKG pathway in dopaminergic neurons.

3. Activities and Findings

The signal transduction cascade involved in neuronal plasticity and memory can be divided into two categories, early phase and long-lasting / late phase (The latter of which requires synthesis of new proteins). We investigate the molecular mechanism underlying neuronal plasticity, learning and memory using a variety of methods including biochemistry, molecular biology, behavioral analysis and gene manipulation technology.

Memory can be observed only by the observation of the behavior of animals. Although the neuronal plasticity is believed to be the cellular basis of the memory, memory itself requires the neuronal network based on the neuronal cell interaction, the brain and the whole body. We chose mice as an intact animal model for the study on memory. Gene manipulation of mice is well established and it is possible to generate gene-deficient mice and transgenic mice in our lab. We utilized the gene-modified mice to examine the molecular mechanisms underlying learning and memory.

3.1 G-substrate and neuroprotection

G-substrate was identified as a substrate of PKG by Paul Greengard's group in the early 1980s. Since then, efforts to clone G-substrate cDNA have been unsuccessful despite numerous efforts. We successfully cloned rat, mouse and human G-substrate cDNA. We have also generated a variety of analysis tools e.g. cDNA probes, antibodies and G-substrate mutant mice. We have also identified the protein phosphatase inhibitory activity of G-substrate upon phosphorylation of the protein by PKG. The shuttling of G-substrate between the nucleus and cytosol in Purkinje cells may also suggest roles of G-substrate in both compartments.

G-substrate is localized in restricted cells within the retina (Nakazawa et al., 2007) and substantia nigra (Chung et al., 2007) as well as its high expression in cerebellar Purkinje cells. In both retinal amacrine cells and substantia nigra A10 cells, G-substrate plays a role in neuroprotection against NMDA-induced injury (Ca^{2+}-induced injury). The retina project is being carried out in collaboration with Dr. Nakazawa in the Department of Ophthalmology, Tohoku University Medical School while the substantia nigra project is being carried out in collaboration with Dr. Isacson at Harvard Medical School.

Unlike retinal amacrine cells and substantia nigra A10 cells, cerebellar Purkinje cells lack NMDA receptors. However, Purkinje cells are highly susceptible to hyperexcitation and other types of toxicity. G-substrate in Purkinje cells may play a role in both neuroprotection and in motor learning.

3.2 ERK2 controls spatial memory

Extracellular signal-regulated kinases (ERKs) are members of the mitogen-activated protein kinase (MAPK) superfamily of enzymes and form a major signal transduction pathway mediating extracellular stimuli to the nucleus. MAPK and cAMP systems are intensively studied in long-term memory (LTM) of various organisms. Originally identified as regulators of cell division and differentiation, the ERK signaling pathway has also been reported to play a major role in synaptic plasticity and learning and memory, e.g. ERK activation is required for the induction of hippocampal long-term potentiation (LTP). However, the efforts to reveal the physiological role of ERKs has not been addressed using genetically modified mice. We started this project with the generation of the mice. This work has been carried out in the collaboration with Drs Takishima and Satoh of National Defense Medical College.

To study the function of ERK2 we used gene targeting to generate a series of ERK2 alleles (Fig. 1). ERK2 protein expression in floxN/floxN mice is reduced to 60-80% of that in wild-type mice. The availability of viable and fertile ERK2 hypomorphic mutant mice (floxN/floxN) allowed us to examine how reduced ERKs affect behavior. Examination of brain structures using light microscopy revealed that reduced ERKs did not affect cerebellar lobules, cell layer structure, or spine morphology and numbers.

ERK2 knockdown (KD) mice showed impairment in contextual fear memory when measured 48 h after training, even though they appeared normal when measured 2 h after training. This suggests that LTM was selectively impaired in these mice (Fig. 2). Mutant mice also showed impaired learning in a 4/8-arm radial maze task. In addition, slower learning was observed in Morris water maze (Fig. 3) and an 8/8-arm radial maze tasks. Given that ERK1 expression in the mutants was not altered, our results indicate that ERK2, not ERK1, plays an essential role in LTM formation. This may suggest a complicated and idiosyncratic involvement of ERK isoforms in the cellular mechanisms that underlie learning and memory.

Even though we showed that ERK2-KD mice clearly have impaired LTM, more convincing results can be obtained with knockout mice. However, null ERK2 gene knockout leads to an embryonically lethal phenotype. To overcome the situation, we have developed a system to conditionally knock out the gene. With this approach, we have established floxed ERK2 mice, which enable us to generate conditional ERK2 knockout mice with the combination of mice expressing Cre DNA recombinase (see Fig. 1, flox(f) and null(**Δ**)). Along with ERK2-KD, conditional ERK2-KO mice will make it possible for us to decipher the physiological role of ERK2 in the nervous system.

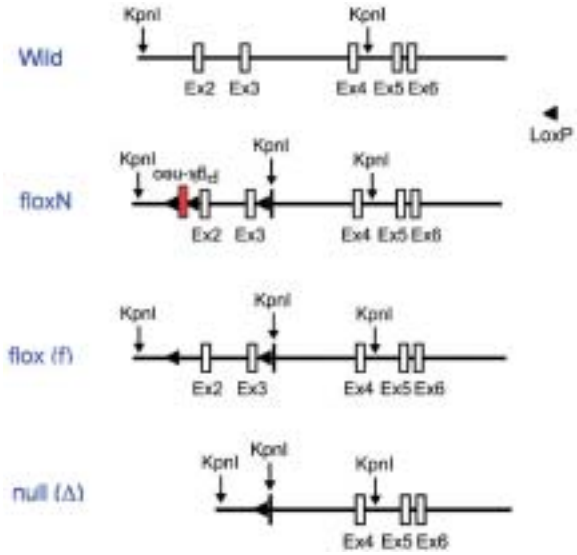


Figure 1. Schematic diagram of targeted knockdown of the mouse Erk2 gene. The initiation codon (Met) is in Ex2. The floxN allele contains 3 loxP sequences and a Pgk-neo cassette selection marker. These insertions reduce ERK2 protein expression in floxN/wild and with floxN/floxN mice to ~ 80% and ~60%, respectively, of that in mice harboring the wild-type allele. The floxN allele is obtained during the course of generating the flox (f) allele, as shown above. The null(**Δ**) allele is obtained by removing Exon 2 and Exon 3 flanked by 2 loxP sequences with Cre DNA recombinase. Finally, to obtain mice that lack ERK2 in specific cells, we will cross mice harboring the flox(f) allele with mice expressing Cre DNA recombinase in specific target cells. Triangles indicate the locations of loxP sequence.

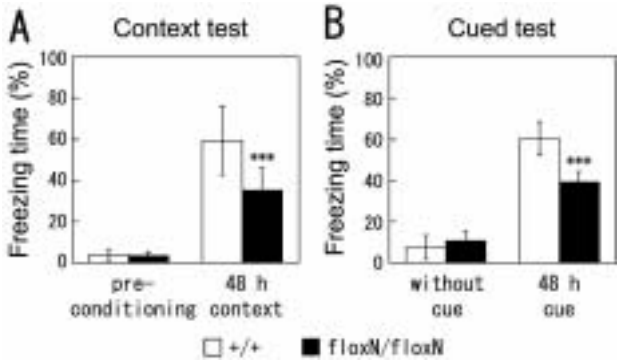


Figure 2. ERK2 knockdown mice showed impaired memory performance in both context and cued tests compared to wild-type littermates.

A, Freezing response was measured in the context prior to shock (basal freezing) and in the conditioning chamber (contextual fear response) 2 h after conditioning ($ERK2^{+/+}$, n= 12; $ERK2^{floxN/floxN}$, n= 12). Freezing response was measured in the context prior to shock (basal freezing) and in the conditioning chamber (contextual fear response) 48 hours after conditioning ($ERK2^{+/+}$, n= 12; $ERK2^{floxN/floxN}$, n= 12).

B, Freezing response (for the same set of mice) was measured in an alternative context without auditory cue (basal freezing) or with a cue 48 hours after conditioning.

For all figures, asterisks represent statistically significant differences (***: $p < 0.001$) in t-test.

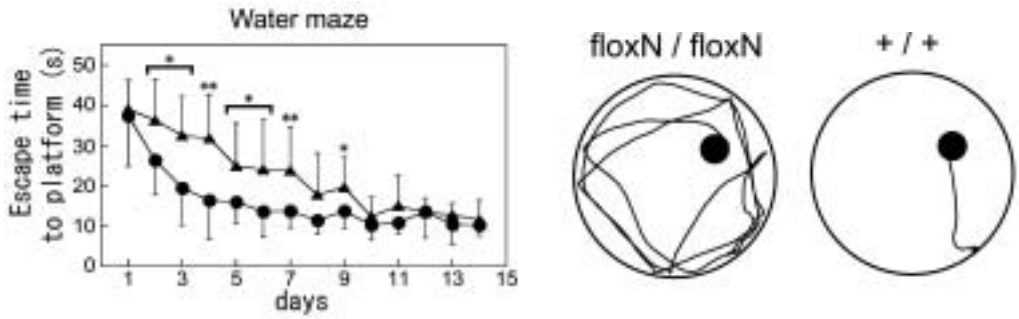


Figure 3. ERK2 knockdown mice showed partially impaired learning in the Morris water maze compared to wild-type littermates. Left panel, ERK2 knockdown mice (filled triangles) showed longer escape latencies from day 2 to day 7, and day 9, but the difference diminished after day 10 compared to wild-type littermates (filled circles). $ERK2^{+/+}$, n=12; $ERK2^{floxN/floxN}$, n=12. Asterisks represent statistically significant differences (*: $p < 0.05$; **: $p < 0.01$) in t-test. Right panel, Representative movement traces of an ERK2 knockdown mouse and a wild-type mouse on day 5. Black circles represent the platform.

3.3 ICER and long-term memory

Long-lasting neuronal plasticity as well as LTM is an event that requires new protein synthesis through nuclear transcriptional activation. The importance of gene regulation through CRE-mediated transcription is well established for long-term memory in a variety of animals including *Aplysia*, *Drosophila*, *C. elegans*, and vertebrates. A strong connection between CREB and neuronal plasticity underlying learning and memory has been demonstrated. In mammals, the outcome of CRE-mediated gene transcription depends on the competitive binding of CREB and CREB competitors. These factors are products of the CREB/CRE modulator (CREM)/activating transcription factor 1 (ATF-1) gene family. Despite the accumulated evidence, the role of CREM-related inducible genes, such as ICER, on neuronal plasticity and memory consolidation remains unknown.

Although basal ICER levels in the brain are relatively low, dramatic upregulation of ICER has been demonstrated in responses such as neuronal excitation. This suggests that ICER may have a role in the regulation of higher-order brain functions. We observed that ICER levels in the brain increased transiently after kindled seizures and after fear conditioning (Fig. 4).

ICER is a member of cAMP response element-binding transcription repressor family. To examine the role of ICER in LTM and kindling, we generated two lines of ICER mutant mice: ICER-overexpressing (OE) and ICER knockout (KO) mice (Figs. 5 and 6). Comprehensive behavioral test battery revealed no robust changes in locomotor activity, sensory and motor functions and emotional responses in these mice. Currently, intensive investigation of the ICER-OE and-KO mice is being carried out with interest mainly in long-term memory, kindling and neuronal plasticities.

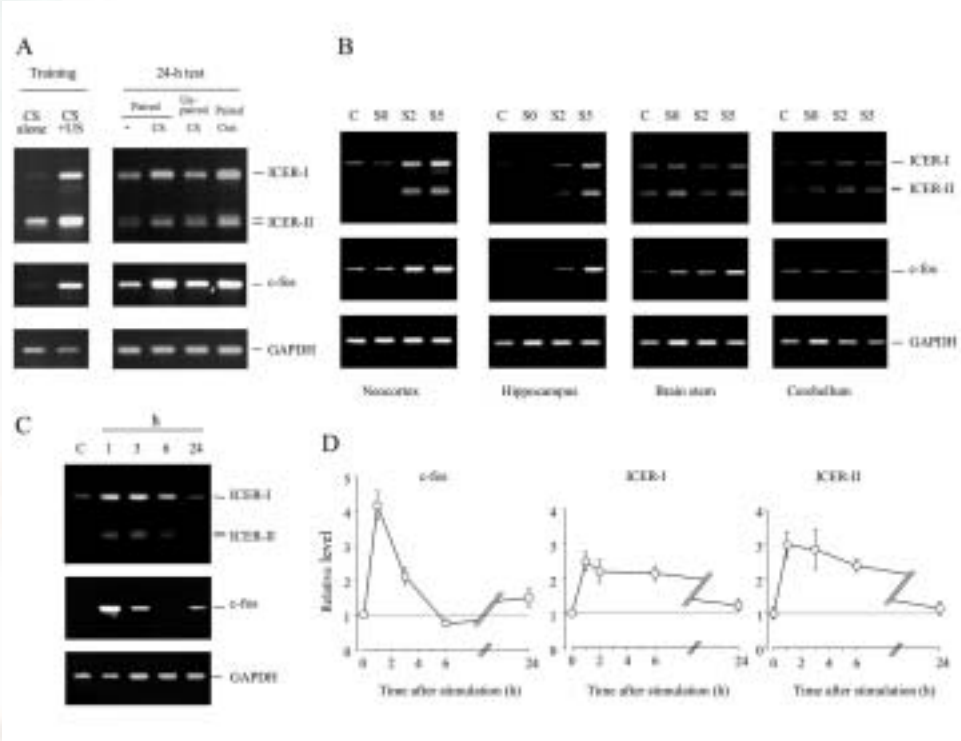


Figure 4. Upregulation of ICER mRNAs in the brain after fear conditioning and after kindling stimulation. Changes in the mRNA level of ICER, c-fos and GAPDH were examined by semi quantitative RT-PCR. **A**, ICER and c-fos mRNAs were detected in the amygdala 3 h after conditioning (training) and after 24-h retention test (24-h test). Increased levels of ICER and c-fos were observed after training in mice that received pairing of CS and US (CS+US) as compared with mice that received CS without US (CS alone). After 24-h tone retention test, increase in the levels of ICER and c-fos mRNAs in the conditioned mice (Paired/CS) was higher than that in conditioned mice that were not presented with the CS during test (Paired/-) and in the pseudo conditioned mice (Unpaired/CS). ICER and c-fos mRNAs were also increased after context-dependent test (Paired/Ctxt). **B**, ICER and c-fos mRNAs were detected 3 h after amygdala stimulation in the neocortex, hippocampus, brain stem and cerebellum of mice showing no convulsion (S0), head nodding (S2) and fully generalized seizure (S5) after the stimulation. Electrode-implanted but non-stimulated mouse was used as a control (C). The increased level of ICER and c-fos mRNAs in the neocortex and hippocampus were in parallel with the severity of convulsion. Upregulation of ICER and c-fos mRNAs was not obviously observed in the brain stem and cerebellum. **C**, ICER and c-fos mRNAs were detected in the forebrain of fully kindled mice at 1, 3, 6 and 24 hours after the stimulation. Non-stimulated kindled mice were used as control (C). Signal intensity in each sample was normalized with that of the non-stimulated control, and presented as a mean±SEM from 3-4 independent samples. ICER-I and II mRNAs were increased 1 to 3 h after kindling stimulation then declined to the baseline by 24 h after stimulation. **D**, Time course of a decline of ICER mRNAs was slower than that of c-fos mRNA.

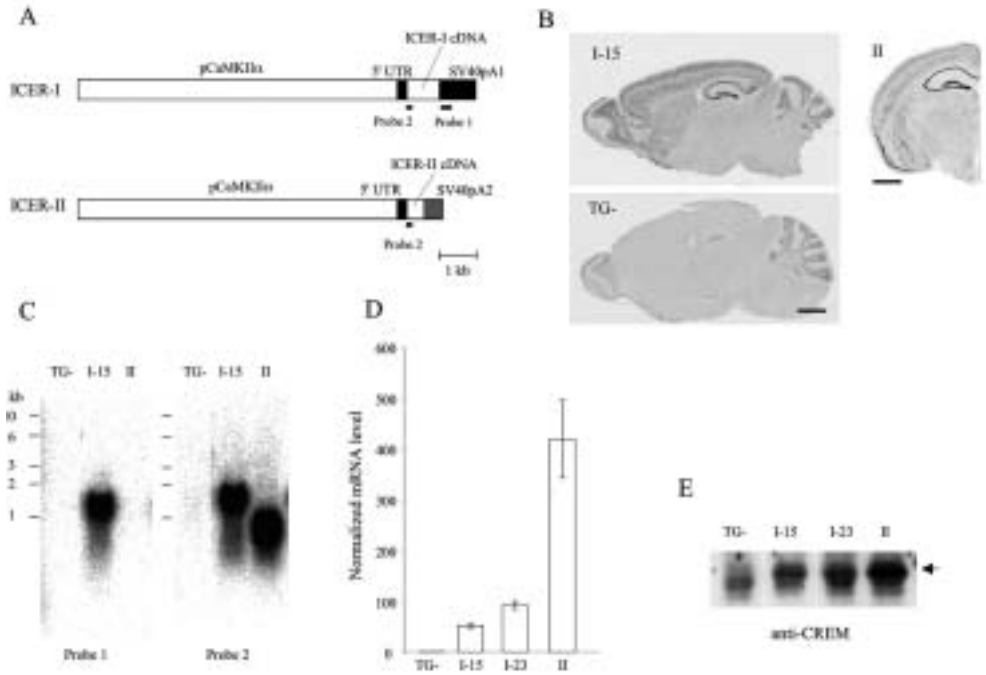


Figure 5. Characterization of ICER-OE mice. **A**, Transgene constructs for ICER-transgenic mice generation were composed of a promoter region of Ca^{2+}/calmodulin-dependent protein kinase IIα gene (pCaMKIIα), 5'-untranslated region (5'-UTR) derived from pNN265, 3'-untranslated region containing poly(A)$^+$signal sequence (SV40pA1 or SV40pA2) and either ICER-I or ICER-II cDNA. The probes for detecting transgenic ICER mRNAs are indicated by thick lines (Probe 1 and Probe 2). **B**, Expression of transgenic ICER mRNA in mouse brain. Sagittal sections from an ICER-I-overexpressing (OE) mouse (line I-15) and its non-transgenic (TG−) littermate were hybridized with digoxigenin-labeled cRNA Probe 1. The transgene-derived ICER-I mRNA was expressed preferentially in forebrain structures (scale bars, 500 μm). A coronal section from an ICER-II-OE mouse was hybridized with digoxigenin-labeled cRNA Probe 2. ICER mRNA was highly expressed in layers II-III and V-VI of neocortex, hippocampus, piriform cortex, and amygdala (scale bars, 500 μm). **C**, Northern blot analysis of total RNA prepared from the forebrain of TG− and ICER-OE mice (lines I-15 and II). ^{32}P-labeled Probe 1 hybridized to ICER-I-OE mRNA (left panel: I-15) and ^{32}P-labeled Probe 2 hybridized to both ICER-I and ICER-II-OE mRNAs (right panel: I-15 and II). Endogenous ICER mRNA in TG− mice was below the detection limit of Probe 2 under the condition used (TG−). **D**, Quantitative RT-PCR detection of ICER mRNA in the hippocampus of each transgenic line. Each value was calculated from 4-6 independent samples and is presented as mean±SEM of mRNA levels normalized to mRNA levels of TG− mice. **E**, Western blot analysis of protein lysates prepared from the forebrains of TG− and ICER-OE mice (lines I-15, I-23, and II); blots were probed with anti-CREM antibody. An arrow indicates the 19-kDa band corresponding to ICER protein.

Neural Computation Unit
G0 Cell Unit
Unit for Molecular Neurobiology of Learning and Memory
Electron Microscopy Unit
Molecular Genetic Unit
Information Processing Biology Unit
Molecular Neurobiology Unit
Mathematical Biology Unit
Developmental Neurobiology Unit
Theoretical and Experimental Neurobiology Unit
Neurobiology Research Unit
Human Developmental Neurobiology Unit
Brain Mechanisms for Behaviour Unit
Computational Neuroscience Unit
Trans-Membrane Trafficking Unit
Cellular and Molecular Synaptic Function Unit
Developmental Signalling Unit
Education and Training Activities

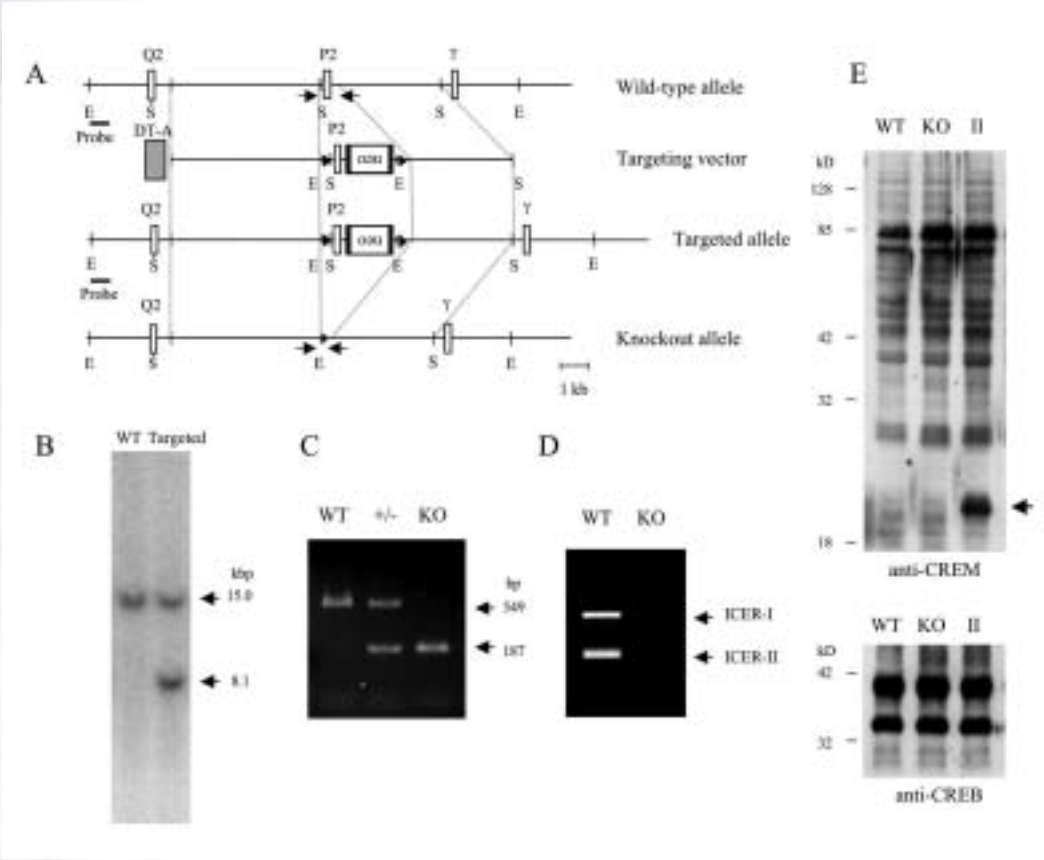


Figure 6. Generation of ICER-KO mice.

A, For construction of the targeting vector, two loxP sequences (arrowheads) were inserted into the 5'- and 3'-flanking regions of the P2 exon of the CREM gene. For negative and positive selection of recombinant ES clones, the PGK-neo cassette (neo) flanked by frt sequences and the diphtheria toxin A gene (DT-A), respectively, were inserted into the targeting vector. The P2 exon flanked by two loxP sequences was deleted by microinjecting a Cre DNA recombinase-expressing vector into the fertilized eggs carrying the targeted genomic allele. A thick line indicates the location of the probe for Southern blot analysis. Restriction sites: E, EcoRI; S, SacI.

B, Southern blot analysis of genomic DNA prepared from wild-type (WT) and recombinant (Targeted) ES cells. ^{32}P-labeled DNA probe detected a single 15.0 kb-EcoRI fragment in WT cells and an additional 8.1 kb-fragment in the Targeted cells.

C, PCR-based genotyping of ICER-KO mice; the primer pair (arrows) specific for the P2 exon distinguished WT, heterozygous (+/-), and homozygous (KO) mice.

D, PCR products produced from using an ICER-specific primer-pair were amplified from brain cDNA prepared from the RNA of WT mice but not from that of KO mice.

E, Western blot analysis of lysates prepared from hippocampi of WT, KO, and II mice. Blots were probed with either anti-CREM or anti-CREB antibody. We detected no apparent difference in CREM isoform and CREB expression levels in all groups of mice. An arrow indicates the additional band corresponding to ICER protein in ICER-II-OE mice.

3.4 Comprehensive identification of mRNAs in the post-synaptic density

Considerable evidence suggests that the formation of long-term memories requires a critical period of new protein synthesis. Recently, the notion that some of these newly synthesized proteins originate through local translation in neuronal dendrites has gained attention (for review, see Sutton and Schuman, 2006). Locally synthesized proteins may; contribute to enhanced signal transmission through an increased number of glutamate receptors; induce morphological changes of spines mediated through cytoskeletal proteins, or aid in the insertion of newly synthesized proteins (a "tag") in the synapse for reception of proteins synthesized in the soma (for review, see Reymann and Frey, 2007). A combination of these events is hypothesized to control neuronal plasticity and possibly memory. However, the identity of the mRNA species present in the post-synaptic region remains elusive.

It is very useful to know and analyze the set of proteins transcribed at the synapse rather than the 30,000 proteins expressed in the whole cell in order to reveal neuronal function in learning and memory. Thus we conducted a comprehensive identification of mRNA species in the post-synaptic density (PSD). We identified approximately 2,000 species of mRNAs enriched in the PSD fraction (Suzuki et al., 2007), 777 of which were determined to specifically localize at the synapse (see the list of mRNAs in Suzuki et al., 2007). We have also characterized some of these novel mRNAs and gene products (Tian et al., 2003; Wang et al., 2005; Jin-Ping et al., 2005; Suzuki et al., 2005; Lu et al., 2005; Tian et al., 2006). Furthermore, we are currently generating genetically modified mice for several genes whose mRNAs are located in PSD. The comprehensive identification of mRNA in the PSD was carried out in collaboration with Dr. Tatsuo Suzuki of Shinshu University.

The cDNA library for mRNA present in the PSD represents a valuable asset for the field of neuronal plasticity and memory. The library can be used to reveal the molecular network initiated by synaptic activation. The cDNA library is also essential for identifying memory-related molecules in genetically modified mice that have deficits in long-term memory, G-substrate KO mice and ERK2 knockdown mice. These mice, in combination with the cDNA library are powerful tools that will aid the advance of our research.

4. Publications

4.1 Journals

Suzuki T., Tian QB., Kuromitsu J., Kawai T., Endo S. (2007) Characterization of mRNA species that are associated with postsynaptic density fraction by gene chip microarray analysis. Neurosci. Res, 57(1), 61-85, (in English).

Maruyama E., Ogawa K., Endo S., Tsujimoto M., Hashikawa T., Nabetani T., Tsugita A. (2007) Brain-derived neurotrophic factor induces cell surface expression of short-form tenascin R complex in hippocampal presynapses, Int. J. Biochem. Cell. Biol., 39(10), 1930-1942, (in English).

Chung CY., Koprich JB., Endo S., Isacson O. (2007) An endogenous Ser/Thr protein phosphatase inhibitor, G-substrate, reduces vulnerability in models of Parkinson's disease. J. Neurosci, 27(31), 8314-8323, (in English).

Satoh Y., Endo S., Ikeda T., Yamada K., Ito M., Kuroki M., Hiramoto T., Imamura O., Kobayashi Y., Watanabe Y., Itohara S., Takishima K. (2007) ERK2 knockdown mice show deficit in long-term memory; ERK2 has a specific function in learning and memory. J. Neurosci, 27(40), 10765-10776, (in English).

Lu Y., Tian QB., Endo S., Suzuki T. (2007) A role for LRP4 in neuronal cell viability is related to apoE-binding, Brain Res, 1177, 19-28, (in English).

Nakazawa T., Shimura M., Ryu M., Nishida K. Pagès G., Pouysségur J., Endo S. (2008) ERK1 plays a critical protective role against N-methyl-D-aspartate-induced retinal injury. J. Neurosci. Res, 86(1), 136-144, (in English).

Suzuki T., Du F., Tian QB., Zhang J., Endo S. (2008) Ca^{2+}/calmodulin-dependent protein kinase IIα clusters are associated with stable lipid rafts and their formation traps PSD-95, J. Neurochem, 104(3), 596-610, (in English).

4.2 Book(s) and other one-time publications

Endo S. (2007) Japanese Journal of Pathophysiology, Japanese Society of Pathophysiology, 16, 32-35.

Sakamoto T. (2008) Comparative studies of hippocampal functions, Nakanishiya Publishers, pp119-133.

4.3 Oral presentations

Sakamoto T., Endo S. GABA receptors in the deep cerebellar nuclei are essential for mouse eyeblink conditioning, Cold Spring Harbor Laboratory Meeting; Synapses: From Molecules to Circuits & Behavior, New York, USA, April 18-22, 2007.

Endo S. Introduction to Brain Science, Miyako High School, Okinawa, Japan, November 16, 2007.

Endo S. Genetic and Molecular Dissection of Memory, 2nd KNU-OIST Workshop 2008, Deagu, Korea, March 13, 2008.

4.4 Posters

Borlikova G., Arai M., Kojima N., Endo S. Overexpression of ICER, endogenous antagonist of CREB, impairs long-term context-dependent fear memory, 7th IBRO World Congress of Neuroscience, Melbourne, Australia, July 12-17, 2007, (in English).

Borlikova G., Arai M., Kojima N., Endo S. Comprehensive behavioral characterization of mice lacking ICER, an endogenous antagonist of CREB, The 30th Annual Meeting of the Japan Neuroscience Society, Yokohama, Japan, September 10-12, 2007, (in English).

Arai M., Borlikova G., Kojima N., Endo S. Overexpression of ICER, and endogenous antagonist of CREB, impairs fear memory, The 30th Annual Meeting of the Japan Neuroscience Society, Yokohama, Japan, September 10-12, 2007, (in English).

Sakamoto T., Arasaki T., Endo S. GABA receptors play roles in acquisition and retention of mouse eyeblink conditioning in the cerebellar nuclei, The 30th Annual Meeting of the Japan Neuroscience Society, Yokohama, Japan, September 10-12, 2007, (in English).

Sakamoto T., Arasaki T., Endo S. GABA receptors in the deep cerebellar nuclei play an important role in acquisition and retention of mouse eyeblink conditionig, The 37th Annual Meeting of Society of Neuroscience, San Diego, USA, November 3-7, 2007, (in English).

Imamura O., Sato K., Endo S., Takishima I. Functions of ERK2 in neuronal stem cells using conditional ERK2 knockout mice, BMB 2007, Yokohama, Japan, December 11-15, 2007, (in English).

Uechi Y., Umikawa M., Suzuki M., Endo S., Kariya K. Generation and characterization of specific antibodies for small G-proteins, Rap2A, 2B and 2C, BMB 2007, Yokohama, Japan December 11-15, 2007, (in English).

Kato K., Miyamoto K., Kuwamura M., Okada T., Osuka S., Itohara S., Endo S., Hirabayashi Y. Characterization of neuronal function in ST3Gal IV knockout mice, BMB 2007, Yokohama, Japan, December 11-15, 2007, (in English).

5. Intellectual Property Rights and Other Specific Achievements

Endo S. Non-human animal model with Erk2 gene knockdown (Japan Patent pending 2007-258186)

Keiko K, Hirabayashi Y, and Endo S. Non-human animal model with ST3galIV gene deletion (Japan Patent, under processing)

Endo S. Non-human animal model with ICER gene deletion and overexpression (Japan Patent, under processing)

6. Meetings and Events

6.1 OIST-Korea Workshop “Neuroscience and Beyond”

Date: February 21-23, 2007

Venue: OIST Seaside House

Co-organizers: Dr. Hee Kyung Jin (Kyungpook National University, Korea)

Speakers: OIST- Drs Doya, Endo, Maruyama, Masai, Naito, Sinclair, Stiefel, Tripp, Wickens and 3 Post-docs

University of the Ryukyus- Drs. Umikawa, Yamamoto, Yanagi

Kyungpook National University (Korea)- Drs. Bae, Han, Jin, Moon, Suk and 2 graduate students

Seoul National University- Drs. Lee C., Lee S.-H. and a graduate student

KNU-OIST Workshop 2008 "Stem Cell Biology and Neuroscience"

Date: March 13, 2008

Venue: Kyungpook National University, Daegu, Korea

Co-organizers: Dr. Hee Kyung Jin, and Jae-sung Bae (Kyungpook National University, Korea)

Speakers: OIST- Drs Doya, Endo, Price, Wickens
Kyungpook National University (Korea)- Drs. Bae, Jin, Kim.
Seoul National University- Drs. Lee C., Lee S.- H., Bong- Kiun Kaang
POSTEC- Dr. Suh
Korea University- Dr. Baik
Catholic University (Korea)- Dr. Oh
Yonsei University- Dr. Kim
Mount Sinai School of Medicine- Dr. Schuchman

VIII. Electron Microscopy Unit

Principal Investigator: Akira Tonomura

Research Theme: Holography Electron Microscopy Dedicated to Nanotechnology

Abstract

The aim of this project is to understand microscopic behaviors of materials through high-precision electron phase measurement using our coherent electron wave techniques. Achievements of this year have been made mainly for nanoscale magnetism research using our new microscope that has become available this fiscal year. The accomplishment includes:

1. Observation of magnetic domain structures in a diluted magnetic semiconductor (Ga, Mn)As. The analysis of flux profile across domain walls using 10 nm resolution electron holography provided new evidence for generation of hole-mediated ferromagnetism.

2. Observation of magnetic flux closure in circularly-chained nickel nanoparticles. An interaction of a thermal fluctuation of magnetic moments in each individual nanoparticle and dipole magnetic coupling with neighboring particles was analyzed by precise measurement of the magnitude of circulating magnetic flux as a function of temperature.

3. Confinement of magnetic domain walls in ridge-and-valley specimen geometry. The specimen was prepared using our original self-organized template method.

1. Staff

Researchers: Tsuyosi Matsuda, Kei-ichi Fukunaga, Joong Jung Kim, Shuichi Mamishin
Technical staff: Yukinori Tsukada
Research advisers: Keiichi Namba (Osaka University), Daisuke Shindo (Tohoku University)
Research administrator/secretary: Chiharu Murota

2. Partner Organizations

Nothing to be reported.

3. Activities and Findings

3.1 Magnetic Domain Structure in (Ga, Ms)As diluted magnetic semiconductors

Gallium manganese arsenide (Ga, Mn)As is a III-V dilute magnetic semiconductor (DMS) and a promising model system for future spintronic devices because the magnetic functionality can be integrated in high-speed GaAs-based device structures. In-plane magnetized GaMnAs(001) layers are known to exhibit unique magnetic anisotropy that depends strongly on specimen temperature. Although it is important to understand micromagnetics of DMS domain structures, no study has been reported using magnetic transmission electron microscopy such as Lorentz microscopy and electron holography in the order of nanometer resolution. One reason lies in difficulty of working with very small magnetizations. In order to obtain sufficient phase shift originating from its in-plane magnetic induction, a relatively thick (500 nm) electron-transparent specimen with a uniform thickness has to be prepared over a large field of view. We prepared such a specimen by selective chemical etching. An MBE-grown, low-defect $Ga_{0.96}Mn_{0.04}As$/GaAs/AlAs multilayer specimen was specifically designed for this experiment. The first domain observation by Lorentz microscopy was performed using a 1 MeV cold FEG-TEM. A temperature range of 8 and 60 K at the sample was made available using a helium-cooled specimen holder.

Figure 1(a) shows an example of a magnetic domain strucature observed at 12K. This segmented domain wall structure takes a form of triangular domains extending from a central kinked domain wall. Its magnetization configuration is summarized schematically in Fig. 1(b). To the best of our knowledge, this type of structure has not been identified in any other materials to date. The composite domain walls are segmented by four different types of local domain walls, suggesting that the structure is closely related with cross-tie domain walls.

We observed spontaneous reorganization of segmented walls by varying temperature as shown in Fig. 2. The domain structure spontaneously reorganized at raising temperatures, even under a condition of zero-field. The change was explained in terms of temperature-dependent magnetocrystalline anisotropy.

Detailed analyses of domain wall profiles were performed by electron holography at 10 nm spatial resolution using our new 300 kV transmission electron microscope (OIST coherent 300 kV FE TEM). Figure 3 shows the wall phase images (right-hand panels) acquired at 30.5, 25.4, and 9.8 K, respectively, together with a larger area overview (left-hand panels) obtained by Fresnel-mode Lorentz imaging. In the phase – reconstructed images, the local **B** directions are parallel to a tangent of equiphase lines, and the magnitude of **B** is inversely proportional to the spacing of equiphase lines. As expected the magnitude of **B** decreases with temperature increase. The direction of **B** rotates gradually across the wall boundary implying the Néel-type walls in the studied (Ga, Mn)As film. For the walls denoted as (i) in Fig. 3, **B** rotates from the near [100] and [010] directions towards the [1$\bar{1}$0] direction with temperature increase; i.e., we acquired direct images of a transition from the near-90° in-plane domain wall at low-temperatures to a near-180° wall at higher temperatures. The width of walls is obtained by differentiating phase images with respect to x ([110]) and y ([1$\bar{1}$0]) directions [see Figs. 4(a–4(e)] and by fitting the measured phase gradient profile with a hyperbolic-tangent function. We obtained [1$\bar{1}$0]-and [110] oriented domain wall width ~ 45 ± 10 nm and 85 ± 15 nm, respectively, for measurement at 8 K. Asymmetry of the [110]-and [1$\bar{1}$0]-oriented wall has been directly demonstrated. The domain wall width is a function of magnetocrystalline anisotropy energy and exchange stiffness. Values for quantities calculated based on a kinetic-exchange model explain well the present result.

The works were done in collaboration with University of Nottingham (UK) and Institute of Physics, Czech Academy of Science.

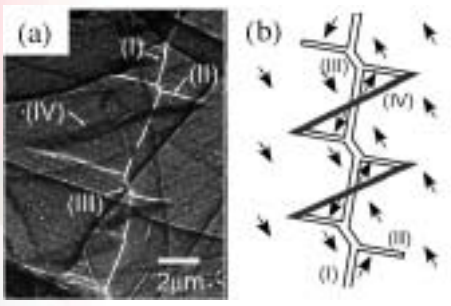


Fig. 1 (a) A Lorentz microscope image showing a segmented domain structure and (b) its corresponding schematic illustration.

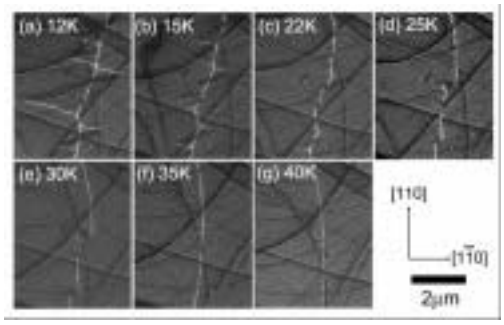


Fig. 2 Spontaneous reorganization of domain walls at varied temperatures.

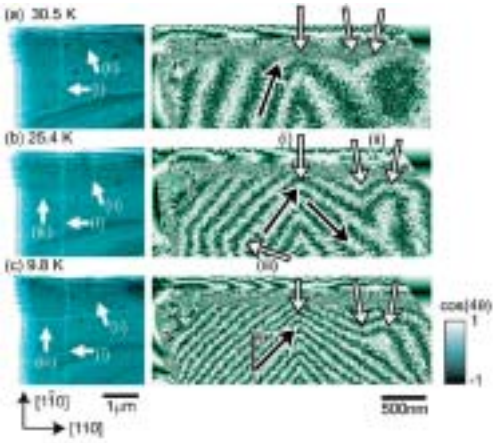


Fig. 3 Lorentz images (left) and electron phase images (right) obtained at (a) 30.5 K, (b) 25.4 K and (c) 9.8 K.

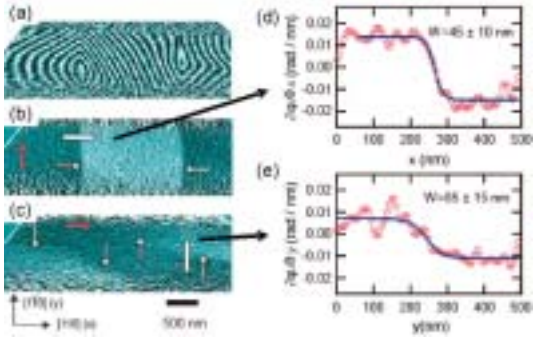


Fig. 4 (a) Electron phase, (b) B_x and (c) B_y mapping obtained at 8 K. The wall widths were determined to be 45 and 85 nm from phase gradient profiles (d-e).

3.2 Long-range magnetic ordering in circularly chained nickel nanoparticles

Recently assembles of nanoscale particles are extensively studied for applications to high-density recording media. Synthesis of polymer-coated metallic nanoparticles suitable for high-throughput production is especially important, where the mono-dispersed size distributions are guaranteed because coalescence of particles is suppressed by coating layers. It is also frequently observed that circular ring structures of such nanoparticles are spontaneously formed in solution.

Nickel nanoparticles of 30 nm are expected to exhibit superparamagnetism at temperatures higher than 150 K, because thermal agitation makes the direction of magnetization unstable. On the other hand, magnetic dipole coupling in one-dimensional arrays stabilizes the direction of magnetization. Long-range order parameter in such linear chains is thus expected to be a function of temperature. Electron holography is an established method to measure such order parameter without breaking their circular symmetry.

We observed the electron phase difference between inside and outside of rings as shown in Fig. 5. The result implies that magnetic flux closure occurs in a ring. The phase difference is proportional to the magnetic order parameter. We measured the phase difference as a function using a liquid-nitrogen cooling specimen holder. The phase shift was found to decrease more rapidly than that of bulky nickel as a function of temperature. We also performed Monte-Carlo simulation taking into account moment decay of bulky nickel as a function of temperature and also the dipolar coupling. The result indicated that the temperature variation of the order-parameter was explained in terms of weakened dipolar coupling field ascribed to decay of bulky magnetization and an enhanced thermal fluctuation as shown in Fig. 6.

The work was done in collaboration with Kyushu University and Simon Fraser University (Canada).

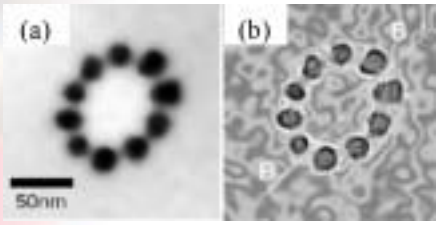


Fig. 5 (a) A reconstructed phase image of an Ni ring, and (b) a cosine display of the phase difference images on which a corresponding Laplacian-filtered phase image is imposed.

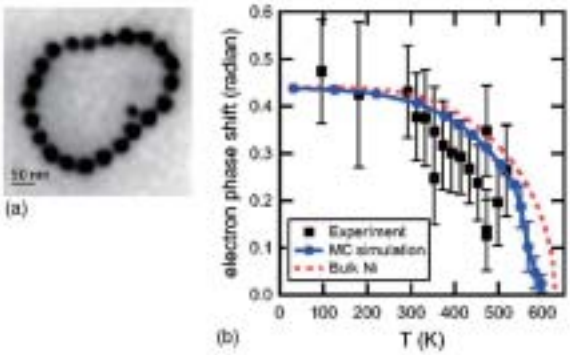


Fig. 6 (a) A phase image of an Ni ring and (b) observed electron phase shift (■), calculated phase shift for bulky Ni sphere (dashed line), and calculated phase shift obtained from the Monte Carlo simulation (●) as a function of temperature.

3.3 Anisotropic cross-tie walls and their confinement in self-organized undulating Fe film

Control of in-plane magnetic anisotropy is a key issue in magnetic storage technology. We investigated magnetic domain structure and domain wall widths within self-organized undulating Fe film by using Lorentz microscopy and electron holography.

The SiO_2 (15 nm)/ Fe (20nm)/ SiO_2 (15 nm) trilayers were deposited onto faceted NaCl(110) substrates. The Fe film exhibited a ridge-and-valley structure with an undulation period of approximately 30 nm as schematically shown in Fig. 7. The specimen exhibited strong in-plane uniaxial anisotropy with an easy axis parallel to the ridge. The domain walls in undulating Fe film were found to be cross-tie walls. Vortices (V) and antivortices (AV) in cross-tie walls induce angular deviations of the moment as small as 20° from an easy axis and effectively reduce wall energy (Fig. 8). Domain wall widths were determined to be 18 ± 4 nm electron holography (Fig. 9). The obtained widths, nearly a half of the undulation period, were narrower than those observed with a flat Fe film. For comparison, stable magnetization distributions and internal structures of the walls were examined by numerical simulations based on Landau-Lifshitz-Gilbert equation (LLG) as shown in Fig. 9(c). The walls are found to be confined between adjacent ridges, i.e. within a half undulation period, due to strong pinning of magnetization along an easy axis near ridges.

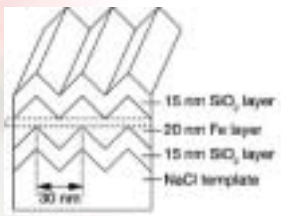


Fig. 7 A specimen structure.

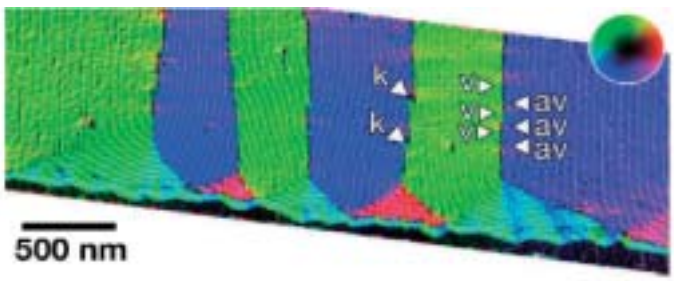


Fig. 8 An induction map in undulating Fe film obtained by using electron holography, on which contour lines (π/2 step) are superimposed.

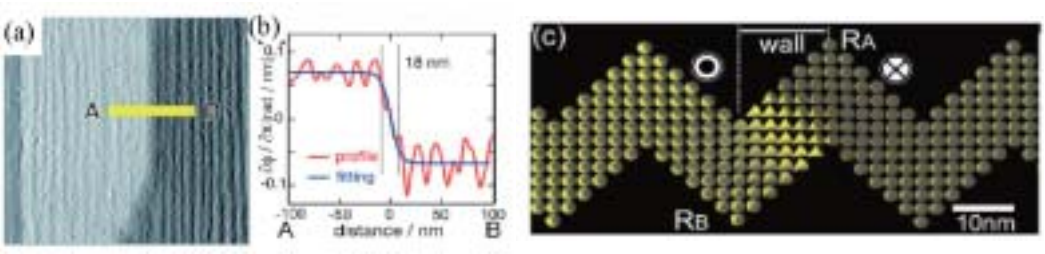


Fig. 9 (a) A differential phase image and (b) a line profile of phase gradient. The domain wall width is measured to be 18 nm. (c) Magnetization distribution on a cross-section plane perpendicular to ridges obtained by LLG micromagnetic simulation.

4. Publications

4.1 Journals

Sugawara, A., Akashi, T., Brown, P. D., Campion, R. P., Yoshida, T., Gallagher, B. L., Tonomura, A., (2007) High-resolution observations of temperature-dependent magnetic domain structures within $Ga_xMN_{1-x}As$ by Lorentz microscopy, Phys. Rev. B, 75, 241306-1-241306-4.

Sugawara, A., Fukunaga, K., Scheinfein, M. R., Kobayashi, H., Kitagawa, H., Tonomura, A., (2007) Electron holography study of the temperature variation of the magnetic order parameter within circularly nickel nanoparticle rings, Appl. Phys. Lett, 91, 262513-1-262513-3.

Sugawara, A., Kasai, H., Tonomura, A., Brown, P. D., Campion, R. P., Edmonds, K. W., Gallagher, B. L., Zemen, J., Jungwirth, T., (2008) Domain walls in the (Ga, Mn) as diluted magnetic semiconductor, Phys. Rev. Lett, 100, 047202-1-047202-4.

Fukunaga, K., Sugawara, A., (2008) Anisotropic cross-tie walls and their confinement in self-organized undulating Fe film, J. Appl. Phys, 103, 053909-1-053909-6.

Tonomura, A., Nori, F., (2008) Disturbance without the force, Nature, 452, 298-299.

4.2 Book(s) and other one-time publications

Nothing to be reported.

4.3 Oral presentations

Tonomura, A., Quantum phenomena visualized by electron waves, Conf. in Honor of C. N. Yang's 85th Birthday, Singapore, October 31-November 3, 2007.

Tonomura, A., Quantum phenomena visualized by electron waves, Symposium on Neutrino Processes and Stellar Evolution, Univ. of Tokyo, November 14-15, 2007.

Tonomura, A., Electron phase microscopy for observing microscopic objects, Symposium on Japan Fine Ceramics Center, Nagoya, January 25, 2008.

Tonomura, A., Magnetic flux observed using electron microscope, Japan Society of Applied Physics, the 55th Spring Meeting, Chiba, March 28, 2008.

Sugawara, A., Akashi, T., Kasai, H., Gallapher, B. L., Jungwirth, T., Lorentz microscopy and electron holography study on magnetic domain walls in (Ga, Mn) As, Japan Society of Applied Physics, the 55th Spring Meeting, Chiba, March 29, 2008.

4.4 Posters

Nothing to be reported.

5. Intellectual Property Rights and Other Specific Achievements

Nothing to be reported.

6. Meetings and Events

Nothing to be reported.

IX. Molecular Genetic Unit

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

Abstract

Because many species of salamander have large cell size, the salamander is thought to be a good material to study the nervous system (especially for imaging studies, electrophysiology, and single-cell analysis). After screening several species of salamander, *Ambystoma mexicanum* has been selected as the experimental material. We have started the salamander project. We constructed cDNA libraries of the brain, retina, and spinal cord of the salamander. We have already done single-run sequencing of the 5'-end of 200,000 clones from these libraries. After clustering single-run sequences, we are carrying out full-length sequencing of the cDNAs included in the clusters and will make a salamander cDNA database.

1. Staff

Molecular Genetic Unit

Technical staff: Shoko Takehara, Saori Goda, Shin-ichi Yamasaki, Miho Hirai

2. Partner Organizations

Okinawa Institute of Science and Technology PC

Type of partnership: Joint research
Name of principal researcher: Dr. Takayuki Naito
Name of researchers: Setsuko Nakanishi, Kiyotaka Akiyama
Research theme: Salamander project

Nagoya University, Department of Chemistry

Type of partnership: Joint research
Name of principal researcher: Prof. Daisuke Uemura
Research theme: Marine microorganism project

3. Activities and Findings

3.1 Salamander project (See also Naito Unit)

We constructed cDNA libraries from brain, retina, and spinal cord of *Ambystoma mexicanum* by using vector-capping method developed by Dr. S. Kato (DNA Res. 2005 Feb 28;12(1):53-62). This method enabled us to construct cDNA library containing full length cDNA at high rate. Table 1 shows the quality of the libraries.

Table1 Quality of three libraries

Library	Number of clone	Sequence with good quality	Nucleotide at 5' end of insert				Average size of insert (kb)*	Ratio of clone with insert (%)*	Ratio of full-length cDNA clone (%)*
			G	A	T	C			
Brain	100208	80864	56040 (74%)	5201 (7%)	10313 (14%)	4196 (6%)	1.3	88	89
Retina	53755	36668	24410 (75%)	2197 (7%)	4596 (14%)	1265 (4%)	1.1	94	63
Spinal cord	50589	39141	25943 (74%)	3789 (11%)	3026 (9%)	2286 (7%)	1.7	80	68

* 96 clones of each library were analyzed.
Each insert was determined whether full length or not by the homology analysis of their orthologs. Ratio of clones with full length cDNA equals number of clones with full length cDNA/number of clones with foreign inserts.

We have completed first run sequence of 5'-end of 100,000 clones from brain library, 50,000 clones from retina library, and 50,000 clones from spinal cord library. These clones were assembled into 22,804, 11,875, and 11,377 contigs in brain, retina, and spinal cord, respectively (Table 2). Because alternative splicing forms are divided into different contigs, the number of contigs is not equivalent to the number of genes. In order to know the number of genes we cloned, all contigs were sorted by BLAST (Basic Local Alignment Search Tool). About 13,000 clusters were constructed by the sorting. That number can be thought to be nearly equivalent to the number of the genes we cloned. We have already revealed full length sequences of about 5,000 of 13,000 clusters and are going ahead with analysis of the remainder.

Table 2 Progress of sequence analysis

First run sequence	Brain	3rd run completed (100,000 clones)
	Retina	1st run completed (50,000 clones)
	Spinal cord	1st run completed (50,000 clones)
Contig	Brain	22,804
	Retina	11,875
	Spinal cord	11,377
Cluster	Three libraries	13,050

In these 13,000 clusters, about 6,000 were shown to be orthologous to human genes. Among them, 187 of the orthologs are known to play key roles in neural activity, and they are categorized as metabolic enzymes of neurotransmitter, G protein-coupled receptors (GPCRs), neuropeptides, ligand-gated channels, and voltage-gated channels. The genes expressed highly in the brain in these categories are shown in Table 3.
Moreover, 454 orthologs of human transcriptional factor genes were found.

Table 3 Neural function related genes expressed highly in the salamander brain

Gene name	Number of clones (/80864 clones)	Category
4-aminobutyrate aminotransferase	2	metabolic enzyme of neurotransmitter
catechol-O-methyltransferase	2	
glutamic acid decarboxylase	2	
tyrosine hydroxylase	2	
glycoprotein hormones, alpha subunit	120	neuropeptide
arginine vasopressin	49	
somatostatin	15	
glucagon receptor	8	GPCR
brain-specific angiogenesis inhibitor	6	
somatostatin receptor	5	
gamma-aminobutyric acid (GABA-A) receptor, subunit alpha 4	5	ligand-gated channel
glutamate receptor, ionotropic, AMPA2 (alpha 2)	3	
gamma-aminobutyric acid (GABA-A) receptor, subunit gamma 2	2	
glycine receptor, alpha 2 subunit	2	
glycine receptor, beta subunit	2	
glutamate receptor, ionotropic, AMPA1 (alpha 1) 2	2	
Potassium channel tetramerization domain-containing 10	12	voltage-gated channel
Chloride intracellular channel protein 4	5	
ATP-sensitive inward rectifier potassium channel 11	2	
Potassium channel tetramerisation domain containing protein 13	2	
Potassium channel tetramerisation domain containing protein 2	2	

We will complete the full-length sequencing of the cDNAs and make a cDNA database for future study.

X. Information Processing Biology Unit

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

Abstract

Our research objectives are (1) to understand how cells detect external information and transmit it to the inside of the cell, and (2) to understand how the nervous system processes the external information as cellular networks to regulate animal behaviors. This year we have made the following findings, using cultured animal cells and the nematode *Caenorhabditis elegans (C. elegans)* as model systems: (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, at physiological expression levels in cultured animal cells as determined by fluorescence resonance energy transfer (FRET) and fluorescence cross-correlation spectroscopy (FCCS). (ii) All the members of the EGFR family have preformed homo- and heterodimeric structures between the family members when analyzed by the bimolecular fluorescence complementation (BiFC) assay. (iii) Cell-surface receptors for neurotrophic factors also seem to function as homo- and heterodimers prior to ligand binding. (iv) In *C. elegans*, mild alkaline pH is perceived by the ASE amphid neurons as well as by the IL2 neurons, whose function was previously unknown. The neuronal networks triggered by the two different sensory neurons 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 as a noxious stimulus. (v) We have also found *C. elegans* mutants that cannot perceive strong alkaline pH. Analyses of these mutants should allow us to understand how the worm can detect the noxious stimulus (nociception). 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 may also be invaluable for the development of pharmaceuticals for human diseases such as schizophrenia and cancers.

1. Staff

Researchers: Aini Suzana Adenan, Satoshi Hasegawa, Stephan Haupt, Kazunobu Hirose, Ichiro Kawasaki, Takashi Murayama, Hideki Nagahama, Katsunori Nakata, and Rong-Hua Tao
Technical staff: Mayuki Fujiwara, Kanako Hisata, and Hiraku Miyagi
Research administrator/secretary: Yuko Toyama

2. Partner Organizations

Department of Chemistry, National University of Singapore
Type of partnership: Collaborative
Name of principal researcher: Thorsten Wohland
Research theme: Investigation of the dimerization of proteins from the epidermal growth factor receptor family by single wavelength fluorescence cross-correlation spectroscopy

University of California, San Diego
Type of partnership: Collaborative
Name of principal researcher: Yishi Jin
Research theme: Genetic and biochemical analyses of synaptogenesis in the nematode *C. elegans.*

Institute of Medical Biology, Singapore
Type of partnership: Collaborative
Name of principal researcher: Sohail Ahmed
Research theme: 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 partnership: Collaborative
Name of principal researcher: Ken-ichi Kariya
Research theme: Total internal reflectance fluorescence microscopic analysis of membrane proteins

Hokkaido University
Type of partnership: Joint
Name of principal researcher: Masataka Kinjyo
Research theme: Optical analysis of membrane protein dynamics

Keio University School of Medicine
Type of partnership: Joint
Name of principal researcher: Takanori Moriki
Research theme: Application of the lambda phage surface display system

3. Activities and Findings

3.1 Project Aims

All forms of life are separated from non-life by cell membranes, and all cells have cell-surface receptor proteins that span the membranes in order to transfer external information, such as environmental changes and cell-cell communications, 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 in humans. (1) 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 receptors, and how the information is processed, transferred to other parts of the cells, and regulates other cellular activities. (2) We wish also to understand information processing at higher levels through cell-cell communications; namely, how the external information is sensed and transmitted through neuronal cells, processed by the nervous system, and how it controls animal behaviors including learning and memory.

3.2 Progress report

3.2.1 Information processing by cells

3.2.1.1 Introduction. The bacterial cell-surface receptor Tar recognizes aspartate molecules in the environment, and brings bacterial cells towards the higher concentrations of the attractant as a nutrient, or the lower concentrations of repellents such as nickel and cobalt ions. This transmembrane signaling by Tar occurs within homodimeric receptor molecules on the cell surface. We have previously shown that the Tar activity is regulated by the ligands, which bind to the extracellular domain of the receptor and lock/freeze the rotational/twist movement of the receptor's transmembrane domains in parallel with the plane of the lipid bilayer. The lock/freezing of the rotation/twist at one position by the attractant seems to inhibit the associated histidine kinase Che A, while the lock/freezing at another position by the repellent seems to activate the kinase activity (the rotation/twist model; Figure 1)

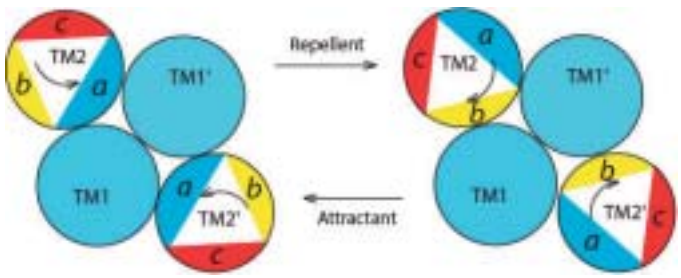


Figure 1. "Rotation/twist" model for the regulation of the Tar receptor activity by ligand binding. Transverse sections of transmembrane domains of the Tar homodimers, in which the transmembrane domain (TM1) does not move during the transmembrane signaling. The attractant aspartate locks/freezes the rotaion/twist of the transmembrane domain 2 (TM2) by bringing the surface 'a' of TM2 towards TM1, while the repellents by bringing the surface 'b' of TM2 towards TM1.

We have also analyzed a molecular mechanism underlying activation of the human epidermal growth factor receptor (EGFR) family of cell-surface receptor tyrosine kinases, also known as ErbB or HER. 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 domain. They form a network of homo- and heterodimers. ErbB2 can only be regulated indirectly, and is thought to be a 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 ligand neuregulin (NRG).

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 still 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 molecules exist 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 rearrange the kinase domains for activation, and 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 PLC γ and PI3 kinase. Indeed, this rotation/twist model (Figure 2) is consistent with the homodimeric structure of the receptor kinase, transmembrane and unactivated extracellular domains that have recently been determined by others.

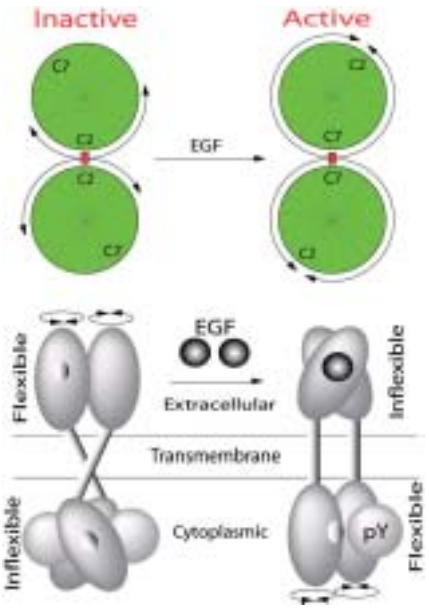


Figure 2. " Rotation/twist " model for the EGFR activation.
Helical wheel representation of the transmembrane region of EGFR (Top). Side views of the EGFR dimers (Bottom). EGFR on the cell surface exists as an inactive dimer with flexible ligand-binding domains. The ligand EGF bound to the extracellular domains locks/freezes the flexible extracellular domains, and induces the rotation/twist of the transmembrane domains in parallel with the plane of the plasma membrane, resulting in the dissociation and rearrangement of the intracellular kinase domains for activation.

Thus, two different cell-surface receptors, bacterial Tar and human EGFR, seem to be similarly regulated by their ligands in order to transmit the external information to the inside of the cell. The ligands regulate the rotation/twist of the receptor's transmembrane domain in parallel to the plane of the plasma membrane. This rotation/twist may be energetically favorable movement of the transmembrane domain, compared to its lateral or vertical movement in the lipid bilayer. Therefore, we are continuing to test the "rotation/twist" model for the activation of other cell-surface receptors including other ErbB receptors and neurotrophic factor receptors as described below.

3.2.1.2 Preformed homo- and heterodimeric structures of EGFR and ErbB2. As described above, we have previously elucidated EGFR has a preformed dimeric structure on the cell surface by chemical cross-linking, cysteine disulfide bridging and sucrose-density gradient centrifugation. By fluorescence resonance energy transfer (FRET) and fluorescence cross-correlation spectroscopy (FCCS), we have detected preformed homo- and heterodimers of EGFR and ErbB2 at physiological expression levels (~10^4 molecules per cell). When EGFR and ErbB2 fused with fluorescent proteins (FPs) 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, irrespective of their expression levels ranging from ~2 x 10^4 to ~3 x 10^6 molecules per cell.

3.2.1.3 Preformed homo- and heterodimers between the EGF/ErbB receptor family. 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 located to the plasma membrane (Figure 3). These provide new insights into an understanding of transmembrane signal transduction mediated by the ErbB receptor family.

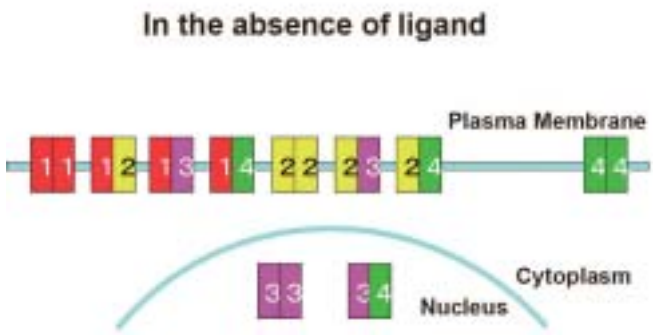


Figure 3. Preformed homo- and heterodimeric structures of the ErbB receptor family and their subcellular localization.

3.2.1.4 EGFR domains required for the homo- and heterodimerization. Three-dimensional 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. Through BiFC analysis of deletion mutants, indeed, we have found that the intracellular domain of EGFR plays a crucial role for the spontaneous dimerization in the absence of ligand. Based on the three-dimensional structures, we have started systematic analysis of the EGFR domain(s) involved in the spontaneous homo- and heterodimerization. By constructing point and deletion mutant molecules, we will try to identify domains and amino-acid residues required for the dimerization.

3.2.1.5 Cell-surface receptors for neurotrophic factors. In the same line of the project, we have also started to analyze monomeric, dimeric or oligomeric structures of the TrkA, TrkB and p75 receptors, which are receptors for nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and all neurotrophins, respectively. Preliminary BiFC analysis of the receptors suggest that these receptors also have homo- and heterodimeric structures prior to the ligand binding. We will continue to collect evidence indicating the spontaneous dimeric structures by using similar approaches to the analysis of EGFR.

3.2.2 Information processing by the nervous system

In the past year, we have focused on the analysis of the nematode *C. elegans* chemotaxis towards mild alkaline pH (3.2.2.1.), and nociception of ammonia gas or strong alkaline pH (3.2.2.2), and the genetic analysis of signaling pathways involved in *C. elegans* synaptogenesis (3.2.2.3).

3.2.2.1 *C. elegans* chemotaxis towards alkaline pH. *C. elegans* responds to water-soluble chemicals such as ions and amino acids, and senses acid pH (H^+) as an aversive stimulus and alkaline pH (OH^-) as an attractant. 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 plate for the worm's response to alkaline pH. On the assay plate with a pH gradient from pH 6.0 to pH 9.5, wild-type animals moved towards alkaline pH along the gradient (Figure 4). *dyf-3*, 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.

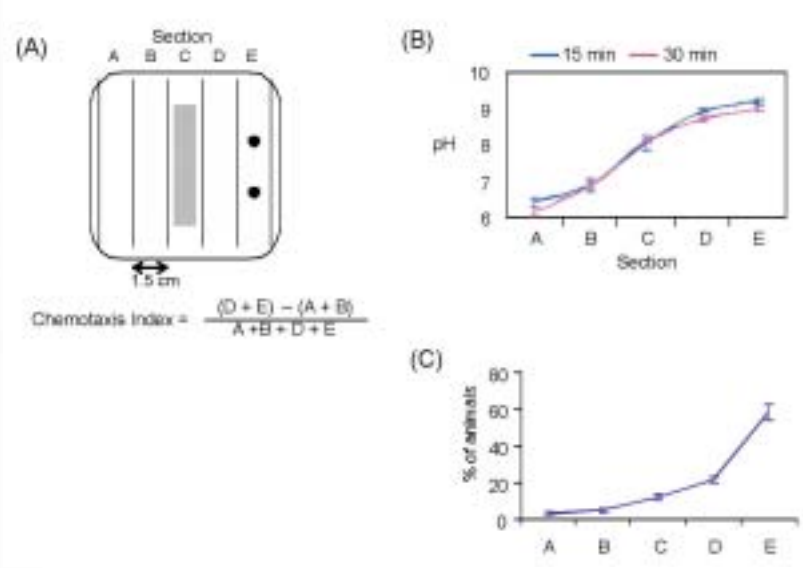


Figure 4. Behavioral assay for *C. elegans* chemotaxis towards mild alkaline pH. (A) Assay agar plate. Animals are placed on the center (shaded area) of the plate. 5% NH^4OH solution, 2 µl each, is spotted onto the two dot points of section E. (B) Alkaline pH gradients formed in 15 or 30 minutes. (C) Distribution pattern of wild-type animals on the pH gradient.

ASE-specific rescue of the gustatory defective *dyf-3* mutant induced normal orientation towards alkaline pH, suggesting that ASE functions are sufficient for this mild alkaline chemotaxis (Figure 5).

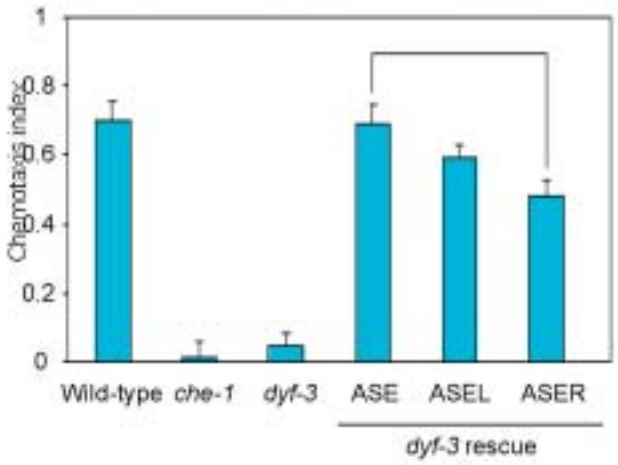


Figure 5. ASE specific rescue of *dyf-3*. Note that *che-1* and *dyf-3* animals did not respond to the pH gradient. The *dyf-3* mutant was completely or partially rescued by expressing DYF-3 in ASE neuron(s).

3.2.2.2 Nociception in *C. elegans*. We have previously found *C. elegans* avoids ammonia gas. At that time, however, we did not know whether the worm senses the gas molecules or extremely higher pH as a noxious stimulus. To elucidate a molecular mechanism underlying the nociception in *C. elegans*, we designed the similar assay to the mild alkaline chemotaxis describe above. An ASE defective *che-1* mutant avoided higher pH ranges than pH 10.0. These suggest that while ASE neurons may be required for chemotaxis towards mild alkaline pH ranges, some other neurons than ASE may be responsible for the avoidance of strong alkaline pH. 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 chemosensory organs, and has normal structure in IL2 chemosensory endings, the results imply that IL2 chemosensory neurons may be one of nociceptor neurons involved in strong-alkaline pH avoidance. Using the plate assay, we have screened mutants insensitive to higher pH ranges than pH 11.0, and found some of them were also insensitive to ammonia gas. This suggests that ammonia gas may be perceived by *C. elegans* as extremely high pH although the existence of nociceptors for ammonia gas itself cannot be ruled out. We are currently trying to identify sensor molecules for the extremely high pH, and neuronal networks responsible for the nociception and avoidance behavior using the paradigm we developed.

3.2.2.3 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. 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. RPM-1 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, *ju587*, of *rpm-1*, and have identified that this gene functions downstream of MKK-4. We are further characterizing the role of the suppressor gene at the molecular level in collaboration with Yishi Jin at University of California, San Diego.

4. Publications

4.1 Journals

Kannan, B., Thankiah, S., Ahmed, S., Maruyama, I., Wohland, T. (2007) Spatially resolved total internal reflection fluorescence correlation microscopy using electron multiplying charge-coupled device camera. Anal. Chem., 76, 4463.

Liu, P., Thankiah, S., Koh, M. L., Hwang, L. C., Ahmed, S., Maruyama, I. N., 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., 93, 684.

Pan, X., Fok, M., Foo, W., Lim, W., Liu, P., Yu, H., Maruyama, I., Wohland, T. (2007) A multifunctional fluorescence correlation microscope for interacellular and microfluidic measurements. Rev. Sci. Instrum., 78, 53711.

Ou, G., Koga, M., Blacque, O. E., Murayama, T., Ohshima, Y., Schafer, J. C., Li, C., Yoder, B. K., Leroux, M. R., Scholey, J. M. (2007) Sensory ciliogenesis in *Caenorhabditis elegans*: assignment of IFT components into distinct modules based on transport and phenotypic profiles. Mol. Biol. Cell, 18, 1554.

Kannan, B., Hong, Y., Thankiah, S., Liu, P., Maruyama, I., Wohland, T. (2007) A system for spatially resolved total internal reflection-fluorescence correlation spectroscopy using an EMCCD camera. Biophys. J., (Suppl.) 92, 1532.

Wohland, T., Liu, P., Thankiah, S., Maruyama, I., Ahmed, S. (2007) Single wavelength fluorescence cross-correlation spectroscopy (SW-FCCS) as a tool to determine biomolecular interactions in vivo. Biophys. J. (Suppl.) 92, 1534.

Tao, R.-H., Maruyama, I. N. (2007) Cell-surface and nuclear localization of preformed ErbB receptor homo- and heterodimers in living cells. FASEB J., 21, lb67.

Murayama, T., Maruyama, I. (2007) Neurons responsible for *C. elegans* chemosensation to pH. Neurosci. Res., 58, S216.

Nakata, K., Maruyama, I., Jin, Y. (2007) UEV-3, a ubiquitin conjugating enzyme E2 variant, functions in the p38 MAP kinase pathway for synaptogenesis. Neurosci. Res., 58, S39.

Moriki, T., Maruyama, I., Ikeda, Y., Murata, M. (2007) ADAMTS13 epitopes required for binding to von Willebrand factor. Jpn. J. Thromb. Hemostas., 18, 472.

4.2 Book(s) and other one-time publications

Hasegawa, S., Maruyama, I. (2007) Water in cells. Neurosci. News, 163, 8.

4.3 Oral presentations

Maruyama, I., Tao, R.-H. Ligand-induced activation of preformed EGF/ErbB receptor homo- and heterodimers: a molecular mechanism. European Life Scientist Organization Annual Meeting (ELSO 2007), International Congress Center Dresden, Dresden, Germany; September 1-4, 2007.

Nakata, K., Maruyama, I., Jin, Y. UEV-3, a ubiquitin conjugating enzyme E2 variant, functions in the p38 MAP kinase pathway for synaptogenesis. Neuro2007 (Joint Meetings of 30th Annual Meeting of the Japan Neuroscience Society, 50th Annual Meeting of he Japanese Society for Neurochemistry, and 17th Annual Meeting of the Japanese Neural Network Society), Pacifico Yokohama, Yokohama, Japan; September 10-12, 2007.

Moriki, T., Maruyama, I., Ikeda, Y., Murata, M. Identification of ADAMTS13 Epitopes Required for Binding to von Willebrand Factor. 30th Congress of Japanese Society on Thrombosis and Hemostasis, Shima Kanko Hotel, Mie, Japan; November 15-17, 2007.

Maruyama, I. Application of FCCS to analysis of the EGF/ErbB receptor activation. 10th Workshop on Fluorescence Correlation Microscopy, Hokkaido University, Sapporo, Japan; November 26-28, 2007.

Tao, R.-H., Maruyama, I. Preformed EGF/ErbB receptor homo- and heterodimers in living cells. 80th Annual Meeting of the Japanese Biochemical Society and 30th Annual Meeting of the Molecular Biology Society of Japan (BMB2007), Pacifico Yokohama, Yokohama, Kanagawa, Japan; December 11-15, 2007.

Maruyama, I. Preformed dimeric structures of epidermal growth factor receptors determined by fluorescence cross-correlation microscopy. 5th Riken Symposium on "Fluorescence Correlation Microscopy and Signal Transduction", Ohkochi Memorial Hall, Wako, Saitama, Japan; February 22, 2008.

4.4 Posters

Tao, R.-H., Maruyama, I. N. Cell-surface and nuclear localization of preformed ErbB receptor homo- and heterodimers in living cells. American Society for Biochemistry and Molecular Biology, 2007 Annual Meeting, Washington, DC, USA; April 28- May 2, 2007.

Murayama, T., Maruyama, I. Chemotactic response to alkaline pH in *C. elegans*. 16th International *C. elegans* Meeting, UCLA, CA, USA; June 27-July 1, 2007.

Nakata, K., Maruyama, I., Jin, Y. UEV-3, a ubiquitin conjugating enzyme E2 variant, functions downstream of the RPM-1-DLK-1-p38 MAP kinae pathway in synapse formation. 16th International *C. elegans* Meeting, UCLA, CA, USA; June 27-July 1, 2007.

Kawasaki, I., Hanazawa, M., Gengyo-Ando, K., Mitani, S., Maruyama, I., Iino, Y. ASB-1, a germline-specific isoform of mitochondrial ATP synthase b subunit, is required to maintain the rate of germline development in *Caenorhabditis elegans*. 16th International *C. elegans* Meeting, UCLA, CA, USA; June 27-July 1, 2007.

Maruyama, I., Tao, R.-H. Ligand-induced activation of preformed EGF/ErbB receptor homo- and heterodimers: a molecular mechanism. European Life Scientist Organization Annual Meeting (ELSO 2007), International Congress Center Dresden, Dresden, Germany; September 1-4, 2007.

Murayama, T., Maruyama, I. Neurons responsible for *C. elegans* chemosensation to pH. Neuro2007 (Joint Meetings of 30th Annual Meeting of the Japan Neuroscience Society. 50th Annual Meeting of he Japanese Society for Neurochemistry, and 17th Annual Meeting of the Japanese Neural Network Society), Pacifico Yokohama, Yokohama, Japan; September 10-12, 2007.

Miyagi, H., Maruyama, I. FCS analysis of EGF-EGFR interaction on the living cell surface. 10th Workshop on Fluorescence Correlation Microscopy, Hokkaido University, Sapporo, Japan; November 26-28, 2007.

Tao, R.-H., Maruyama, I. Preformed homo- and heterodimers between four members of the ErbB receptor family. 47th Annual Meeting of the American Society for Cell Biology, Washington Convention Center, Washington, DC, USA; December 1-5, 2007.

Moriki, T., Maruyama, I. N., Yamaguchi, Y., Igari, A., Ikeda, Y., Murata, M. Identification of ADAMTS13 Epitopes Required for Binding to von Willebrand Factor Using Lambda Phage Surface Display (Publication Number: 2707). 49th American Society of Heamatology Annual Meeting and Exposition, Georgia World Congress Center, Atlanta, Georgia; December 8-11, 2007.

Tao, R.-H., Maruyama, I. Preformed EGF/ErbB receptor homo- and heterodimers in living cells. 80th Annual Meeting of the Japanese Biochemical Society and 30th Annual Meeting of the Molecular Biology Society of Japan (BMB2007), Pacifico Yokohama, Yokohama, Kanagawa, Japan; December 11-15, 2007.

Murayama, T., Maruyama, I. Neurons responsible for *C. elegans* chemosensation to alkaline pH. 80th Annual Meeting of the Japanese Biochemical Society and 30th Annual Meeting of the Molecular Biology Society of Japan (BMB2007), Pacifico Yokohama, Yokohama, Kanagawa, Japan; December 11-15, 2007.

Nakata, K., Maruyama, I., Jin, Y. UEV-3, a ubiquitin conjugating enzyme E2 variant, functions downstream of the RPM-1-DLK-1-p38 MAP kinase pathway in synapse formation. 80th Annual Meeting of the Japanese Biochemical Society and 30th Annual Meeting of the Molecular Biology Society of Japan (BMB2007), Pacifico Yokohama, Yokohama, Kanagawa, Japan; December 11-15, 2007.

4.5 Invited Lectures and Seminars

Maruyama, I. Molecular targeting of the epidermal growth factor receptor for cancer therapy. Department of General Medicine, Nara Medical University, Nara, Japan; October 31, 2007.

Maruyama, I. Innate immunity and antigen recognition by Toll-like receptors. Undergraduate Program in Autoimmune Diseases, Nara Medical University, Nara, Japan; November 1, 2007.

Maruyama, I. Molecular mechanism underlying transmembrane signaling. Niigata University, Niigata, Japan; December 17, 2007.

Maruyama, I. The *C. elegans* nervous system. Undergraduate Program, Ryukyu University School of Medicine, Okinawa, Japan; December 20, 2007.

Maruyama, I. Molecular mechanism of extracellular information processing by cell-surface receptors. Ryukyu University Faculty of Science, Okinawa, Japan; January 25, 2008.

5. Intellectual Property Rights and Other Specific Achievements

none

6. Meetings and Events

6.1 Seminars hosted

Seminar entitled "TRP channels and magnesium homeostasis"

Date: February 6, 2008

Venue: IRP Conference Room

Speakers: Kouichi Iwasaki, Northwestern University, USA

Seminar entitled "mall animal imaging by PET"

Date: July 13, 2007

Venue: IRP Conference Room

Speakers: Norio Arai, Molecular Imaging Research Center,
GE Yokogawa Medical Systems

XI. Molecular Neurobiology Unit

Principal Investigator: Takayuki Naito

Research Theme: Single-cell Biochemistry and Molecular Analysis of Brain Functions

Abstract

In gene-based approach to the study of brain functions, our present major aims are to study activity-dependent gene expression in the nervous system and to identify neuron types by genes expressed within them (NeuMap project). We have carried out the following research in this fiscal year of 2007.

1. Gene expression analysis of single type neuron and small regions of the brain
2. NeuMap
3. Activity-dependent gene expression
4. Molecular profiling of the brain by mass spectrometry
5. Salamander project

These are described in Section 2.

1. Staff

Molecular Neurobiology Unit

Group leader: Setsuko Nakanishi, Kiyotaka Akiyama

Researchers: Nozomu Nakamura, Michael Chandro Roy

Technical staff: Seiko Kuraba, Sayaka Arai, Saori Ishida

Research administrator/secretary: Kaori Yamashiro

2. Partner Organizations

Okinawa Institute of Science and Technology, PC Principal researcher

Type of partnership: Joint research

Name of principal researcher: Dr. Sydney Brenner

Research theme: Salamander project

University of Tokyo, Department of Metabolome, Graduate School of Medicine

Type of partnership: Joint research

Name of principal researcher: Prof. Ryo Taguchi

Research theme: Lipids in the nervous system

Hamamatsu University School of Medicine

Type of partnership: Joint research

Name of principal researcher: Prof. Mitsutoshi Setou

Research theme: Mass Imaging

University of Kuopio, Finland

Type of partnership: Joint research

Name of principal researcher: Dr. Kazuhiko Tatemoto

Research theme: Peptidome in the brain

Nagoya University, Department of Chemistry
Type of partnership: Joint research
Name of principal researcher: Prof. Daisuke Uemura
Research theme: Marine microorganism project

Osaka Bioscience Institute
Type of partnership: Joint research
Name of principal researcher: Dr. Shigetada Nakanishi
Research theme: Study of cerebellum

3. Activities and Findings

3.1 Gene expression analysis of single type neuron and small regions of the brain

In order to examine gene expression profiling of single type neurons and specific brain regions, we adopted the following methods: 1) collection of single type neurons and small regions of the brain by laser capture microdissection (LCM) and isolation of RNA from the collected samples, 2) amplification of isolated mRNA, 3) gene expression profiling by using DNA microarray, 4) quantitative analysis of gene expression by using Real Time PCR.

In Affymetrix GeneChip Mouse Genome 430 2.0, each probe-set consists of 11 probes. Only 26,386 probe-sets, corresponding to 19,249 genes, contain more than 9 probes matched to define cDNAs in ENSMBL or NCBI (ref.seq) database (Table 1). These probe-sets were used for gene expression analysis. Furthermore, we examined the location of probe-set on mRNA according to the following criteria: 1) Representative position of a probe is defined as the 13th nucleotide of the probe, 2) Probe distance" is the distance between 5' end of polyA and representative position of "each probe, 3) "Probe-set distance" is an average of "probe distance".

Table 1 Information of probe-sets

Classification	Number of probe sets
No match	15391
Minus strand match	799
Only 1 - 8 probes match	2424
Total	18614
Genome （>9 probe)	14034 genes in 18614
Above 9 probes match	26386 (45000 - 18614)
Number of genes	19249

We used 3 μg and 1 ng of total RNA of cultured cerebellar granule cells as a starting material for non-amplification and 1st round amplification, respectively. Based on T7 RNA polymerase transcription, mRNA was amplified. The amplified and non-amplified samples were analyzed by using GeneChip Mouse Genome 430 2.0 Array.
Totally 24414 and 20038 probe-sets gave "Present" or "Marginal" calls (P/M call) in non-amplification and amplification samples, respectively. Totally 19262 probe-sets were assigned to "P/M call" in both samples. It indicated a small influence of amplification procedure. Thus, the result of amplified sample was reliable.

We also examined the influence of the location of probe-set (Table 2). If probe-sets were assigned within 300 bp from polyA, more than 90% of probe-sets were commonly assigned to "P/M call" in both samples. The data from the probe-sets designed near to 3' end were more reliable. Therefore, primer sequence for Real-Time PCR should be confined within 300 bp from 3' end of each mRNA.

Table 2 Location of probe-sets and population of the probe-sets assigned to P/M call

The average distance of probe-set from polyA (bp)	No. of P and M/No. of Total probe-sets (%)		1ng/3μg (%)	No. of Probe-sets (cumulative)
	3 μg	1 ng		
100	76	74	98	396
200	68	66	97	1107
300	63	57	91	3862
400	62	55	89	8892
500	63	55	87	10916

These experiments revealed the following facts:

1) The homogeneities of probe-set with P/M calls between two amplified samples were 87% (20138/22961) and 91% (20138/22209), indicating a high reproducibility.
2) The number of probe-set with P/M call was 24414 in non-amplified sample and 20138 in amplified sample. A 79% of probe-sets with P/M call in non-amplified sample was called P/M in amplified sample. More than 90% and 98% of homogeneities were found between two samples in the probe-sets located on the region within 300 bp and 100 bp from polyA, respectively.
3) In case of probe-sets located on the region within 300 bp from polyA, the correlation coefficient R^2 between two amplified samples was 0.995. That indicates a high reproducibility.
4) In case of probe-sets located on the region within 300 bp from polyA, the correlation coefficient R^2 between amplified sample and non-amplified samples was 0.930.

These experimental results demonstrate that it is possible to amplify mRNA using only 1 ng of total RNA as a starting material without losing their varieties.

After testing the described methods, we analyzed gene expression of single type neurons and small regions of the brain. About 1 ng of RNA was extracted from Purkinje cells, cerebellar granule cell layer, hippocampal CA1 and CA3 regions. Expression profiling of each RNA was examined by Genechip Mouse Genome 430 2.0 Array (Affymetrix). Among ~45000 probe-sets, 736 genes were selected and categorized according to neural functional groups: biosynthetic enzymes of neurotransmitters, neuropeptides, G protein-coupled receptors (GPCRs), ligand-gated channels, voltage-gated channels, relative to voltage-gated channel, and heteromeric G protein (Table 3).

Table 3 Number of genes expressed in Purkinje cell, granule cell layer, CA1 and CA3 regions

Genes	No. of Genes	Present or Marginal call			
		Purkinje cell	Granule cell layer	CA1 region	CA3 region
Neurotransmitter synthetic enzyme	19	8	7	8	9
Neurotransmitter catabolic enzyme	10	5	5	6	6
Neuropeptide	62	9	2	8	5
GPCR	386	56	48	75	68
Ligand-gated channel	76	23	19	27	22
Voltage-gated channel	135	48	46	58	58
Relative to voltage-gated channel	28	6	5	8	6
Heteromeric G protein	33	21	19	23	22

Note: a term, “Relative to voltage-gated channel” , is quoted from KEGG (Kyoto Encyclopedia of Genes and Genomes) database.

3.2 NeuMap

Since transcription factor is responsible for cell fate determination, the expression profiling of transcription factor might be useful to classify cell type. We chose 1321 genes coding transcription factor and their expression profiling was performed on cell types and regions (Table 4). We found cell type- or region-specific expression of transcription factors. As shown in Table 4, 86 genes were expressed in cerebellum but not in hippocampus and 104 genes are expressed in hippocampus but not in cerebellum. Purkinje cell specifically expressed 34 genes, Granule layer specifically expressed 28 genes, CA1 region specifically expressed 32 genes, and CA3 region specifically expressed 22 genes. Genes indicated in red and blue colour in Table 4 might be useful to classify cell types.

Table 4 Transcriptional factors expressed in cell type- and region-specific manner

Expression pattern				Number of gene	Cell type and region
Purkinje cell	granule cell	CA1	CA3		
P	P	P	P	529	
P	P	P	A	14	
P	P	A	P	4	
P	P	A	A	24	cerebellum
P	A	P	P	43	
P	A	P	A	12	
P	A	A	P	8	
P	A	A	A	34	Purkinje
A	P	A	A	28	granule
A	P	P	A	21	
A	P	P	P	48	
A	A	P	P	50	hippocampus
A	P	A	P	7	
A	A	P	A	32	
A	A	A	P	22	CA1
A	A	A	A	445	CA3
Total				1321	
“P” indicates Present/Marginal and “A” indicates Absent call.					

3.3 Activity-dependent gene expression in the CNS neurons

Individual neurons can be activated differentially, depending on a variety of stimuli. Neurons predominantly have two types of action to stimuli: 1) membrane action potential and 2) activity-dependent gene expression of transcription factors and effectors. Membrane action potential is immediate transmission of a synaptic signal on a time scale of milliseconds, whereas activity-dependent gene expression is a late phase modification of gene expression underlying synaptic circuitry on a time scale of minutes or even hours. Some of immediate early genes (IEGs), such as Fos, Egr1 (known as Zif268, NGFI-A and Korx24), and Arc (known as Arg3.1) have been identified as critical indicators of synaptic plasticity and memory processing. However, total analysis of activity-dependent gene expression is still remained to be investigated. To investigate dynamics of activity-dependent gene expression in neurons, we have prepared the following CNS neurons: 1) cultured hippocampal neurons from E17 mice, 2) cultured cerebellar granule neurons from P8 mice, 3) organotypic hippocampal slices from P7 mice, and 4) acute hippocampal slices from adult mice.

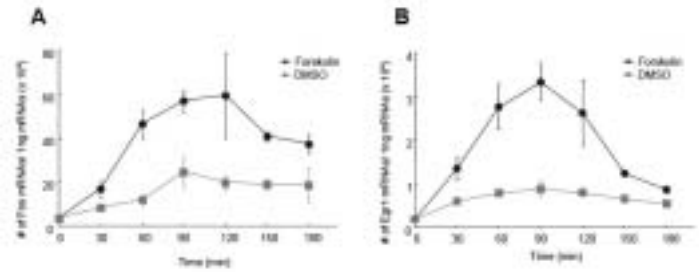


Fig. 1 Time course (min) of forskolin stimulation in cultured hippocampal cells on DIV5

Using real-time RT-PCR, we investigated time courses of gene expression in these cultured cells and slices after various stimuli. The time course of expression of Fos and Egr1 mRNAs in cultured hippocampal cells induced by 10 μm forskolin, a stimulant for cAMP signaling pathway, is shown in Fig. 1.

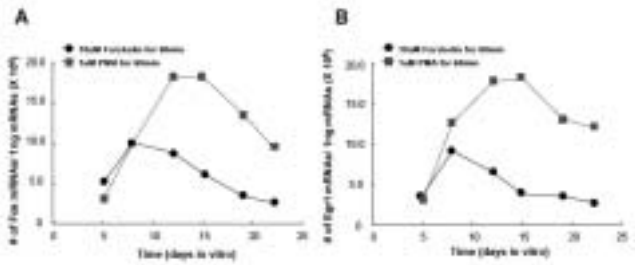


Fig. 2 Time course (days in vitro) of forskolin stimulation in cultured cerebellar granule neurons

Fig. 2 shows the time course of forskolin and PMA stimulation in cultured cerebellar granule cells. The peak expression of Fos and Egr1 induced by forskolin was observed on 8-10 days of *in vitro* culture. Whereas, the peak expression of Fos and Egr1 induced by PMA was observed on 12-15 days of *in vitro* culture.

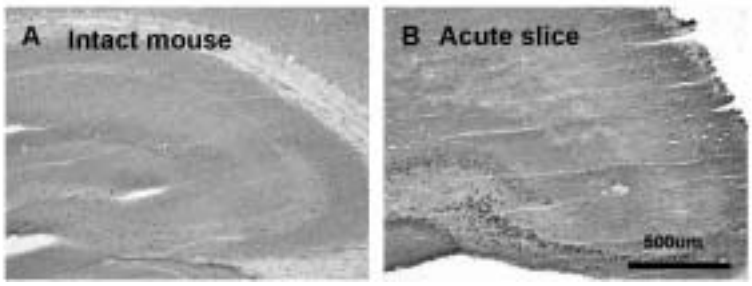


Fig. 3 Expression pattern of Fos protein in intact (A) and acute (B) slice of mouse hippocampus

Furthermore using immunohistochemistry and *in situ* hybridization histochemistry, we have visualized the spatial expression patterns of Fos and Egr1 in these cells and tissues induced by forskolin stimulation. Fos proteins were identified by immunohistochemistry, as shown in Fig. 3.

We will use these neurons for further quantitative and imaging analysis in the next years to investigate dynamics of activity-dependent gene expression. It is important to identify all input into the neurons. We already identified all receptors (all input) on some of the neurons by DNA microarray analysis described above. Based on the expression data of receptors on the neurons, we will study dynamics of activity-dependent gene expression in the neurons.

3.4 Molecular profiling of the brain by mass analysis

We established methods for analysis of the brain chemicals using nano LCMS (liquid chromatography mass spectrometry) and CEMS (capillary electrophoresis mass spectrometry).
Method:
Brain tissue extract was prepared and analyzed by MS, as shown in the flow diagram (Fig. 4).

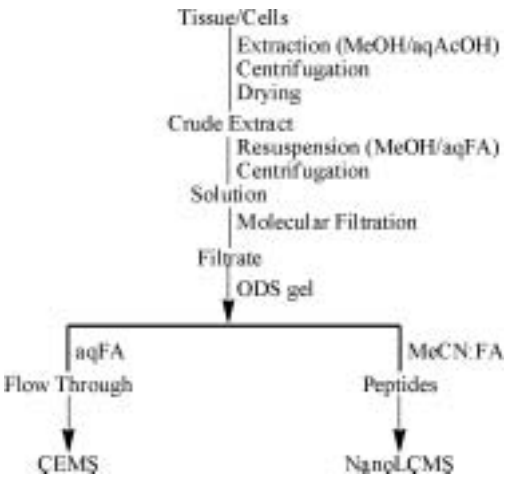


Fig. 4 Flow diagram of the preparation

Application:
Using the above method, analysis of pig cerebrospinal fluid (CSF) and rat CSF showed about 200 peptide signals. Every peptide signal can be sequenced manually and/or using auto de novo sequencing program (PEAKS studio, BSI). Resulting peptide sequences can then be searched against a protein database (UniProt, Swiss-Prot) to get the known protein information and their

functions. At the same time, peptide fragments whose functions are unknown could also be identified. These unknown peptides may turn out to be new functional molecules or markers for diagnosis.

For diagnosis and for the study of changes in physiological states of animals, differential expression study between different samples is important. We carried out differential study between CSF samples using SEIVE program and confirmed that it worked well. We will apply it to study daily rhythm of animal and molecular profiling between normal and disease state of animal. We also developed a combination program to combine SEIVE and PEAKS Studio for this differentiation study. This program will assist us faster identification of interesting peptide and their sequence.

We applied the above CEMS method to analyze water soluble molecules in animal brain tissue. All amino acids, GABA, serotonin, and dopamine were identified in pig striatum. All of these compounds were also found in rat striatum. There are some unidentified compounds in rat striatum sample. We will use the CEMS method to identify monoamines and their derivatives produced in the brain. We will collect intercellular fluid from different parts of the brain. The fluids' molecular signature will then be characterized by the CEMS. Some of these molecules may likely have important functions.

3.5 Salamander project

General matter

Because of large cell size, the salamander is thought to be a good material to study the nervous system, (especially for imaging studies, electrophysiology, and single-cell analysis). After screening several species of salamander, *Ambystoma mexicanum* has been selected as the experimental material. We constructed cDNA libraries of the brain, retina, and spinal cord of the salamander. In 2007, we have carried out mainly sequencing of cDNAs from these libraries, as well as some extent of morphological study. After completing full length sequencing of 15,000 cDNAs, we will make a salamander cDNA database for future study.

Brain atlas

Availability of the atlas is necessary for neurological research. Atlas of *ambystoma mexicanum* was constructed by using coronal, horizontal, and sagittal sections through brain with labels. Some examples are shown in Fig. 5.

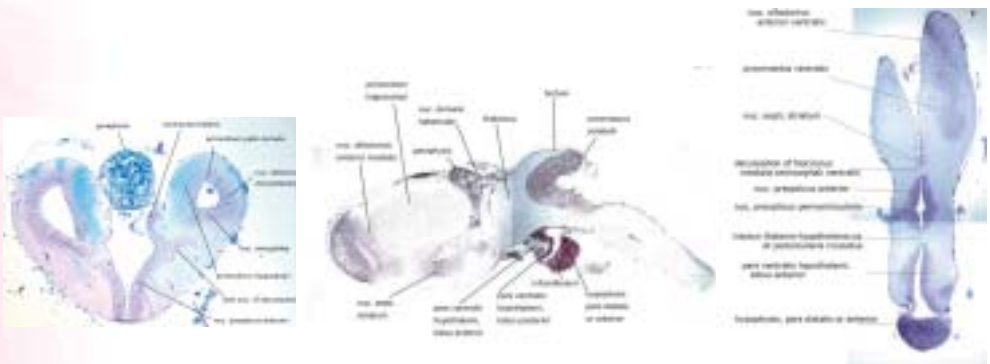


Fig. 5 Coronal section, sagittal section, and horizontal section of Ambystoma mexicanum from left to right. Coronal section and horizontal section were stained with Klüver-Barrera method. Sagittal section was stained with Bodian's method.

Cell types in hypophysis of *ambystoma mexicanum* and projections from brain to hypophysis

To analyze neural networks between brain and hypophysis, it is necessary to establish a brain atlas showing connectivity with hypophysis. Histochemical analysis identified at least five endocrine cell types as putative β-basophile, δ-basophile, weak-basophile, α-acidophile, and ε-acidophile (Fig. 6). Neuroendocrine cells projecting to hypophysis were also identified in both side of the third ventricle, corresponding to the nucleus preopticus (Fig. 6).

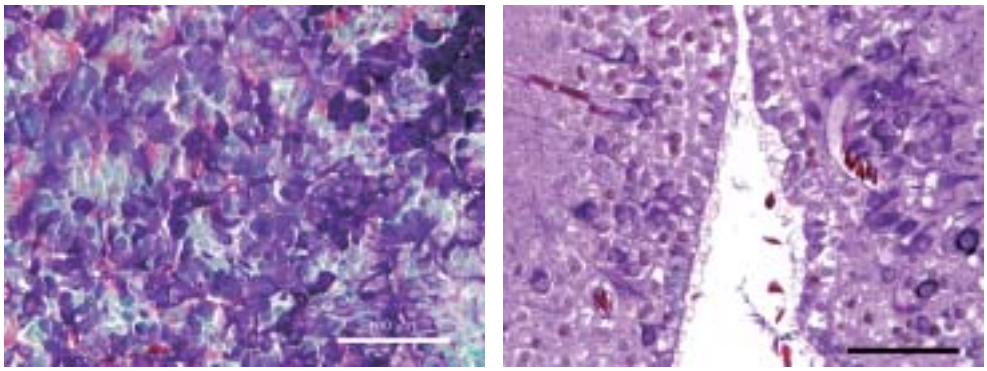


Fig. 6 Five cell types in the hypophysis (left) and neurosecretory cells in the nucleus preopticus (right) projecting to hypophysis were identified in horizontal sections stained with aldehyde fuchsin. Bars, 100 μm.

Neuronal network imaging in the brain of *ambystoma mexicanum*

To elucidate the connectivity between different populations of neurons, multiple axonal tracing methods are important. A preliminary effort to construct a tracing system is going on. Iontophoretic microinjection of biocytin into the habenulae nucleus in vivo demonstrated incorporation of tracer in somata and anterograde transport in axons and axon bundles (Fig. 7).

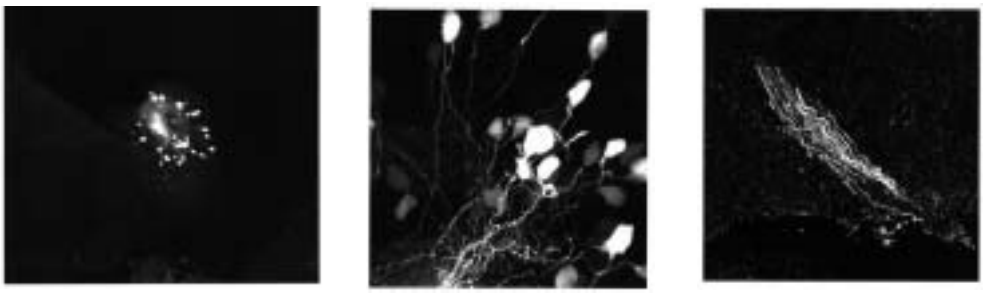


Fig. 7 Iontophoretically microinjected biocytin into habenulae nucleus (left). In higher magnification somata and axons were shown (middle). Axon bundles from habenulae nucleus were traced in a ventral area of diencephalon (right). 50 μm thick sagittal vibratome sections were used.

Ultrastructure of synapse in salamander's retina

To examine whether the ultrastructure of salamander's cells is equivalent to mammalian cells, transmission electron microscopic observation is going on. An example is shown here: the diameter of small synaptic vesicles in salamander's retina was slightly larger than that of murine and the large dense cored-vesicles were more frequently found in salamander's neuron than murine one (Fig. 8).

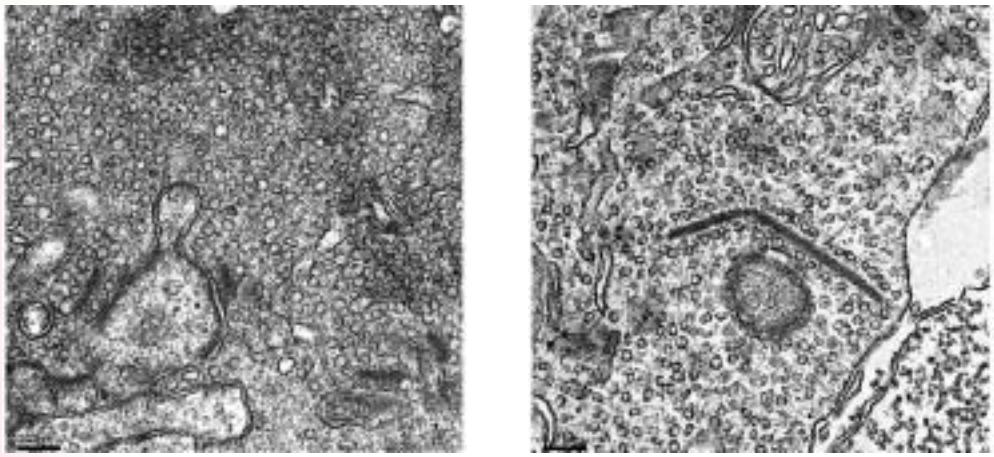


Fig. 8 The diameter of small synaptic vesicles was slightly larger in salamander's retina (left) than in murine retina (right).

Sequencing of cDNAs and construction of cDNA database

We are carrying out sequencing of cDNAs from the cDNA libraries of brain, retina, and spinal cord in collaboration with Brenner Unit. (Details; see Brenner Unit)

4. Publications

4.1 Journals

Akiyama K., Nakanishi S., Nakamura N. H., Naito T. (2008) Gene expression profiling of neuropeptides in mouse cerebellum, hippocampus, and retina, Nutrition, (in press).

Akiyama K., Morita H., Suetsugu S., Kuraba S., Numata Y., Yamamoto Y., Inui K., Ideura T., Wakisaka N., Nakano K., Oniki H., Takenawa T., Matsuyama M., and Yoshimura A. (2008) Actin-related protein 3 (Arp3) is mutated in proteinuric BUF/Mna rats., Mammalian Genome, 19, 41-50.

Nakamura N. H., Akama K. T., Yuen G. S., McEwen B. S. (2007) "Thinking outside the pyramidal cell: unexplored contributions of interneurons and neuropeptide Y to estrogen-induced synapse formation in the hippocampus.", Reviews in the Neurosciences, 18, 1-13.

Kita, M., Roy, M. C., Siwu, E. R. O., Noma, I., Takiguchi, T., Itoh, M., Yamada, K., Koyama, T., Iwashita, T., and Uemura, D.". (2007) A long carbon-chain polyol compound from the symbiotic dinoflagellate Durinskia sp, Tetrahedron Lett., 48, 3423-3427.

Kita, M., Roy, M. C, Siwu, E. R. O., Noma, I., Takiguchi, T., Yamada, K., Koyama, T., Iwashita, T., Wakamiya, A., and Uemura, D. (2007) Relative stereochemistries of the ether rings and sugar moieties in durinskiol A, Tetrahedron Lett., 48, 3429-3432.

4.2 Book(s) and other one-time publications

Nothing to be reported.

4.3 Oral presentations

Naito T. Genes and brain functions, The 12th Conference of Peace through Mind/Brain Science, Hamamatsu, Japan, February 5-7, 2008.

Naito T. Study of neuropeptides using gene expression analysis , The 9th Neuropeptide Y Meeting, Okinawa, Japan, March 16-20, 2008.

4.4 Posters

Akiyama K., Nakanishi S., Naito T. Gene expression analysis of single type-neuron(1)-methodology, The 30th Annual Meeting of the Molecular Biology Society of Japan and the 80th Annual Meeting of the Japanease Biochemical Society, Pacifico Yokohama, Yokohama, Japan, December 11-15 2007.

Nakanishi S., Akiyama K., Naito T. Gene expression analysis of single type-neuron(2)-analysis of murine Purkinje cell and granule cell-, The 30th Annual Meeting of the Molecular Biology Society of Japan and the 80th Annual Meeting of the Japanease Biochemical Society, Pacifico Yokohama, Yokohama, Japan, December 11-15 2007.

5. Intellectual Property Rights and Other Specific Achievements

Nothing to be reported.

6. Meetings and Events

6.1 Lecture at The University of Tokyo

Title: Molecular analysis of brain functions
Date: November 9, 2007
Venue: The University of Tokyo, Faculty of Pharmaceutical Sciences, Tokyo, Japan,
Speakers: Takayuki Naito

6.2 The 9th Neuropeptide Y Meeting

Date: March 16-20, 2008
Venue: Kariyushi Beach Resort Ocean Spa, Okinawa, Japan
Co-organizers: Akio Inui (Kagoshima University), Takayuki Naito(OIST)

6.3 Pre G8 Genome meeting in Okinawa

Date: March 28, 2008
Venue: Loisir Hotel, Okinawa, Japan
Co-organizers: The Okinawa Prefectural Council for Promotion of the
Okinawa Institute of Science and Technology

XII. Mathematical Biology Unit

Principal Investigator: Robert Sinclair
Research Theme: Mathematical Biology

Abstract

Research in biology is a constant source of challenge and opportunity for mathematicians. We are particularly interested in fields which are still underdeveloped mathematically, but are of use in or of relevance to biological research. We believe that new mathematics will arise from work in these areas. For example, statistics typically assumes a large number of data points or some detailed knowledge of the probability distribution of the experimental data. In cases where neither is available, there are currently essentially no well-founded mathematical methods which can be used to analyse the data. We have been attempting to create new mathematical approaches which may be applied to such cases. In addition to our work in this area, we have been collaborating with other units at OIST, especially in the area of neuroscience. Finally, we keep up-to-date with current developments in pure mathematics by remaining active in mathematical research and its applications to areas outside of biology.

1. Staff

Mathematical Biology

Group leader: Dr. Robert Sinclair

Researcher: Dr. Tobias Mühlenbruch

Research administrator/secretary: Mrs. Shino Fibbs

2. Partner Organizations

Nothing to be reported.

3. Activities and Findings

Both Dr. Sinclair and Dr. Mühlenbruch have most recently (before coming to OIST) been working on abstract mathematical problems. We are both investing a large amount of our time in learning biology, becoming familiar with terminology and the types of questions which are of interest to modern biologists. The reader will notice that the list of activities and findings below reflects this state of transition. We have work completed or near completion which has no relation to biology, and work in its initial stages which is heavily motivated by biology. The research actually performed within the unit will become more and more relevant to biology over the next few years.

3.1 "Data Analysis – Extremely Small Sample Sizes" (Dr. Sinclair)

This has been a major thrust, in an attempt to find generalizations of useful concepts such as the standard error of the mean to extremely small data sets (less than ten data points), which do frequently appear in cutting-edge biological research papers. The experimentalist can be faced with the dilemma of having data which is the result of an enormous investment in time and effort but not in sufficient quantity to allow one to apply classical statistical analysis without reservations.

The crucial point is that classical statistical methods almost always assume that (i) the data is representative of the probability distribution it is imagined to be drawn from and/or (ii) this probability distribution is known, perhaps up to some parameters. Where neither of these apply, there is very little one can do. If one applies well-known methods such as bootstrapping to a data set which is not representative, then the quantitative results may still be seriously flawed. The use of any method which uses the standard deviation of the data sample to estimate the standard deviation of the underlying probability distribution will be similarly difficult to justify.

We have focused on the case of estmating the error in the mean of a small number of experimental measurements of some quantity such as mass or voltage.

The starting point of our considerations has been (i) to include from the beginning the fact that all measurements are associated with error, and (ii) to consider only cases in which an error estimate would be possible at all. Regarding point (i), this means that we do not begin with a continuous probability distribution defined upon the real numbers. Instead, we take discrete probability distributions defined upon the finite set of measurable values of the quantity of interest. For example, we consider only those values which are possible readouts of the equipment being used.

Regarding point (ii), one must admit that it is not possible to make any reasonable estimate of error unless one has some idea of the possible range of possible measurements. We therefore make explicit use of the expected range in our formulation of the problem. This is often trivial, since one can be sure that the mass of any biologically relevant object is positive etc.

We have already made solid progress here. It has been possible to generalize the concept of the standard error of the mean to all sample sizes, including n=1, in a manner which appears to be reasonable and applicable to real experiments. We have identified a single underlying principle, making use of the concept of likelihood, from which qualitatively different predictions emerge, in dependence upon the sample size. It thus seems possible to unify large-sample and small-sample error estimation, despite the fact that the formulae which are used do differ.

3.2 “Data Analysis – Fundamental Studies of the Bayesian Paradigm” (Dr. Sinclair)

Bayesian analysis is an extremely successful and practical theory, first put forward more than two hundred years ago. It is of interest to biologists for two reasons. First, it can be applied to problems of inference from experimental data (that was its original application). Second, there are indications that our nervous systems may use some form of Bayesian analysis to make predictions about the world we live in.

Bayesian analysis cannot be performed or even formulated within the framework of classical “frequentist” statistics, since the Bayesian concept of probability is fundamentally different from the statistical concept. In the former, probability is defined in terms of belief. In the latter, probability is defined in terms of the outcomes of an (imagined) infinite number of experiments.

We are attempting to find ways of bridging the divide between these two approaches, in the firm belief that they both have something solid to offer biology. The central theoretical point

of contention has been the role of belief in the Bayesian paradigm. We have therefore made steps to create examples of computations which are of a Bayesian nature, and yet do not involve belief (no "prior information" is put in). It appears that this is possible to a limited extent.

We have been able to show that the influence of the experimenter's belief can be suspended indefinitely (this is to be understood in the context of a so-called hierarchical model _the prior need not be specified at any finite level). We are aware of the fact that such suspension is not always desirable in practical applications. Our primary interest is theoretical.

This work is difficult because of the conceptual and notational differences between the two branches of scientific literature it attempts to bridge, and also because a complete mathematical understanding of the relevant probability spaces is not yet available. Nonetheless, some results of an experimental nature (in the sense of experimental mathematics) have been obtained, and we are in the process of writing a paper. Dr. Sinclair visited Prof. Erkki Somersalo of Helsinki University in late March 2008 to discuss this work.

3.3 "Discriminating between Selection and Inertia – A study of Ancient Tandem Repeats" (Dr. Sinclair)

Imprefect tandem repeat patterns in amino acid sequences can be due to selection (the modification of an ancient non-repeating pattern to form a pattern with a repeat structure) or what one may call inertia (the remnants of an ancient, perfect tandem repeat pattern, perhaps due to slippage, which has become imperfect due to mutations and selection).

We are aware of a number of examples of genes (such as NadC) with gene sequences which typically display very imperfect tandem repeats, but whose apparent repeat patterns also display an unusually high degree of symmetry for a few unrelated species. We hypothesize that these isolated examples of relative perfection within a family grouping are in fact evidence of an ancient perfect tandem repeat structure, which has been more or less lost due to the actions of selection and mutation in most extant species.

mathematical challenge here is to devise logical approaches to this question.
Inferring ancient sequences from modern sequence data is an area in which it is difficult to avoid circular arguments. At present, we can say that the strongest arguments we can make are of a more qualitative rather than quantitative nature. We are able to include chemical and ecological knowledge into our analysis. We have already demonstrated that our ideas can be applied to real data and expect real progress in the near future.

3.4 "Models of Worm Brains" (Dr. Sinclair)

This is a long-term project in collaboration with Dr. Ichiro Maruyama of OIST. Many researchers have proposed mathematical models of the nervous system of C.elegans, but none of these models has been entirely convincing, despite the wealth of information known about the worm's nervous system. We are interested in new approaches which would have predictive power but also give some theoretical understanding of the basic design of nematode nervous systems.

present, it would appear that those activities which do not involve learning may best be modeled by assuming that the nervous system acts as an immediate linear map from what one may call sensors to actuators, some of which have the function of "timers". A timer is any

mechanism which introduces a time scale into the operation of the nervous system. An obvious example would be the sense of hunger, which of course appears some time after eating. The timing of reverse movement associated with the tap reflex is another example. We are interested in confronting such a theoretical model with experiments.

Prof. Stretton of the University of Wisconsin-Madison was invited specifically to discuss this project.

3.5 "Applications of Mathematics to the Neurosciences" (Dr. Sinclair)

We have been involved in a very fruitful collaboration with Dr. Klaus Stiefel of OIST and members of his unit. It has been possible to apply classical results from applied mathematics to problems in the neurosciences. The specific study was of the relationship between form and function for a neuron. We were able to produce quantitative predictions from a simple model which matched the results of a more involved computational project. The two approaches complement each other in the sense that the mathematical model allows one to understand some aspects of the computational studies, but also that the computational studies validate the simplifications made in constructing the mathematical model. One joint paper has recently been submitted.

3.6 "A computational study of the effect of the discrete Laplace operator on directed finite clustered graphs" (Dr. Mühlenbruch)

The discrete Laplace operator on graphs and and related operators are well studied objects in mathematics and physics. Its spectrum encodes properties of the underlying graph. We want to see how properties of the Laplace operator behave if the operator acts on biologically inspired networks. Clustered networks, found in e.g. cortical networks, are of particular interest.

This project started in March 2008. Dr. Mühlenbruch collaborates with Prof. Claus Hilgetag (Jacobs University, Bremen, Germany), and looks forward to interaction with neuroscience units at OIST. Dr. Wickens has already expressed some interest in the outcomes of this and related work.

3.7 "Nearest integer continued fractions and its extension to Hecke triangle groups" (Dr. Mühlenbruch)

E. Artin showed in 1924 that the usual Gauss continued fractions are well suited for coding geodesics on the hyperbolic surface H/GL(2,Z). However, it turned out that we cannot extend this results to the coding of geodesics on the hyperbolic surface H/G(q) where G(q) is the q-th Hecke triangle group, generated by the reflection z -> -1/z and the translation z -> z + 2 cos pi/q, q = 3,4,5,... . On the other hand, the nearest integer fractions, studied by A. Hurwitz in 1889, have some properties which allow us to write the continued fractions directly in terms of the generators of the Hecke triangle group G(3). We investigated how the nearest integer continued fractions can be extended to a continued fraction compatible with the generators of G(q) for arbitrary q and how this extended continued fractions can be used to code the geodesics on the hyperbolic surface. It turns out that there is indeed a mapping between closed geodesics and eventually periodic continued fractions. The mapping is bijective up to one exceptional class of closed geodesics where the mapping is 1-2.

This is part of a larger project with international collaborators, which has already resulted in one paper (January 2007) in the renowned journal Journal fuer die reine und angewandte Mathematik. One further paper has been submitted to the Journal of Number Theory, and another is in an advanced stage of preparation.

3.8 "Conjugate and cut loci of a two-sphere of revolution with application to optimal control" (Dr. Sinclair)

This is work in collaboration with Prof. Minoru Tanaka of Tokai University, and Profs. Bernard Bonnard and Jean-Baptiste Caillau of Bourgogne University. It is an interdisciplinary approach to problems arising from spaceflight (orbital transfer) and control theory (a branch of engineering) applied to quantum mechanical systems, using methods from differential geometry which have been developed by Prof. Tanaka over many years. Dr. Sinclair has collaborated with Prof. Tanaka frequently over the past decade, using computational techniques to support or motivate theoretical results.

This work has already resulted in one joint paper which will appear in the French journal Ann. Inst. H. Poincaré Anal. Non Linéire.

3.9 "The Total Absolute Curvature of Open Curves" (Dr. Sinclair)

This was work in collaboration with Prof. Kazuyuki Enomoto of the Tokyo University of Science and Prof. Jin-Ichi Itoh of Kumamoto University. The object was to classify those open curves in space which are optimal with respect to their total curvature (ignoring its direction). There is an unexpected biological connection here, since proteins are open curves in space, and their curvature is known to be biologically relevant. We hope that the results of this work may be applicable to the theoretical problem of protein folding. The results have direct applications to robot arm design.

A paper will appear in the Illinois Journal of Mathematics in 2008.

3.10 "Cosmetic Design based upon Genetics" (Drs. Sinclair and Mühlenbruch)

We have had several meetings with Dr. Yoshio Okada, Director of the R&D Division of the Bio21 Corporation. So far, the discussions have been of a very introductory nature. We are exploring possibilities of the application of mathematics to cosmetic design in an Okinawan context.

4. Publications

4.1 Journals

Sinclair, R., Tanaka, M. (2007) The cut locus of a two-sphere of revolution and Toponogov's comparison theorem, Tohoku Mathematical Journal, 2nd series, 59(3), 379-399.

4.2 Book(s) and other one-time publications

none

4.3 Oral presentations

Mühlenbruch, T. Maass wave forms, period functions, transfer operators and dynamics of continued fraction, Kobe University, June 25, 2007.

Mühlenbruch, T. Maass wave forms, period functions, transfer operators and dynamics of continued fraction, Osaka City University, June 27, 2007.

Mühlenbruch, T. The dynamics of the nearest continued fraction map and its relations to Maass wave forms, Keio University, October 22, 2007.

4.4 Posters

Torben-Nielsen, B., Sinclair, R., Stiefel, K. M. Systematic Mapping of Neural Function to Morphology, CNS, Toronto, Canada, July 11, 2007.

Torben-Nielsen, B., Sinclair, R., Stiefel, K. M. Systematic Mapping of Neural Function to Morphology, SFN, San Diego, US. Poster, America, November 4, 2007.

5. Intellectual Property Rights and Other Specific Achievements

none

6. Meetings and Events

Both Dr. Sinclair and Dr. Mühlenbruch have made numerous appearances in OIST-internal journal clubs and research meetings. Here we will list only more formal events.

6.1 Inverse Problems and Biology Workshop

Date: April 19-21, 2007

Venue: Seaside House

Co-organizers: Dr. Klaus Stiefel

Speakers: Martin Kreitman, The University of Chicago, Department of Ecology and Evolution
Andreas Dress, CAS-MPG Partner Institute for Computational Biology, Department of Combinatorics and Geometry
Hans-Christian Hege, Zuse Institute Berlin, Visualization and Data Analysis Department
Bob Anderssen, CSIRO (Australia), Mathematical and Information Sciences
Wayne Rossman, Kobe University, Department of Mathematics
Robert Sinclair, OIST
Klaus Stiefel, OIST
Peter Waddell, University of South Carolina, South Carolina Cancer Center
Erkki Somersalo, Helsinki University of Technology, Institute of Mathematics
Daniela Calvetti, Case Western Reserve University, Department of Mathematics

6.2 Joint Seminar with the Department of Mathematics of the University of the Ryukyus:Symbolic Dynamics and Transfer Operators

Date: September 27, 2007

Venue: Department of Mathematics of the University of the Ryukyus

Co-organizers: Prof. Toshiaki Suzuki, University of the Ryukyus

Speakers: Fredrik Stroemberg, TU-Clausthal, Institute for Theoretical Physics

6.3 OIST Special Seminar: Neuropeptides in Nematodes

Date: November 16, 2007
Venue: IRP Conference Room
Co-organizers: Dr. Ichiro Maruyama
Speakers: Antony Stretton, University of Wisconsin-Madison, Department of Zoology

6.4 OIST Special Seminar: Global organization of cerebral cortical networks

Date: March 18, 2008
Venue: IRP Conference Room
Co-organizers: Dr. Jeff Wickens
Speakers: Hilgetag, Jacobs University, Bremen

6.5 OIST Seminar: From Knot Theory to Molecular Biology

Date: September 4, 2007
Venue: IRP Conference Room
Speakers: Dr. Nafaa Chbili, Korea Advanced Institute of Science and Technology
Other remarks: We will co-organise an international PhD-level course with Dr. Chbili in 2009.

6.6 OIST Seminar: Integrating mass spectrometry and behavioral neuroscience in fruit fly models of courtship and aggression

Date: February 27, 2008
Venue: OITC Seminar Room
Speakers: Dr. Joanne Yew, Harvard Medical School, Department of Neurobiology

6.7 OIST Seminar: A Diamond Twin-an application of discrete geometric analysis

Date: March 19, 2008
Venue: IRP Conference Room
Speakers: Prof. Toshikazu Sunada, Meiji University, Department of Mathematics

6.8 OIST Seminar: The thermodynamic formalism: from the statistical mechanics of spin systems to applications in evolutionary biology

Date: March 27, 2008
Venue: IRP Conference Room
Speakers: Prof. Dieter Mayer, Clausthal Technical University, Germany

XIII. Developmental Neurobiology Unit

Principal Investigator: 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. Staff

Researchers: Dr. Yuko Nishiwaki, Dr. Toshiaki Mochizuki, Dr. Fumiyasu Imai, Dr. Yukihiro Yoshimura

Technical staff: Ms. Keiko Iriyama, Ms. Ayako Yuasa-Nagashima, Ms. Nozomi Hanahara

Research administrator/secretary: Ms. Ayako Gima

2. Partner Organizations

RIKEN Brain Science Institute

Type of partnership: Collaborative research

Name of principal researcher: Dr. Hitoshi Okamoto

Research theme: Molecular cloning of zebrafish genes that regulate neuronal differentiation in the brain

3. Activities and Findings

3.1 Mechanism that regulates the spatial and temporal pattern of retinal neurogenesis in zebrafish

In the developing zebrafish retina, neurogenesis is initiated at a few cells adjacent to the optic stalk and progresses to the entire neural retina. Such a pattern of retinal neurogenesis provides a good model for studying the mechanism underlying the spatial and temporal pattern of neurogenesis in the nervous system. Previous studies including ours suggest that at least five signaling molecules regulate the pattern of retinal neurogenesis in zebrafish. Fibroblast growth factors (Fgfs) are expressed in the optic stalk and required for the initial induction of retinal neurogenesis in zebrafish. The Hedgehog signaling pathway is important for the progression of retinal neurogenesis in zebrafish. The activation of Wnt and Notch signaling pathways promotes cell proliferation and inhibits neurogenesis in the zebrafish retina, respectively. Previously, we identified a zebrafish mutant namely *ascending and descending* (*add*), in which retinal progenitor

cells fail to exit from the cell cycle and continue to proliferate (Fig. 1). We showed that the *add* mutant gene encodes histone deacetylase 1 (HDAC1), and that HDAC1 promotes retinal neurogenesis by suppressing both Wnt and Notch signaling pathways. 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 HDAC1-dependent regulation of retinal neurogenesis, we will identify factors interacting with HDAC1 and its downstream targets. As a genetic approach, we searched new mutations that enhance or suppress the hyperproliferation of retinal cells in the *add* mutant. To date, we screened 72 mutagenized genomes and identified two mutations that suppress the *add*-mediated hyperproliferation. We are currently characterizing phenotypes of these mutants.

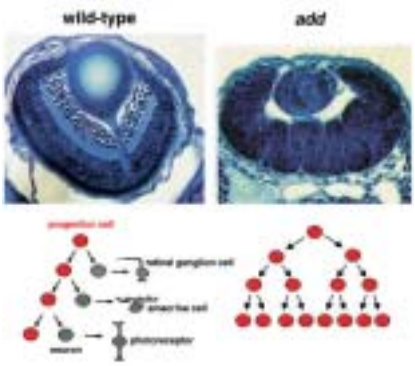


Fig. 1 *add* mutant retina at 2dpf. In the *add* mutant, retinal progenitor cells (red) fail to exit from the cell cycle and continue to proliferate.

3.2 Mechanism that regulates apoptosis during retinal development in zebrafish

After the cell-cycle exit, postmitotic retinal cells differentiate into six major classes of retinal neurons and form the neural network responsible for phototransduction. To elucidate the mechanisms underlying the late stages in neuronal differentiation, we identified zebrafish mutants that show various defects in neuronal differentiation and neural circuit formation in the retina. Among them, five mutants show severe apoptosis in the developing retina. In this class of mutants, differentiating retinal neurons are selectively eliminated prior to their maturation, suggesting that the switch between differentiation and cell death seems deregulated. *pinball eye* (*piy*) is one of these apoptotic mutants and shows extensive apoptosis of retinal neurons. We found that a missense mutation occurred in the small subunit of DNA primase (Prim1) in the *piy* mutant. 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'. Thus, Prim1 is essential for DNA replication. However, this mutation does not affect cell proliferation but rather induces neuronal apoptosis. It was reported that the RNA synthesis by Prim1 is required for the activation of DNA damage response, which may activate Ataxia telangiectacia mutated (ATM), Checkpoint kinase 2 (Chk2) and the tumor suppressor p53. We found that apoptotic induced by the prim1 mutation depends on the ATM-Chk2-p53 apoptotic pathway. The blockade of ATM, Chk2 or p53 significantly suppresses the *piy*-mediated apoptosis of retinal cells. These data suggest that the surveillance system of genomic integrity strongly determine whether zebrafish retinal cells will continue to differentiate normally or undergo apoptosis.

3.3 Mechanism that regulates 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 and identified zebrafish mutants with disrupted visual behaviors such as optokinetic response (OKR). *The eclipse* (*els*) mutant is one of such OKR-defective mutants. We found that the *els* mutant gene encodes cGMP-phosphodiesterase 6 α'-subunit (PDE6α'). 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. The *els* mutant displays no visual response to light, where cones are active, but shows relatively normal OKR to the dim light, where only rods function. These observations suggest that only the cone-specific phototransdution pathway is disrupted in the *els* mutant. In human genetic diseases associated with photoreceptor degeneration, mutations in the PDE6 α and β subunit genes have been reported. However, no mutation in PDE6α' genes have been found in human. To elucidate whether photoreceptor degeneration occurs in the absence of PDE6α' activity, we examined the morphology of the *els* mutant retinas during development. We found that cone photoreceptors are selectively eliminated in the adult *els* mutant retinas, suggesting that cones undergo a progressive degeneration in the *els* mutant retinas. Taken together, these data suggest that PDE6α' regulates not only cone-specific phototransduction but also cone maintenance.

4. Publications

4.1 Journals

Tanaka, H., Maeda, R., Shoji, W., Wada, H., Masai, I., Shiraki, T., Kobayashi, M., Nakayama, R., Okamoto, H. (2007) Novel mutations affecting axon guidance in zebrafish and a role for plexin signaling in the guidance of trigeminal and facial nerve axons, Development, 134, 3259-3269.

Yamaguchi, M., Fujimori-Tonou, N., Yoshimura, Y., Kishi, T., Okamoto, H., Masai, I. (2008) Mutation of DNA primase causes extensive apoptosis of retinal neurons through the activation of DNA damage and checkpoint and tumor suppressor p53, Development, 135, 1247-1257.

4.2 Book(s) and other one-time publications

none

4.3 Oral presentations

Masai, I. Mechanism regulating apoptosis during retinal development, 2007, Annual conference of the Japanese society of Molecular Biology, Yokohama, Japan, December 11-15, 2007.

4.4 Posters

Yamaguchi, M., Fujimori, N., Yoshimura, Y., Kishi, T., Okamoto, H., Masai, I. Mechanism underlying apoptosis during retinal development, 6th symposium “Development, differentiation and regeneration” organized by CREST JST, Tokyo, Japan, November 20, 2007, (in Japanese).

Nishiwaki, Y., Komori, A., Sagara, H., Suzuki, E., Manabe, T., Hosoya, T., Nojima, Y., Wada, H., Tanaka, H., Okamoto, H., Masai, I. Analysis of zebrafish photoreceptor degeneration mutants, which provide a model of hereditary human diseases, 6th symposium “Development, differentiation and regeneration” organized by CREST JST, Tokyo, Japan, November 20, 2007, (in Japanese)

5. Intellectual Property Rights and Other Specific Achievements

Nothing to be reported.

6. Meetings and Events

6.1 Workshop in the 2007 annual conference of Japanese society of Molecular Biology, which is entitled “The latest frontier of retinal research- From development to regeneration”

Date: December 13, 2007

Venue: Yokohama, Japan

Co-organizers: Professor Sumiko Watanabe (Tokyo University)

Speakers: Dr. Kunimasa Ohta (Kumamoto University)
Dr. Ichiro Masai (OIST)
Dr. Sinya Sato (Tokyo University)
Dr. Yoshihiro Ohmori (Harvard Medical Shool)
Dr. Masayuki Akimoto (Agamasaki Hospital)
Dr. Hiroshi Ueda (Nagasaki University)
Dr. Masayo Takahashi (RIKEN CDB)

Other remarks: This is not internal workshop in the OIST.

XIV. Theoretical and Experimental Neurobiology Unit

Principal Investigator: Dr. Klaus M. Stiefel
Research Theme: Single cell computation, Dendritic morphology, Theoretical Neurobiology

Abstract

The goal of this research unit is to shed more light on the function of individual brain cells. For that purpose, we are using a combination of experimental and theoretical approaches.

The experimental approaches are whole-cell patch clamp recordings in slices of the mouse frontal cortex. Data acquisition is done with LabView. The basic question we want to answer is; what type of computer is a neuron? We are studying several aspects of the signal processing of neurons, such as their precision, phase-reset curves as well as their behavior under intrinsic and forced oscillations. As neurons in the cortex are subject to a variety of neuromodulatory (dopaminergic, cholinergic) influences, we are also studying all the afromentioned phenomena in cells subjected to these modulators.

The theoretical approach is to look for correlations between dendritic shape and function. To investigate this relationship, we have developed a method of finding models of neurons with dendritic trees optimized for a given type of computation. This method utilizes genetic algorithms as a search method and L-systems as a method for generating dendritic trees.

In our first year at OIST, we established the research infrastructure to conduct *in vitro* recordings and large-scale computational simulations.

1. Staff

Theoretical and Experimental Neurobiology Unit
Researchers: Maxence LeVasseur, Ben Torben-Nielsen
Research administrator/secretary: Ryoko Uchida

2. Partner Organizations

Mathematical Biology Unit @ OIST
Type of partnership: Collaboration
Name of principal researcher: Dr. Robert Sinclair
Research theme: Dendritic function – structure relationships

Case Western Reserve University, Cleveland
Type of partnership: Collaboration
Name of principal researcher: Dr. Peter J. Thomas
Research theme: Neural sub-threshold oscillations

CNRS, Paris
Type of partnership: Collaboration
Name of principal researcher: Dr. Boris S. Gutkin
Research theme Neural oscillators

Austrian Research Institute for Artificial Intelligence
Type of partnership: Collaboration
Name of principal researcher: Dr. Arthur Flexer
Research theme Data mining of multi-compartmental simulations

3. Activities and Findings

This being our first year at OIST, a large part of our efforts was directed in establishing the research infrastructure for our unit. This was successful, we performed the 1st *in vitro* recordings in late 2007 and routinely ran simulations on our computational cluster since mid 2007.

Experimentally, we started to address the influence of neuromodulators on the precision of neural spiking in the frontal cortex. These studies are conducted with the patch-clamp technique in slices of the mouse frontal cortex. We use a field-programmable gate array (FPGA) to implement the dynamic clamp recording technique. An FPGA is a price of reconfigurable hardware, and to our knowledge, we are the first ones using this technology for electrophysiological recordings.

Theoretically, we started addressing the relationship between the function and structure of dendrites. We use large-scale computer simulations for this purpose which search for optimized dendritic trees in model neurons. A manuscript describing this research has been submitted in April 2008.

4. Publications

4.1 Journals

Stiefel, KM., Sejnowski, TJ. Mapping Function onto Neuronal Morphology., J Neurophysiology, 2007 Jul;98(1):513-26.

Englitz B., Stiefel, KM., Sejnowski, TJ. Irregular Firing of Isolated Cortical Interneurons *in vitro* Driven by Intrinsic Stochastic Mechanisms, Neural Computation, 2008 Jan;20(1):44-64.

Chen E., Stiefel KM., Sejnowski TJ., Bullock TH. Model of traveling waves in a coral nerve network, J Comp Physiol A Neuroethol Sens Neural Behav Physiol. 2008 Feb;194(2):195-200.

4.2 Oral presentations

Stiefel, Klaus M. Time-Critical Neural Dynamics, University of the Ryukyus, Biology Department, Oct 23rd 2007.

Stiefel, Klaus M. Mappig Function to Structure in Dendrites, OIST-Salk Joint Neuroscience Meeting.

Stiefel, Klaus M. Unit Overview, OIST Internal Seminar, April 18th, 2008.

Torben-Nielsen, Ben. Mappig Function to Structure in Dendrites, OIST Internal Seminar.

Stiefel, Klaus M. NEURON tutorial, OCNC 2007.

Stiefel, Klaus M. Dendritic function, OCNC 2007.

4.3 Oral presentations

none.

4.4 Posters

Torben-Nielsen, Ben., Sinclair, Robert., Stiefel, Klaus M. Systematic Mapping of Neural Function to Morphology, CNS Meeting, Toronto, Canada, July 7-12th, 2007.

Torben-Nielsen, Ben., Sinclair, Robert., Stiefel, Klaus M. Systematic Mapping of Neural Function to Morphology, SFN Meeting, San Diego, CA, USA, November 3-7th, 2007.

5. Intellectual Property Rights and Other Specific Achievements

none.

6. Meetings and Events

6.1 Inverse Problems in Biology

Date: April 20 – 22, 2007
Venue: OIST Seaside House
Co-organizers: Dr. Robert Sinclair
Speakers: Please see http://www.irp.oist.jp/mbu/inverse/

6.2 Okinawa Computational Neuroscience Course 2007

Date: June 25 – July 13, 2007
Venue: OIST Seaside House
Co-organizers: Drs. Kenji Doya, Erik DeSchutter, Jeff Wikens
Co-sponsors: Japanese Neural Networks Society
Speakers: Please see http://www.irp.oist.jp/ocnc/2007/index.html
Other remarks: 4th time this course was organized

6.3 OIST – Salk Joint Neuroscience Meeting

Date: November 8 – 10, 2007
Venue: Salk Institute, La Jolla, CA
Co-organizers: Dr. Terry Sejnowski
Co-sponsors: Crick-Jacobs Center
Speakers : Please see http://www.irp.oist.jp/tenu/oistsalk.html
Other remarks: Following the Society for Neuroscience Meeting

6.4 Hardware and software for large-scale biological computing in the next decade

Date: December 11 – 14, 2007
Venue: OIST seaside house
Co-organizers: Drs. Kenji Doya, Erik DeSchutter
Speakers: Please see http://www.irp.oist.jp/hpc-workshop/index.html

XV. Neurobiology Research Unit

Principal Investigator: Jeff Wickens
Research Theme: Cellular mechanisms of learning and adaptive behaviour

Abstract

The overall aim of the Neurobiology Research Unit is to elucidate the cellular mechanisms of learning and adaptive behaviour in the brain. We focus on the mammalian basal ganglia, a set of brain structures implicated in reward and intentional action. Previously we found that reward causes physical changes in the neural connections of the basal ganglia, which are associated with learning. Now we aim to discover the mechanisms that govern these changes in neural connections, concentrating on the actions of neurochemicals - such as dopamine - that are released by rewards. Our hypothesis is that molecular signaling networks activated by dopamine define precise rules for activity-dependent synaptic plasticity. We are investigating synaptic plasticity in live brain tissue using electrophysiological, molecular and computational approaches at multiple levels of analysis. In the first full year of operation of the unit we have initiated studies using 2-photon microscopy to measure events taking place at individual dendritic spines. Conventional patch-clamp recording is being used to measure synaptic plasticity. Lines of transgenic mice have been established in which different types of cells can be definitively identified. Using these approaches we are investigating (i) cell-type specific differences in synaptic plasticity, (ii) molecular mechanisms involved in induction of synaptic plasticity, and (iii) precise timing requirements for synaptic plasticity. The long term aims of these experiments are to determine rules for induction of synaptic plasticity and to elucidate their underlying cellular mechanisms.

1. Staff

Researchers: Dr Tomomi Shindou
Dr Cathy Vickers
Dr Mayumi Ochi-Shindou
Dr Karen Rommelfanger
Dr Adam Ponzi
Technical staff: Mr Kiyoshi Baba
Ms Michelle Callahan
Dr Saori Miura
Mr Prageeth Saraka Wimalaweera
Research assistant/graduate student: Mr Takashi Nakano (based in Doya Unit)
Research administrator/secretary: Ms Yukako Suzuki

2. Partner Organizations

University of Otago, New Zealand
Type of partnership: Joint Research
Name of principal researcher: Jeff Wickens
Names of researchers: Dr Brian Hyland, Dr Brent Alsop, Dr Alison Mercer, Dr Gail Tripp
Research theme: Cellular and Behavioural Mechanisms of Hyperactivity and Movement Disorders

Institute of Biomedical Engineering, National Cheng Kung University, Taiwan
Type of partnership: Research Collaboration (currently being set up)
Name of principal researcher: Dr Jason Chen
Name of researcher: Mr Yu-Ting Li
Research theme: Research on neuroplasticity using wireless dopamine sensing and microPET

3. Activities and Findings

The Neurobiology Research Unit was established January 29, 2007. During the first 14 months of operation, the laboratory interior was completed and the equipment was commissioned. Staff have been recruited and the Unit has grown rapidly. Initial research activity has focussed on experimental studies of synaptic plasticity in the corticostriatal pathway, and theoretical studies of striatal network dynamics and reinforcement mechanisms important in learning. Activity has been conducted in the following main areas:

3.1 Measurement of cell-type specific differences in synaptic plasticity

We have initiated studies of synaptic plasticity in striatal cells using electrical field stimulation and electrophysiological recording in brain slices. These experiments involve conventional whole-cell recording techniques in transgenic mice that express fluorescent markers in specific cell types. Transgenic mouse lines have been established, which selectively express green fluorescent protein (GFP) in subtypes of striatal spiny cells, so that we are now able to definitively identify the neurons from which records have been obtained. In the first phase of this research we are developing plasticity-inducing stimulation protocols for mouse brain slices. We have started experiments to test the hypothesis that dopamine differentially regulates synaptic plasticity in dopamine D1 versus dopamine D2 receptor expressing subtypes of striatal neuron, by comparing the effects of the same induction protocols on such neurons identified by intracellular labeling and by expression of GFP.

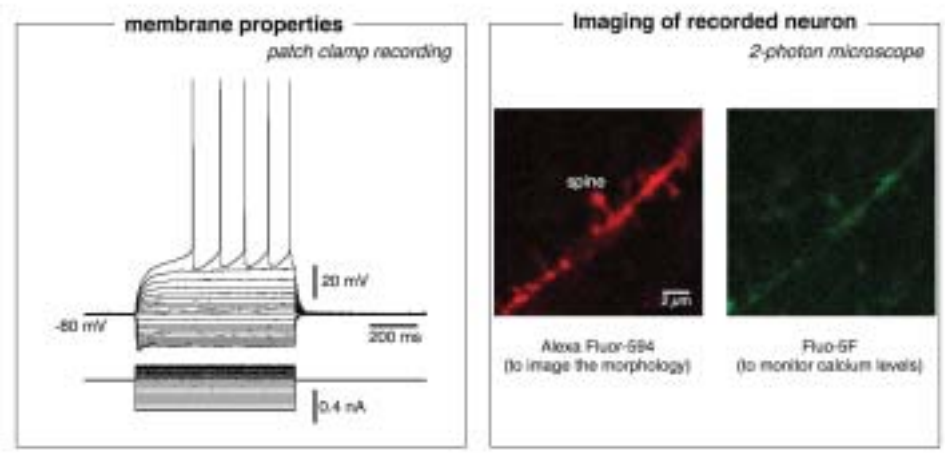


Fig. 1. Combined patch-clamp recording and 2-photon imaging of recorded striatal neuron. Left, electrophysiological response of striatal spiny neuron to current pulses. Right, morphological (red) and functional (Ca^{2+} imaging, green) images of the recorded neuron, showing segment of spiny dendrite and dendritic spines. Shindou and Wickens, unpublished.

3.2 Investigation of molecular mechanisms involved in synaptic plasticity

The molecular mechanisms underlying plasticity operate at the level of individual dendritic spines, which in the striatum may receive synaptic inputs from glutamatergic and dopaminergic synapses. Intracellular Ca^{2+} levels play a key role in the induction of synaptic plasticity. We have initiated studies using 2-photon microscopy to measure intracellular Ca^{2+} concentration in individual dendritic spines. To date we have established methods for performing 2-photon imaging simultaneously with whole-cell patch clamp recording. Figure 1 shows an electrophysiological recording made simultaneously with our first 2-photon images from a single cell. The electrophysiological characteristics are typical of a spiny projection neuron of the striatum. The morphology revealed by Alexa Fluor-594 shows a densely spiny dendrite. The same cell has also been filled with Fluo-5F, a calcium indicator dye. A dendritic spine is indicated on the figure.

In our preliminary experiments we simulated the effect of a glutamatergic synapse on spine Ca^{2+} by uncaging glutamate in a small (micron order) volume adjacent to the spine. Figure 2 illustrates the localised increase in Ca^{2+} concentration brought about by 2-photon stimulation in the presence of caged glutamate. The axis of the spine is repeatedly scanned along the line shown on the image, and the ratio of the fluorescence of the two dyes is plotted over time in the graph. Uncaging of glutamate produces an excitatory postsynaptic current (uEPSC) in the electrophysiological recording, and an increase in the Ca^{2+} concentration in the spine head. We have now initiated studies in which dendritic spine Ca^{2+} concentration is measured during application of synaptic plasticity-inducing stimulation protocols.

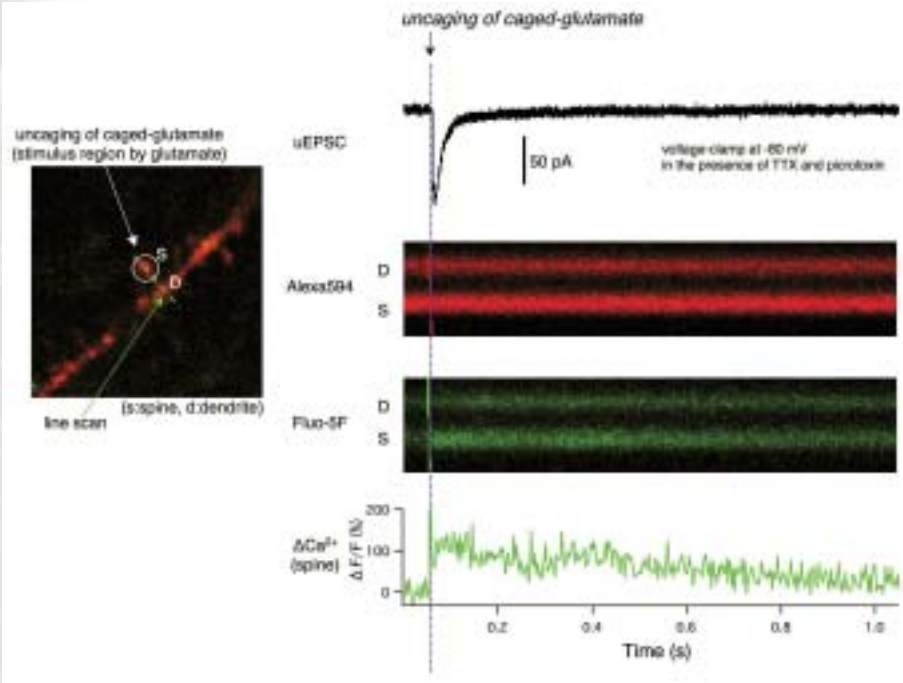


Fig. 2. Demonstration of effect of uncaging glutamate close to a dendritic spine on the intracellular Ca^{2+} concentration. The glutamatergic stimulation causes an increase in the calcium concentration in the head of the dendritic spine. Same cell as in Fig. 1.

3.3 Measurement of timing requirements for induction of synaptic plasticity

Synaptic plasticity mechanisms are thought to operate at the level of individual synapses. We hypothesize that the selection of particular synapses for modification during learning involves activity-dependent plasticity with precise timing requirements. Among other factors, the timing of presynaptic and postsynaptic activity is likely to be crucial, as is the timing of neuromodulator release. We have commenced studies of spike-timing dependent plasticity in the corticostriatal pathway. A particular focus of interest is the action of dopamine on this pathway, as a cellular mediator of behavioural reinforcement. To mimic the release of dopamine induced by reinforcing stimuli we are using flash-photolysis of a caged dopamine compound. This allows us to expose the synapses to a pulse of dopamine, with unprecedented precision in timing and concentration. We are combining these treatments with protocols that induce spike-timing dependent plasticity in corticostriatal synapses. To date we have verified the release of dopamine produced by uncaging, and determined that uncaging can be achieved without cellular damage. We are now using whole cell recording from striatal neurons to measure synaptic strength before and after the application of plasticity-inducing stimulation. By systematically varying the timing of the dopamine pulse aim to measure the precise temporal requirements for dopamine's actions on synaptic transmission in the striatum. These experiments will provide the first definitive measures of the effects of delay on a cellular mechanism for reward-related learning, and test the hypothesis that there is a delay of reinforcement gradient at the cellular level.

3.4 Theoretical and computational studies of the basal ganglia

Our electrophysiological experiments are being conducted in parallel with computational modeling of the network of phosphatases and kinases present in the dendritic spines of the spiny neurons of the striatum (in collaboration with the Doya Unit). In addition we have been developing computer simulations of network activity in striatal inhibitory networks (in collaboration with the Arbuthnott Unit). At a more general level, we have contributed to the development of a neurobiological hypothesis of altered reinforcement mechanisms in attention deficit hyperactivity disorder (in collaboration with the Tripp Unit).

4. Publications

4.1 Journals

Pitcher, T.L., Wickens, J.R., Reynolds, J.N.J. (2007) Differences in striatal action potentials between two behahaviorally distinct rat strains, Neuroscience, 146, 135-142.

Wickens, J.R., Horvitz, J.C., Costa, R.M., Killcross, S. (2007) Dopaminergic mechanisms in actions and habits, Journal of Neuroscience, 27, 8181-8183.

Wickens, J.R., Budd, C.S., Hyland, B.I., Arbuthnott, G.W. (2007) Striatal contributions to reward and decision-making: making sense of regional variations in a reiterated processing matrix, Annals of the New York Academy of Sciences, USA, 1104, 192-212.

Arbuthnott, G.W., Wickens, J.R. (2007) Space, time and dopamine, Trends in Neuroscience, 30, 62-69.

Shindou, T., Arbuthnott, G.W., Wickens, J.R. (2008) Actions of adenosine A_{2A} receptors on synaptic connections of spiny projection neurons and fast-spiking interneurons in the neostriatal inhibitory network, Journal of Neurophysiology, 99, 1884-1889.

Tripp, G., Wickens, J.R. (In Press) Dopamine transfer deficit: A neurobiological hypothesis of altered reinforcement mechanisms in ADHD, Journal of Child Psychology and Psychiatry and Allied Disciplines, doi:10.1111/j.1469-7610.2007.01851.x.

Tripp, G., Wickens, J.R. (In Press) Reply to Commentary on "Dopamine transfer deficit: A neurobiological hypothesis of altered reinforcement mechanisms in ADHD.", Journal of Child Psychology and Psychiatry and Allied Disciplines.

4.2 Book(s) and other one-time publications

Wickens, J.R., Arbuthnott, G.W., Shindou, T. (2007) Simulation of GABA function in the basal ganglia: Computational models of GABAergic mechanisms in basal ganglia function, Progress in Brain Research, 160, 313-329.

4.3 Oral presentations

Wickens, J.R. Cellular mechanisms of learning and decision making in the basal ganglia, 2nd KNU-OIST Workshop "Stem Cell Biology & Neuroscience", Daegu, Korea, March 13, 2008.

Wickens, J.R. Dopamine release and actions in the striatum and prefrontal cortex, Champalimaud Neuroscience Workshop “Neural bases of reward and decision making”, Lisbon, Portugal, September 4, 2007.

Wickens, J.R. Synaptic plasticity and behaviour, Okinawa Computational Neuroscience Course, Onna, Okinawa, June 25, 2007.

4.4 Posters

Young, K.R., Hyland, B.I., Wickens, J.R. "Altered dopamine clearance in the striatum in the genetically hypertensive rat model of attention deficit hyperactivity disorder.", Society for Neuroscience, USA, November 1, 2007.

Shindou, T., Arbuthnott, G.W., Wickens, J.R. Adenosine A_{2A} receptors in the striatal inhibitory network, Society for Neuroscience, USA, November 1, 2007.

Young, K., Hyland, B.I., Wickens, J.R. Electrochemical measurements of dopamine clearance in the striatum in an animal model of ADHD, International Basal Ganglia Society, Egmond aan Zee, Netherlands, September 2, 2007.

Hyland, B.I., Wickens, J.R., Perk, C. Pattern of context-specific responses of striatal neurons to reward-predicting stimuli is consistent with a role in gating dopamine cell activity, International Basal Ganglia Society, Egmond aan Zee, Netherlands, September 2, 2007.

5. Intellectual Property Rights and Other Specific Achievements

Nothing to be reported.

6. Meetings and Events

6.1 Seminar

Date: August 6, 2007

Venue: Uruma City, OISTPC

Speakers: Dr Mike Paulin, University of Otago

6.2 Seminar

Date: July 18, 2007

Venue: Uruma City, OISTPC

Speakers: Dr Brian Hyland, University of Otago

6.3 Seminar

Date: December 12, 2007

Venue: Uruma City, OISTPC

Speakers: Dr Adam Ponzi, RIKEN BSI

XVI. Human Developmental Neurobiology Unit

Principal Investigator: Dr Gail Tripp
Research Theme: Reward mechanisms in human behaviour and neuropsychiatric disorders

Abstract

The overall aim of the research conducted by the Human Developmental Neurobiology Unit is to investigate reinforcement mechanisms in human behaviour and neuropsychiatric disorders. Initially, research will focus on attention deficit hyperactivity disorder (ADHD), a common and debilitating disorder first evident in childhood. In the longer term the research will address other behavioural disorders (such as substance abuse and gambling) and disorders of the brain's neuromodulatory systems (such as Parkinson's disease).

The research hypothesis is that there is an abnormal response to reinforcement in children with ADHD. Using innovative signal detection methodology we hope to clarify the nature and extent of this altered sensitivity to reinforcement in children with ADHD. The results of these studies will contribute to a greater understanding of the mechanisms underlying altered sensitivity to reinforcement and the etiology of ADHD. This knowledge will contribute to the development of more effective behavioural and pharmacological interventions for this chronic and debilitating disorder. The research findings may also offer new directions for identifying which children require assessment for ADHD.

1. Staff

Researchers: Dr Emi Furukawa (started March 2008)
Dr Keiko Ito (started April 2008)
Technical staff: Ms Yuko Hokama (started January 2008)
Mr Naoya Miyagi (started January 2008)
Mr Paul Boshears (started February 2008)
Research administrator/secretary: Ms Mika Matsuda

2. Partner Organizations

University of Otago
Type of partnership: Joint Research
Name of principal researcher: Dr Gail Tripp
Name of researchers: Dr Jeff Wickens, Dr Brian Hyland, Dr Brent Alsop, Dr Alison Mercer
Research theme: Cellular and behavioural mechanisms of hyperactivity and movement disorders

University of Otago
Type of partnership: Joint research
Names of principal researcher: Dr Gail Tripp and Dr Elizabeth Schaughency
Research theme: Identification and diagnosis of ADHD

3. Activities and Findings

3.1 Preparatory Research Activities

With the recent arrival of research staff to the Human Developmental Neurobiology Unit we have been engaged in a number of preparatory research activities including:

3.1.1 Human Subjects Ethics Committee guidelines: The unit PI and research technicians have been working closely with the Research Support Team on the development of appropriate ethical guidelines and regulations for the Human Subjects Ethics Committee currently being established by OIST.

3.1.2 Identifying and sourcing culturally appropriate assessment instruments: Advancing our understanding of the etiology of ADHD requires careful and accurate diagnosis of the disorder. We are working to identify and source the most appropriate Japanese instruments to achieve this as well as identifying assessment measures appropriate for translation into Japanese. This involves networking with other research groups in Japan and has already led to some possible collaborative research activities.

3.1.3 Outreach activities: Our research subjects are normally developing children and children exhibiting symptoms of attention deficit hyperactivity disorder (ADHD). Recruiting children and families to our research will be facilitated by a high level of community awareness regarding our unit and research activities. To this end we are preparing appropriate descriptive materials and initiating contact with relevant community and prefectural groups.

3.1.4 Staff training: In preparation for data collection here in Okinawa unit staff are participating in a number of training activities and seminars. These include seminars on the etiology and assessment of ADHD, training and practice in the administration of relevant assessment instruments, and in the case of the unit PI learning Japanese.

3.2 Collaborative Research Activities

In addition to her research activities at OIST the unit PI maintains an active programme of related research at the University of Otago, New Zealand. This research is currently funded by a three year grant from the Health Research Council of New Zealand. The study Co-PI (Dr Brent Alsop) and Research Fellow (Dr Paula Sowerby) have both visited OIST twice in the past 12 months for planning meetings and to work on manuscripts from the study. Dr Tripp recently visited the University of Otago to work with Drs Alsop and Sowerby.

Data collection on several other projects initiated at the University of Otago is complete and the unit has hosted visits by Ms Shelley Taylor and Mr Tom Robinson to work on data analysis and manuscript preparation from these data.

4. Publications

4.1 Journals

Tripp, G., Wickens, J. R. (In press) Dopamine transfer deficit: A neurobiological theory of altered reinforcement mechanisms in ADHD. Journal of Child Psychology and Psychiatry. Currently available online.

Tripp, G., Wickens, J. (In press) Response to Williams commentary. Journal of Child Psychology and Psychiatry.

4.2 Book(s) and other one-time publications

Sowerby, P., Tripp, G. (In press) Evidence based assessment of Attention-Deficit/Hyperactivity Disorder (ADHD). In J.L. Matson, F. Andrasik, & M. L. Matson. (Eds). Assessing Childhood Psychopathology and Developmental Disabilities. New York; Springer.

4.3 Oral presentations

Tripp, G. Dopamine Transfer Deficit and Delay Aversion, 18th Eunethydis Meeting, Calgiari, Sardinia, October 4 - 7, 2007.

4.4 Posters

Nothing to be reported.

5. Intellectual Property Rights and Other Specific Achievements

Nothing to be reported.

6. Meetings and Events

6.1 OIST Special Seminar: Attention Deficit Hyperactivity Disorder

Date: February 7, 2008
Venue: Okinawa Prefectural Nambu Medical Centre
Co-sponsors: Okinawa Prefectural Nambu Medical Centre
Speakers: Professor Joseph Sergeant, Department of Clinical Neuropsychology, Vrije Universiteit, Amsterdam.

6.2 OIST Seminar: Feedback learning in ADHD: Can reward resolve the problem

Date: April 25, 2008
Venue: IRP conference room
Speakers: Dr Marjolein Luman, Department of Clinical Neuropsychology, Vrije Universiteit, Amsterdam.

XVII. Brain Mechanisms for Behaviour Unit

Principal Investigator: Professor Gordon Arbuthnott
Research Theme: Cell circuits involved in motor control

Abstract

We are preparing to record electrical activity from striatal neurons in culture and in brain slices by methods that have two different aims: to study the synaptic efficacy of single cortico-striatal synapses, and to image the group of cells responding to a particular cortical region and study their dynamics. This year has also seen the equipment built, the culture methods established, and the first results of a joint project with University of Ottawa published as posters. This Ottawa project aims to allow us to mix striatal and cortical cells of different developmental ages so as to include - or exclude - individual groups of interneurons in the cultures. We began by characterizing the cholinergic interneurons thought to be involved in parkinsonism, and now have methods to study cortico-striatal dynamics with and without them.

1. Staff

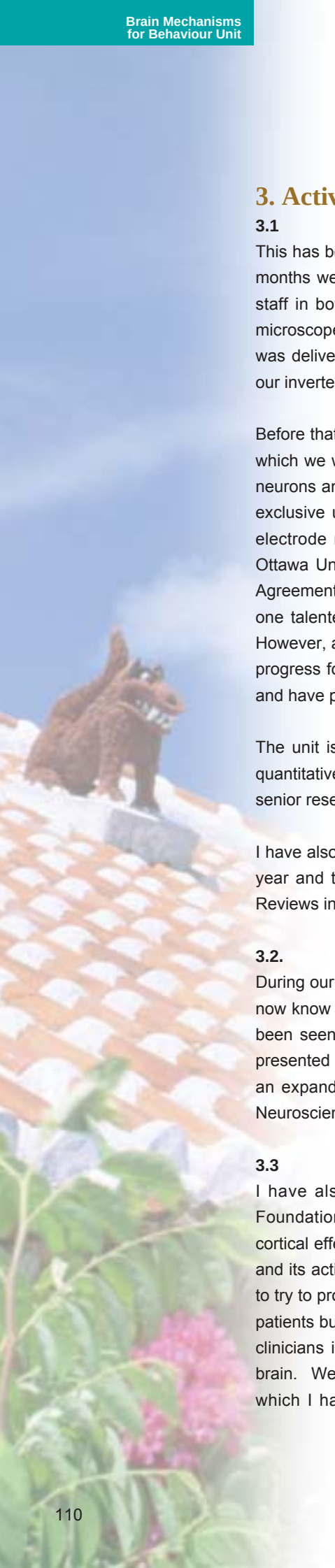
Brain Mechanisms for Behaviour
Group leader: Dr. Marianela Garcia Munoz
Researchers: Actively searching for suitable candidates
Technical staff: Shakuntala Pandian M.Sc. joining in June
Research administrator/secretary: Ms. Hiroko Chinone

2. Partner Organizations

University of Ottawa
Type of partnership: Joint research
Name of principal researcher: Professor W. A. Staines
Names of researchers: Professor A. Krantis, Professor Geoff Mealinq, Dr. Sara Schock
Research theme: Isolating genetically marked neurons for culture

University of Otago
Type of partnership: Joint research
Name of principal researcher: Dr. Beulah Leich
Research theme: Determining the location of L-type Calcium channels (CaV1.3) on striatal membranes

University of Otago
Type of partnership: Ongoing research funded in New Zealand
Name of principal researcher: Professor Gordon Arbuthnott
Names of researchers: Dr. Brian Hyland and Dr. Cyril Dejean
Research theme: Cortical effects of deep brain stimulation in the subthalamic nucleus



3. Activities and Findings

3.1

This has been a year of development. Development of staff, equipment and space. In the first few months we helped the Wickens Unit to set up 5 rooms of recording equipment. We helped train staff in both Units and developed the room in the Biocentre for the reception of the 2 photon microscope and general Biochemical/Molecular biological equipment. The 2 photon microscope was delivered with faulty shutters which took some time to repair but by then we had developed our inverted microscope set-up for our initial experiments with cultured primary neurons.

Before that project could bear fruit we needed training in culture of the cryo-preserved neurons on which we wanted to base our culture systems. They are a little more fragile than acutely isolated neurons and so we had to upgrade the quality control in the common P2 facility and negotiate the exclusive use of one laminar flow cabinet so that we could initiate the projects involving multi-electrode recording from the cultures. The experiments are in collaboration with the group in Ottawa University that first developed the cryo-preservation methods. The final “Joint Research Agreement” was signed just in time to start the project within this financial year. The delays lost us one talented post-doctoral student and resulted in under expenditure from our planned budget. However, a promising post doctoral candidate has now been recruited and there are signs of great progress for the new year. We also have the possibility of recruiting another promising candidate and have priced the next year's joint research accordingly.

The unit is expanding rapidly and a student who is experienced in both electrophysiology and quantitative anatomical methods will join us in June. We are also actively trying to find another senior researcher to work on the 2 photon microscope with us.

I have also completed two papers on the work I finished as I left Edinburgh. One is published this year and the other is in press. We have also been asked to write a review of the project for Reviews in the Neurosciences.

3.2.

During our visit to the University of Ottawa the first exciting technical breakthrough was made. We now know how to culture striatal cells with and without the cholinergic interneurons that have long been seen as critical to the motor output from the striatum. That resulted in two of the posters presented this year. One on the method to the International Basal Ganglia Society in Holland and an expanded version including some of our first multi-electrode array results to the Society of Neuroscience in San Diego.

3.3

I have also supervised and written up work in New Zealand funded by the Neurological Foundation of New Zealand. This project has provided an intriguing correlation between the cortical effects of the kind of deep brain stimulation, used in the treatment of Parkinson's disease, and its actions in 'un-freezing' rats treated with dopamine antagonist drugs. We are working hard to try to produce more direct evidence that this is the effective mechanism in the therapy in human patients but, although similar cortical effects have been reported in patients, the consensus among clinicians is still to expect the therapeutic action to involve the basal ganglia loops through the brain. We have the opportunity to convince them otherwise in a conference next December to which I have been asked to contribute. By then we hope to have data to bear on whether

activating the same layer of cortex – without directly stimulating the basal ganglia loops will be as effective as its activation from the presently favored subthalamic nuclear site.

3.4.

A successful visit by Dr Beulah Leitch, a specialist in electron microscopic immunohistochemistry, this year has set the scene for further collaborations with her in Otago during the coming year. In Okinawa she tested many methods for the visualization of voltage sensitive calcium channels in tissue from mice and we have plans to extend that study both in OIST and in Otago.

3.5.

We continue to collaborate with the Wickens Unit and have published with them this year, as well as initiated very successful joint meetings where research ideas are discussed weekly.

4. Publications

4.1 Journals

Wright, A. K., Arbuthnott, G. W. (2007) Influence of subthlamic nucleus upon the damage to the dopamine system following lesions to the globus pallidus in rats, Eu. J. Neuroscience, 26, 642-648.

Li, S., Arbuthnott, G.W., Jutras, M.J., Goldberg, J.A., Jaeger, D. (2007) Resonant antidromic cortical circuit activation as a consequence of high-frequency subthalamic deep-brain stimulation, J. Neurophysiol, 98, 3525-3537.

Wickens, J.R., Budd, C.S., Arbuthnott, G.W., Hyland, B.I. (2007) Striatal contributions to reward and decision-making: making sense of regional variations in the reiterated processing matrix. NYAS, 1104, 192-212.

Shindou,T., Arbuthnott, G.W., Wickens, J.R. (2008) Actions of Adenosine A2A Receptors on Synaptic Connections of Spiny Projection Neurons in the Neostriatal Inhibitory Network, J.Neurophysiol, 99, 1884-1889.

Wright, A.K., Miller, C., Williams, M., Arbuthnott, G.W. (2008) Microglial activation is not prevented by tacrolimus but dopamine neuron damage is reduced in a rat model of Parkinson's disease progression, Brain Res, In Press.

4.2 Book(s) and other one-time publications

Wickens, J.R., Arbuthnott, G.W., Shidou, T. (2007) "GABA and the Basal Ganglia - from Molecules to Systems." / Simulation of GABA function in the basal ganglia: Computational models of GABAergic mechanisms in basal ganglia function, Progress in Brain Research, 160 pp 315-331.

4.3 Oral presentations

Arbuthnott, G.W. Neuroscience for mathematicians, OCNC 2007 (Okinawa, Japan), June 26th, 2007.

Cyril Déjean., Brian Hyland., Gordon Arbuthnott. Cortical effects of subthalamic stimulation correlate with behavioural recovery from dopamine inactivation, Society for Neuroscience Annual Meeting (San Diego, USA), November 7, 2007.

Arbuthnott, G.W. Slowly progressive dopamine cell loss; a model on which to test neuroprotective strategies for Parkinson's disease? OIST/SALK joint workshop (La Jolla, USA), November 9, 2007.

Arbuthnott, G.W. Brain mechanisms for behaviour, The Senior High School Affiliated to Showa Pharmaceutical University (Okinawa, Japan), March 12, 2008.

4.4 Posters

A.K. Wright., G.W. Arbuthnott. The influence of subthalamic nucleus upon the damage to the dopamine system following lesions of globus pallidus in rats, International Basal Ganglia Society (IBAGS) Egmond aan Zee, The Netherlands, Sept, 2-6 2007.

G.W. Arbuthnott., M. Garcia-Munoz., B. Tinner., K. Vaillancourt., A. Krantis., W. A. Staines. Cholinergic interneurons develop in striatal cultures of mixed gestational age, International Basal Ganglia Society (IBAGS) Egmond aan Zee, The Netherlands, Sept, 2-6 2007.

T. Shindou., G.W. Arbuthnott., J.R. Wickens. Actions of adenosine A_{2A} receptors in the striatal inhibitory transmission, International Basal Ganglia Society (IBAGS) Egmond aan Zee, The Netherlands, Sept, 2-6 2007.

G.W. Arbuthnott., M. Garcia-Munoz., B. Tinner., K. Vaillancourt., A. Krantis., W.A. Staines. Preserving cholinergic interneurons in cryopreserved striatal neuron cultures, Society for Neuroscience, San Diego, USA, Nov, 2-7 2007.

T. Shindou., G.W. Arbuthnott., J.R. Wickens. Actions of adenosine A_{2A} receptors in the striatal inhibitory transmission, Society for Neuroscience, San Diego, USA, Nov, 2-7 2007.

XVIII. Computational Neuroscience Unit

Principal Investigator: Erik De Schutter

Research Theme: Modeling Cellular and Molecular Mechanisms of Neural Information Processing

Abstract

During the first year we successfully started research in the new unit. The STEPS and Neurofitter software packages were released and further improved and expanded. Anomalous diffusion caused by trapping by spines was modeled in different types of neurons. Several projects on cerebellar Purkinje cell modeling ran in parallel. These included studies of the effect of synaptic plasticity on pattern recognition and of the role of calcium in activity homeostasis and in induction of synaptic plasticity, both projects using an old Purkinje cell model. A major effort to use Neurofitter to create a new Purkinje cell model based on electrophysiological data has made good progress. In addition, we contributed to the investigation of a cerebellar Golgi cell model and the mechanisms of firing rate correlation.

1. Staff

General services

Research administrator/secretary: Tsuyuki Nakabayashi (from May 2007)

Molecular modeling

Technical staff: Stefan Wils

Cellular modeling

Researcher: Sungho Hong (from September 2007)

Technical staff: Haroon Anwar (from November 2007), Ivan Raikov, Werner Van Geit

Network modeling

Researcher: Thomas Sangrey (from April 2008)

In addition the molecular modeling group benefited from the work done by Katri Hituri from Tampere of Technology (Finland) during her visit to the unit (January - April 2008).

2. Partner Organizations

University of Antwerp, Belgium

Type of partnership: Scientific collaboration and graduate program

Name of principal researchers: D. Snyders, J.-P. Timmermans

Name of researchers: K. Bhuvanasundaram, R. Maex, Q. Robberecht, K. Tahon, K. Veys

Research theme: Cerebellar physiology, multiple themes

ATR, Japan

Type of partnership: Scientific collaboration

Name of principal researcher: M. Kawato

Name of researcher: H. Ogasawara

Research theme: Molecular modeling of cerebellar signaling pathways

Tampere University of Technology, Finland
Type of partnership: Scientific collaboration
Name of principal researcher: M.-L. Linne
Name of researcher: K. Hituri
Research theme: Stochastic modeling of IP3 receptors

Duke University, United States of America
Type of partnership: Scientific collaboration
Name of principal researcher: G. J. Augustine
Name of researcher: F. Santamaria
Research theme: Molecular modeling of diffusion in dendrites

University College London, United Kingdom
Type of partnership: Scientific collaboration
Name of principal researcher: M. Häusser
Name of researcher: A. Roth, V. Steuber
Research theme: Purkinje cell physiology and modeling

Emory University, United States of America
Type of partnership: Scientific collaboration
Name of principal researcher: D. Jaeger
Name of researcher: N. Schultheiss
Research theme: Modeling of deep cerebellar nuclei neurons

University of Pavia, Italy
Type of partnership: Scientific collaboration
Name of principal researcher: E. D'Angelo
Name of researcher: S. Solinas
Research theme: Modeling of cerebellar Golgi cells

3. Activities and Findings

3.1 General software services

3.1.1 Semi-structured model of scientific data cataloging and management

It is difficult to manage the efficient access and analysis of scientific data that has been generated and/or used in various phases of scientific experiments and simulations. Current data management and analysis techniques do not measure up to the challenges of collaborative science which involves usage of distributed resources and experimental data obtained from multiple labs.

Our approach to organizing experimental data obtained from external sources,needed to constrain our computational models, is an attempt to combine the advantages of file systems, databases and wiki systems, while avoiding their respective disadvantages. A wiki is a collaborative interactive web system, that permits the reader to actively change and enhance the content of the website. The most famous public wiki system is Wikipedia, a community encyclopedia where users can easily edit and extend the content.

We have constructed a simple wiki interface combined with facilities for editing and search by meta data properties that are linked to the data files. Wikis are particularly well-suited for collaborative creation of content, but typically take a considerable amount of effort to organize and structure the content. We have extended an existing wiki system, so that a data file residing on a file server can be annotated in one of two ways. The first way is to link the file with a free-form text page, which is standard wiki functionality. The second way permits meta data to be specified via structured web forms, where the user chooses from a list of predefined fields and enters values for each field. This idea is based on the semi-structured model of databases. In this model, there is no separation between the data and the schema, and the amount of structure used depends on the purpose and can be easily changed. The main advantages of this model are that it can represent diverse information from multiple data sources that cannot be constrained by a fixed schema and that it can provide enough structure for querying and searching.

We expect that by adopting the semi-structured model, we can catalog diverse sets of experimental data, and maintain an evolving set of meta data that can be easily searched and extended as necessary. We plan on releasing the software to the scientific community in the hope that it will be useful for organizing scientific data in general.

3.2 Molecular mechanisms of synaptic plasticity

3.2.1 Software development of STEPS

STEPS is a software system developed by us that stochastically simulates reaction-diffusion processes in 3D environments. The basic algorithm has been implemented some time ago, but to make this project successful, we also need to pay attention to more 'mundane' aspects of software design. Only by making the software well documented, mature in functionality and easy to use can we promote adoption of the software outside of the lab.

In the context of addressing these issues, we decided to integrate STEPS with the Python scripting language. This language is becoming increasingly popular within the broader scientific programming community (Cannon et al., 2007). It is easy to learn and a number of third party modules are available in Python for data processing and visualization. This required a partial reordering of the STEPS code to bundle its core computational routines in Python extension modules.

We have also been working on developing a user tutorial and on improving its robustness. We have benchmarked STEPS against MCell (Stiles et al. 2000). Finally, we implemented a large number of small improvements, such as the ability to specify rate functions rather than rate constants and support for improved output generation. We maintained the build/distribution system and dealt with compatibility issues as STEPS is used on more and more systems.
A paper describing STEPS and it performance is being finalized.

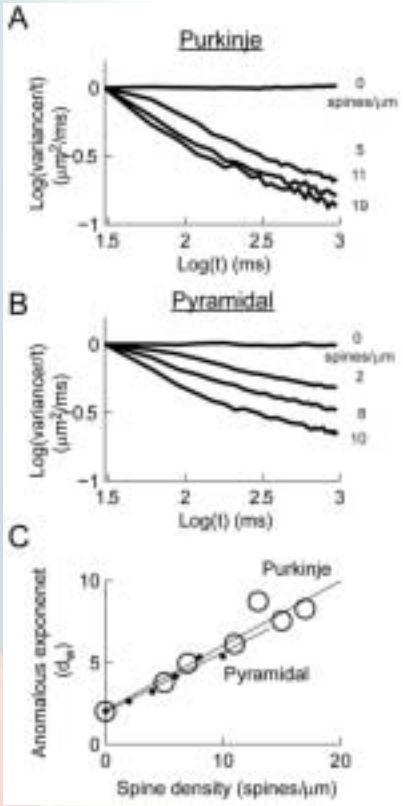


Figure 1: Anomalous diffusion calculated from multiple simulations performed in Purkinje and pyramidal cel l models with increasing spine densities. A-B) Several examples of the logarithmic transform of the calculated value of the spatial variance along the simulated dendrites.C) Anomalous exponent (dw) against spine density.

3.2.2 Electrical field computations in STEPS

STEPS currently only computes the reactions between and the diffusion of molecules, which enables it to simulate a fairly broad set of signaling pathways. Of course, in neuroscience such pathways are tightly coupled with local and global electric excitability of the cell; changes in membrane potential can trigger signaling pathways but are also affected by their outcome. Coupling a reaction-diffusion simulation with simulation of the membrane potential would therefore further increase the power of STEPS.

We analyzed several ways to couple these two types of simulation which did not require new approaches. We rejected coupling STEPS with a third party simulator, such as GENESIS (Bower and Beeman, 1998) or Neuron (Hines and Carnevale, 1997), because the required mesh transforms were cumbersome and probably inaccurate. Similarly, we rejected computing the interaction between the electrodiffusive motion of charge carriers and electric field because it is too computationally intensive. Instead, we started developing an extension of the cable equations for a three dimensional, unstructured mesh. The main part of this work was executed under a contract with Dr. R. Cannon (Textensor, Inc). In this approach, each vertex point of the STEPS mesh has a capacitance associated with it, which is zero for internal points, and is coupled with neighboring mesh points through resistances. This method allows us to re-use well-known constants from electrophysiology, while still retaining the level of detail provided by the 3D meshes.

A reference implementation of this algorithm in Java/Fortran has been shown to pass some of the Rallpack tests (Bhalla et al., 1992). Currently we are working on porting this reference implementation to a C++ module for STEPS.

3.2.3 Stochastic modeling of the signaling pathways involved in cerebellar LTD

This work is still in very early stages. We have ported a deterministic kinetic model of the IP3-receptor (Doi et al. 2005) to the STEPS environment and are currently evaluating its stochastic behavior. In collaboration with the team of Dr. M. Kawato (ATR, Japan) we are investigating why a deterministic model of the full signaling pathway, previously developed by Dr. Kawato and colleagues (Kuroda et al., 2001), fails in stochastic mode.
This model is also being ported to STEPS.

3.2.4 Anomalous diffusion in neuronal dendrites

In collaboration with Drs G.J. Augustine and F. Santamaria we have continued our investigation of anomalous diffusion in Purkinje cell spiny dendrites (Santamaria et al., 2006). They compared the influence of dendritic structure on the diffusion of intracellular chemical signals in Purkinje and CA1 pyramidal cells. They combined flash photolysis of caged Rhodamin dextran and two-photon fluorescent microscopy to track changes in concentration gradients along the dendrites of both cell types. We found that the apparent diffusion coefficient measured in spiny dendrites of Purkinje cells decays as a function of time. This reduction is due to molecular trapping by dendritic spines, that generate a distinct type of diffusion process, called anomalous diffusion. Diffusion in pyramidal cells was also anomalous and the value of the anomalous exponent was shown to depend linearly on spine density, confirming our modeling predictions (Figure 1). Our analysis also suggests that anomalous diffusion in pyramidal cells is present even with low numbers of spines, which could be due to a larger obstruction by intracellular components in pyramidal cells compared to Purkinje cell smooth dendrites. We conclude that spines cause anomalous diffusion in multiple types of neurons and that this process could be used by neurons to regulate the movement of molecules along dendrites.

A paper is being finalized.

3.3 Cellular mechanisms regulating firing and synaptic properties of neurons

3.3.1 Software development of Neurofitter

The increase in available computational power and the higher quality of experimental recordings have turned the tuning of neuron model parameters into a problem that can be solved by automatic global optimization algorithms.

Neurofitter is a software tool that interfaces existing neural simulation software and sophisticated optimization algorithms with a new way to compute the error measure (Van Geit et al., 2007). This error measure represents how well a given parameter set is able to reproduce the experimental data.

The Neurofitter error is based on the phase-plane trajectory density method (LeMasson, 2001), which is insensitive to small phase differences between model and data. This technique transforms the traces into V-dV/dt plots (see Figure 2). The 3D histograms of these plots are used to calculate an

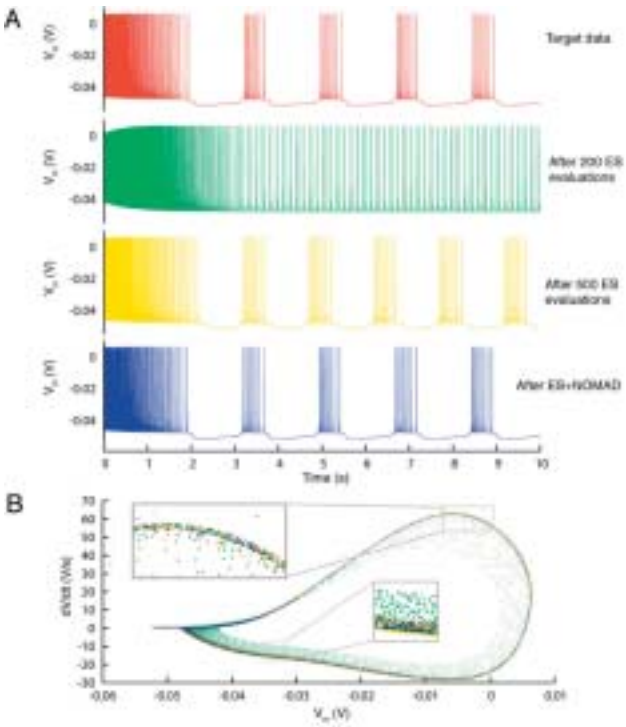


Figure 2: (A) Somatic membrane voltage traces of target data (red), the best model found by an ES optimization after 200 (green) and 500 (yellow) evaluations, and by a Mesh Adaptive Search that started with the best solution found by ES (blue). (B) Phase-plane trajectory of the traces in A (same color code). The density of the trajectory is not visible.

error value that shows how well the experimental and model data match. Optimization algorithms are used to find parameter values that have a low error value. These algorithms include Evolutionary Strategies, Particle Swarm optimization and Mesh Adaptive Search. Neurofitter enables the effortless combination of many different time-dependent data traces into the error measure, allowing the neuroscientist to focus on what are the seminal properties of the model.

Over the last year we turned Neurofitter from a research tool (Achard and De Schutter, 2006) into a released software package (under GPL). We cleaned up and expanded the Neurofitter code, provided a new user interface and detailed user instructions (Van Geit et al., 2007).

3.3.2 Software development related to NeuroML

As computational models of neurons become more complex, their software implementations become more difficult to define, comprehend, and communicate. Consequently, for scientific understanding of biological nervous systems to progress, it is crucial to have software tools that support easy exchange and understanding of computational models (Cannon et al., 2007).

Significant development effort has been invested in NeuroML, which is intended as a common format for exchange between neuron modeling software. The aims of the language are to be human-readable and to support efficient simulation of the models (Goddard 2004).

We have attempted to utilize ChannelML, one of the component languages of NeuroML, for the purpose of having a simple, simulator-independent way of specifying ion channel dynamics, and for automated generation of efficient simulation code for the NEURON environment. So far, we have ChannelML descriptions of the De Schutter and Bower (1994) and Akemann et al. (2006) models, and a basic software library for processing ChannelML descriptions and generating efficient simulation code has been released.

However, major issues remain with ChannelML as a platform for the development of future models. In our experience, the language has very restrictive semantics for specifying channel dynamics. Over a dozen extensions to the format were needed to express the two Purkinje cell models. No mechanism was provided for extending the language or for specifying equations that do not fit the predefined patterns. We are preparing a report on our experiences with ChannelML and NeuroML, and a proposal for modifications to the standard that will facilitate the development of Purkinje cell models. Future efforts in this direction will include the development of additional software libraries that support the extensions proposed in our report, and that can generate code for several simulation environments, in order to encourage more widespread usage of the format.

3.3.3 A modeling study of the effect of cerebellar LTD on pattern recognition by Purkinje cells

Many theories of cerebellar function assume that long-term depression (LTD) of parallel fiber synapses enables Purkinje cells to learn to recognize parallel fiber activity patterns. We studied the LTD-based recognition of parallel fiber patterns in a biophysically realistic Purkinje cell model (De Schutter and Bower, 1994). With simple-spike firing as observed in vivo, the presentation of a pattern resulted in a burst of spikes followed by a pause (Steuber et al., 2007).

Surprisingly, the best criterion to distinguish learned patterns was the duration of this pause (Figure 3). Moreover, our simulations predict that learned patterns elicited shorter pauses, thus increasing Purkinje cell output, opposed to the predictions of standard cerebellar learning theory

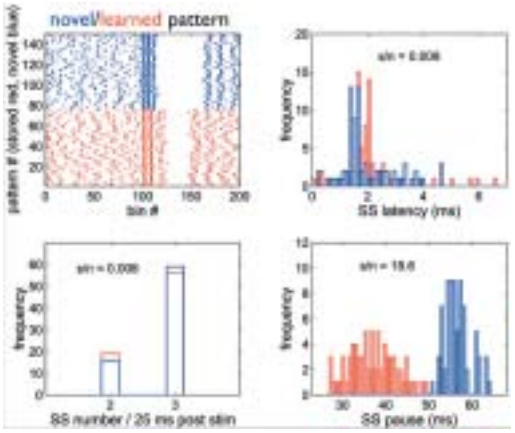


Figure 3: Response of a Purkinje cell model to a parallel fiber pattern. Top left: response consists of burst/pause sequence, raster plots show responses to novel patterns (blue) and learned patterns (red). Other panels: three criteria are compared to recognize learned from novel patterns, measured as a signal-to-noise ratio (s/n). Latency of the burst or number of spikes do not distinguish novel from learned patterns, but the duration of the pause does (lower right), with significantly shorter pauses for a learned pattern.

(Ito, 1984). In collaborations we tested this prediction using Purkinje-cell recordings in vitro (with Dr. M. Häusser, London, UK) and in vivo (with Dr. C. I. De Zeeuw, Rotterdam, the Netherlands). In vitro, we found a shortening of pauses when decreasing the number of active parallel fibers or after inducing LTD. In vivo, we observed longer pauses in LTD-deficient mice. Our results suggest a novel form of neural coding in the cerebellar cortex based on changes in duration of pauses in simple spike firing.

3.3.4 Calcium, synaptic plasticity and intrinsic homeostasis in Purkinje cell models

Intrinsic homeostasis designates the regulation of a neuron's activity through the dynamic expression of ion channels. Such mechanisms allow the cell to keep a stable input/output relationship for years while its basic constituents are constantly renewed. It is generally assumed that there is a correspondence between the conductance space used by homeostasis mechanisms and the parameter landscape of fairly realistic neuron models (Marder and Goaillard, 2006). Some models and experiments have hypothesized that a mechanism for homeostatic control works via the regulation of the average cytoplasmic calcium concentration. This raises many questions for Purkinje cells, for which calcium plays an important role in the induction of synaptic plasticity. Indeed, LTD of the parallel fiber synapses requires a sustained elevation of the calcium concentration in the spine. This high level of calcium is usually obtained by a conjunctive activation of parallel fibers and climbing fibers in a delimited time window.

To address the role of calcium in the Purkinje cell, we have generated 148 new Purkinje cell models, using a Neurofitter based parameter search around the 1994 model (De Schutter and Bower, 1994; De Schutter and Achard, 2006; Van Geit et al., 2007). We demonstrated that, while the somatic membrane voltage was robust, the somatic calcium behavior was very variable from model to model, in agreement with experimental results (Swensen and Bean, 2005). The models showed similar behavior in the smooth dendrite. Therefore calcium seems an unlikely candidate for being the activity-sensor in this cell.

Conversely, the calcium signal in the spiny dendrites was very stable, which is interesting as this is the site where LTD is induced. To test whether the LTD mechanisms were preserved for these different models, we used a Purkinje cell spine model of calcium signal transduction pathways (Doi et al., 2005). In all our models, an LTD induction protocol lead to a sustained calcium release from internal stores, hence LTD induction was preserved. LTD mechanisms can therefore be seen as intrinsic properties of the Purkinje cell. Additionally, if the variability observed in calcium signals

at the somatic level was also present in the spines, then LTD induction was not preserved anymore.

We conclude that the induction of synaptic plasticity, which is very important for cerebellar function, is strongly correlated with the normal electrical activity of the Purkinje cell.

This work has been submitted for publication.

3.3.5 Development of a new Purkinje cell model

We are building a new detailed model of the cerebellar Purkinje cell. The model we currently use (De Schutter and Bower, 1994) has been very successful (Steuber et al., 2007) but is showing its age, as it is over 15 years old. The new model will not only incorporate many new electrophysiological findings, but also extend the modeling technology in a significant way. Though it will still be a large, compartmental single cell model, it will contain much more molecular detail with fully characterized channel subunits and eventually include channel modulation and regulation.

To achieve this goal we obtained Purkinje cell electrophysiological recordings from two different laboratories, the Häusser group in London, UK and the Turner group in Calgary, Canada, and are using Neurofitter to fit channel conductances to these data. The Neurofitter method was previously validated by fitting the parameters of the old Purkinje cell model to output generated by the model itself (Achard and De Schutter, 2006). Fitting to real experimental data, however, is a more complicated task, since underlying mechanism used in the model, like for example the ion channel kinetics, must match with those present in the recorded cell. We started with channel kinetics from the old model, using the dendritic morphology provided by the experimenters. But we soon found out that this mixture of ion channels (and their kinetics) was unable to match the characteristics of the experimental data. Therefore we started using more recent channel equations described in Akemann and Knöpfel (2006). These performed better but required extensive hand-tuning of the kinetic parameters of some ion channels. At this stage we can report partial success in creating a new Purkinje cell model that faithfully fits data from the Häusser group.

An interesting discovery is that, while multiple models with different combinations of channel conductances can reproduce identical electrophysiological output (Achard and De Schutter, 2006).

the channel kinetics seem to be severely constrained by the data. We will investigate this observation in more detail in FY2008.

3.3.6 Reducing the morphological complexity of a cerebellar Purkinje cell

One of the big challenges in modeling a detailed cerebellar Purkinje cell is to handle the computational load of its extensive dendritic morphology. Although our available computational resources are adequate, dealing with such a detailed model can be time consuming and cost ineffective, especially during parameter searches. A possible solution would be to effectively reduce the morphology. A few methods have been presented by Burke et al. (2000) to collapse the dendritic trees into unbranched dendrite. These methods are based on the somatofugal electrotonic distance transform, the somatofugal voltage attenuation transform and the signal propagation delay.

Our goal is to reduce the morphological complexity while keeping the somatic firing properties, influenced by the dendritic load, as constant as possible. We collapsed dendritic trees with spiny and aspiny branches to unbranched dendrites using the somatofugal electrotonic distance transform, while keeping the smooth dendrites unchanged (Figure 4). For this specific Purkinje cell, the number of compartments in transformed cell was reduced by a factor 14. We compared the voltage attenuation for somatic injection and transients at synapses between the reduced and original morphology. Though small differences due to local changes in input impedance were present, the overall characteristics of signal attenuation and spike propagation were quite consistent between the two morphologies. This reduced morphology will be used to tune model parameters using Neurofitter (Van Geit et al. 2007), see above.

3.3.7 Models of channel phosphorylation in Purkinje cells

We are interested in the role of signal transduction in the cerebellar Purkinje cell. Of particular interest are the processes of protein phosphorylation and dephosphorylation, which are among the most important and common cellular signaling mechanisms (Widmer 2003). It is thought that phosphorylation can cause long-term changes in the structure of key proteins that form neuron channels and receptors, and therefore affect how the neuron responds to stimuli. Understanding these phosphorylation-induced structural changes is important and necessary for constructing realistic computer models of neuronal function.

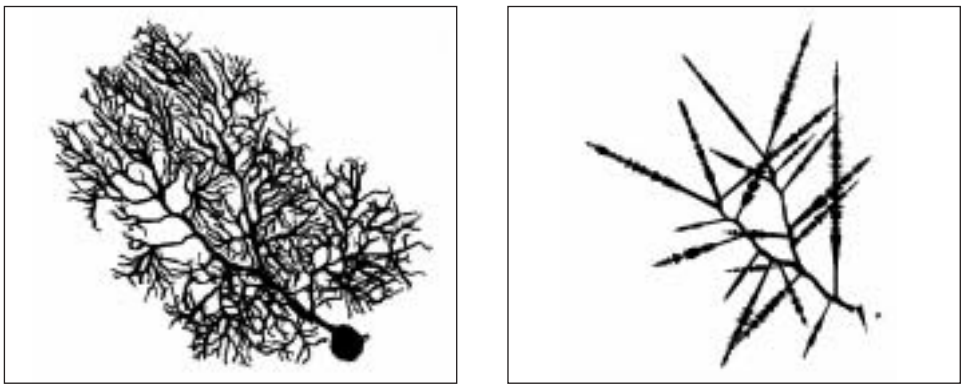


Figure 4: Morphology of original cerebellar Purkinje cell (obtained from Dr. Turner, Canada) with ~1400 compartments (left) versus reduced morphology with ~ 100 compartments (right).

Thus far, we have implemented a prototype software system that allows us to study statistical methods for modeling the effects of phosphorylation in neurons. The purpose of that system was to provide a testing ground for algorithms and data structures that we consider using in our approach to modeling the effects of phosphorylation. We have completed and released over a dozen software modules that comprise the core of the system, along with implementations of four previously existing neuron models that have been modified to utilize a statistical method proposed by Schneidman et al. (1998). We have extended two of those models with a simple model of phosphorylation based on data by Misonou (2006). Some small-scale simulation experiments have been conducted to confirm the correctness of the implementation. The next step will involve further studies of methods to model phosphorylation, and implementing such methods within NEURON, a commonly used, well-established software environment for neuron modeling and simulation.

3.3.8 Neuronal homeostasis and its functional implications in Purkinje cells

Active mechanisms in neurons are under constant influence from intracellular processes. Interestingly, there is a lot of variability in biophysical parameters even when dynamical behaviors of the neurons are almost identical (Golowasch et al. 2002; Achard and De Schutter, 2006).

However, studies of activity homeostasis have focused only on one or several dynamical properties of a neuron, such as the firing rate. In a neural system, neurons are rarely autonomous but transform synaptic inputs into spike outputs. Therefore, it is interesting to study how this functional property of a neuron changes due to ion channel modulation.

One of the possible strategies is to use functional characterization methods that statistically infer an input/output relationship of a neuron. For example, a phase response curve (PRC) describes how an input generates a phase shift in an output spike train (Winfree, 1980). We calculated PRCs of a recent Purkinje neuron model proposed by Akemann and Knöpfel (2006), which contains a fast acting and high-threshold K+ current, Kbin, interacting with resurgent Na+ currents to allow a high firing rate without sodium block (Khaliq et al., 2003). Our result shows that Kbin contributes to generating a relatively flat PRC, which resembles the PRCs of Purkinje neurons obtained from experiments (Phoka et al., 2008). With Kbin, Purkinje neuron can reliably encode its integrated synaptic current into a phase shift independently of the relative timing of the input. This example shows how one can study modulation of ion channels in terms of its functional implications, which we plan to extend to general cases.

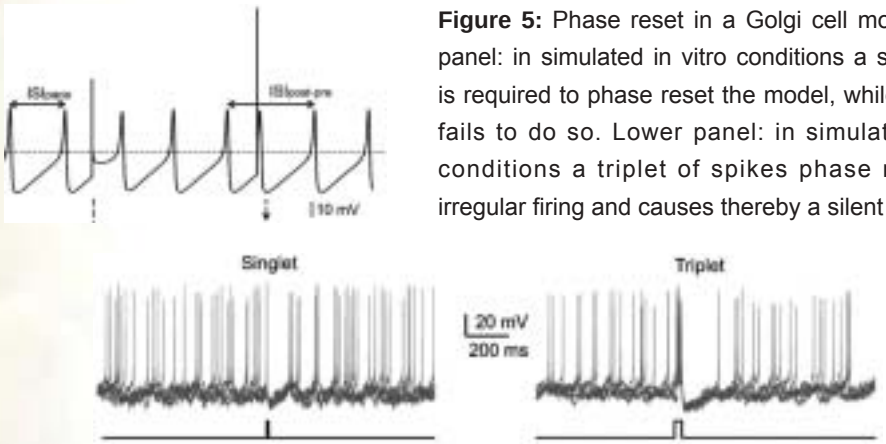


Figure 5: Phase reset in a Golgi cell model. Upper panel: in simulated in vitro conditions a single spike is required to phase reset the model, while an EPSP fails to do so. Lower panel: in simulated in vivo conditions a triplet of spikes phase resets the irregular firing and causes thereby a silent period.

3.3.9 Impact of intrinsic Golgi cell properties on in vivo responses

We have investigated in collaboration with Dr. D'Angelo (Pavia, Italy) the properties of a realistic cerebellar Golgi cell model that they developed (Solinas et al., 2008a) based on their previous electrophysiological studies (Forti et al., 2006). We investigated Golgi cell excitability, in particular the mechanisms causing spontaneous pacemaker activity and subthreshold oscillations.

In a next study (Solinas et al., 2008b) we showed that the pacemaker cycle can be suddenly reset by spikes in both real cells and in the model. Phase-reset required activation of SK channels and was shown to require a much larger charge transfer than can be caused by synaptic input, therefore firmly linking it to spikes (Figure 5). By adding balanced synaptic noise, we could turn the pacemaking into the spontaneous irregular discharge observed in vivo (Vos et al., 1999). In this constellation the phase-reset mechanism required a short burst of spikes to be activated and

could then replicate the response to sensory stimulation observed in vivo (Vos et al., 1999; Volny-Luraghi, 2002). Therefore the model suggests that the silent period following the initial burst, observed during the normal in vivo response pattern to sensory input, may be largely caused by the intrinsic phase reset of Golgi neurons. We conclude that Golgi cell intrinsic properties exert a profound impact on time-dependent signal processing in the cerebellar granular layer.

3.3.10 Relating firing rate correlation and single neuron computation

Firings of neurons usually have small pairwise correlations and its sources and functional consequences in relation to the population code have been under active debate. One candidate is a common input model, which postulates that the correlation in firings is mainly generated by an input correlation. In this project, we focus on the consequences of this common input model when it contains neurons with various computational properties, and therefore we try to understand how the computation of a neural population interacts with those of individual neurons.

Recently, de la Rocha et al. (2007) derived a relationship between correlation and gain from the common input model. Also, it was shown that the gain is modulated by the statistics of an input in two different ways, depending on the intrinsic computation of a neuron (Higgs et al., 2006).

Therefore, the relationship between an input and output correlation might significantly depend on how an individual neuron transforms the input, and indeed this is true even for very simple Hodgkin-Huxley (HH) type neuron models (Lundstrom et al., 2007). By computing correlations from the simulated data of these models, we have found that, if each neuron performs integration on an input, the result qualitatively follows the result of de la Rocha et al. (2007), but if the intrinsic computation of each neuron is differentiation, there is a non-trivial correlation that cannot be predicted by the firing rate and gain (Figure 6B). This indicates the statistics of a population activity also depends heavily on the intrinsic computation of an individual neuron.

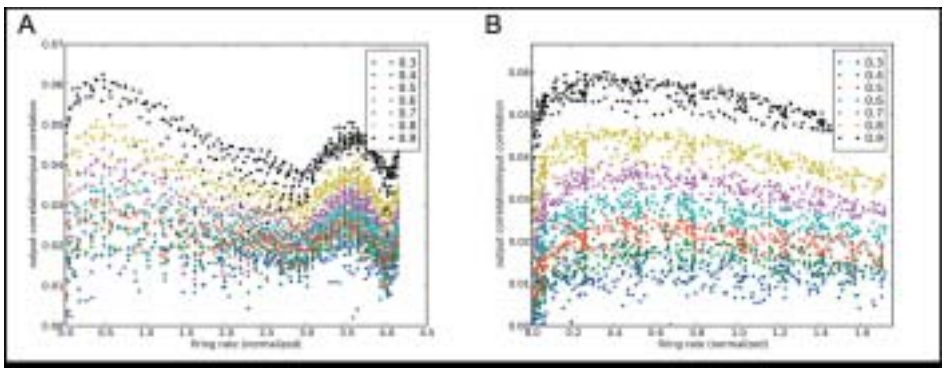


Figure 6: Ratios of input and output correlation with various input correlations. A. An HH neuron. B. An HH neuron with low sodium conductance. In both figures, the color represents an input correlation. Each point corresponds to a stimulus statistics, (mean, variance) of the injected Gaussian white noise current. Legends: variance of the input.

3.4 Information processing in the olivocerebellar system

No work was performed on this topic in FY2007 because of a delay in hiring of personnel.

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Steuber, V., Mittmann, W., Hoebeek, F. E., Silver, R. A., De Zeeuw, C. I., Häusser, M., and De Schutter, E. (2007) Cerebellar LTD and pattern recognition by Purkinje cells. Neuron 54, 121–136.
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Van Geit, W., Achard, P., and De Schutter, E. (2007) Neurofitter: a parameter tuning package for a wide range of electrophysiological neuron models. Front. Neuroinf. 1, 1.
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4. Publications

4.1 Journals

Steuber, V., Mittmann, W., Hoebeek, F. E., Silver, R. A., De Zeeuw, C. I., H_usser, M., De Schutter, E. (2007) Cerebellar LTD and pattern recognition by Purkinje cells, Neuron, 54, 121-136.

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Solinas, S., Forti, L., Cesana, E., Mapelli, J., De Schutter, E., D'Angelo, E. (2008) Fast-reset of pacemaking and theta-frequency resonance patterns in cerebellar Golgi cells: Simulations of their impact in vivo, Frontiers in Cellular Neuroscience, 1, 4, Electronic.

4.2 Book(s) and other one-time publications

Nothing to be reported.

4.3 Oral presentations

Achard, P., De Schutter, E. Activity-homeostasis preserves synaptic plasticity in Purkinje cell but calcium is not the activity-sensor, Computational Neuroscience Meeting, Toronto, Canada, July 7-9, 2007.

De Schutter, E. Decoding Purkinje cell simple spike trains: patterns and pauses, 7th IBRO World Congress of Neuroscience, Melbourne, Australia, July 12-17, 2007.

De Schutter, E. Decoding Purkinje cell simple spike trains: patterns and pauses, Neuro2007, Yokohama, Japan, September 10-12, 2007.

De Schutter, E. Plasticity I, OIST_Salk Joint Neuroscience Meeting, San Diego, USA, November 8-10, 2007.

De Schutter, E. The Purkinje neuron model parameter landscape: implications for homeostasis and synaptic plasticity, CAS-MPG Partner Institute for Computational Biology, Shanhai, China, November 16, 2007.

De Schutter, E. A European collaboration on cerebellar LTD and pattern recognition, 1st International Conference on Cognitive Neurodynamics, Shanghai, China, November 17-21, 2007.

De Schutter, E. Using Neurofitter for automated parameter fitting to electrophysiological data, Cosyne Workshops, Snowbird, Utah, USA, March 3-4, 2008.

De Schutter, E. Calcium, synaptic plasticity and intrinsic homeostasis in Purkinje neuron models, Calgary University, Canada, March 6, 2008

4.4 Posters

Van Geit, W., Achard, P., De Schutter, E. Neurofitter: a parameter tuning package for a wide range of electrophysiological neuron models, Computational Neuroscience Meeting, Toronto, Canada, July 7-9, 2007.

Van Geit, W., Achard, P., De Schutter, E. Neurofitter: a parameter tuning package for a wide range of electrophysiological neuron models, Society for Neuroscience Meeting, San Diego, USA, November 3-7, 2007.

Achard, P., De Schutter, E. Calcium, synaptic plasticity and intrinsic homeostasis in Purkinje neuron models, Society for Neuroscience Meeting, San Diego, USA, November 3-7, 2007.

Hituri, K., Achard, P., Wils, S., Linne, M.-L., De Schutter, E. Importance of stochasticity in simulation of the function of inositol-1,4,5-trisphosphate receptor, Society for Neuroscience Meeting, San Diego, USA, November 3-7, 2007.

Wils, S., De Schutter, E. Reaction-diffusion in complex 3D geometries: mesh construction and stochastic simulation with STEPS, Society for Neuroscience Meeting, San Diego, USA, November 3-7, 2007.

Achard, P., De Schutter, E. Calcium. synaptic plasticity and intrinsic homeostasis in Purkinje neuron models, Cosyne Meeting, Salt Lake City, USA, February 28 - March 2, 2008.

Hong, S., Muria, M., Fairhall, A. L., Dawson, G. Network Analysis of EEG Coherence in Autism Spectrum Disorder, Cosyne Meeting, Salt Lake City, USA, February 28 - March 2, 2008.

5. Intellectual Property Rights and Other Specific Achievements

n.a.

6. Meetings and Events

6.1 Olfaction targeted

Date: April 26, 2007
Venue: IRP seminar room, OIST
Speakers: Dr. Peter Mombaerts (The Rockefeller University)

6.2 Okinawa Computational Neuroscience Course 2007

Date: June 25 - July 13, 2007
Venue: Seaside House, OIST
Co-organizers: K. Doya (main organizer), K. Stiefel, J. Wickens
Co-sponsors: Nara Institute of Science and Technology
Japanese Neural Network Society

Speakers: Ad Aertsen, Universität Freiburg, Germany
Gordon Arbuthnott, OIST, Japan
Tom Bartol, Salk Institute, USA
Hagai Bergman, Hebrew University of Jerusalem, Israel
Nathaniel Daw, New York University, USA
Sophie Deneve, ENS Paris, France
Erik De Schutter, OIST, Japan
Markus Diesmann, RIKEN Brain Science Institute, Japan
Kenji Doya, OIST, Japan
Michael Häusser, University College London, UK
Shin Ishii, Nara Institute of Science and Technology, Japan
Dieter Jaeger, Emory University, USA
Mitsuo Kawato, ATR Computational Neuroscience, Japan
Eve Marder, Brandeis University, USA
Klaus Stiefel, OIST, Japan
David Terman, Ohio State University, USA

Gail Tripp, OIST, Japan
Jeff Wickens, OIST, Japan

6.3 Workshop: Hardware and software for large-scale biological computing in the next decade

Date: December 11-14, 2007
Venue: Seaside House, OIST
Co-organizers: K. Doya, K. Stiefel
Speakers: Phil ANDREWS (San Diego Supercomputer Center, USA)
Alan GARA (IBM, USA)
Robert GROSSMAN (University of Illinois Chicago, USA)
Seth Copen GOLDSTEIN (Carnegie Mellon Univ., USA)
Mike HINES (Yale University, USA)
Tetsuya SATO (Earth Simulato Center, Japan)
Felix SCHUERMANN (EPFL Lausanne, Switzerland)
Masakazu SEKIJIMA (AIST, Tokyo, Japan)
John SHALF (Lawrence Berkeley National Lab., USA)
Thomas STERLING (Louisiana State University, USA)
Arthur TREW (Edinburgh University, UK)
Tadashi WATANABE (RIKEN, Japan)
John WAWRZYNEK (Univ. California Berkeley, USA)

6.4 Seminar: The positive feedback mechanisms that transduce short-lived to long-lasting signals for cerebellar LTD

Date: January 10, 2008
Venue: IRP seminar room, OIST
Speakers: Dr. Keiko Tanaka (Duke University Medical Center)

6.5 Seminar: Unraveling the "Black Box" of the cerebellum

Date: February 18, 2008
Venue: IRP seminar room, OIST
Speakers: Dr. Marylka Uusisaari (RIKEN Brain Science Institute)

6.6 Seminar: Parallel Simulation of Large Neuronal Networks on Clusters of Multiprocessor Computers

Date: March 14, 2008
Venue: IRP seminar room, OIST
Speakers: Dr. Hans Plesser (Norwegian University of Life Sciences)

6.7 Seminar: Visual processing and short tern synaptic depression

Date: March 31, 2008
Venue: IRP seminar room, OIST
Speakers: Dr. Mark van Rossum (University of Edinburgh)

XIX. Trans-Membrane Trafficking Unit

Principal Investigator: Fadel A. Samatey
Research Theme: Structural and Functional Study of Membrane Proteins

Abstract

Our aim is to understand the function, at the molecular level, of macro-molecular complexes made of membrane proteins.

Biological Membranes contain specialized machineries made of membrane proteins. Membrane proteins play crucial roles by assuring transmission of signals and translocation of small molecules and proteins. They are involved in highly regulated trans-membrane traffic that is vital to cells. To give a few examples, trans-membrane trafficking occurs in ion channels and neurotransmitter receptors in the nervous system, in molecular-motors enabling the rotation of bacterial flagella and in the bacterial secretion systems involved in disease. To understand the function of these membrane proteins at the molecular level, knowledge of their three-dimensional structures is essential.

In a molecular complex, each protein is essential for the function of the complex. Thus, to understand the function, it is necessary to study all the proteins together. However, for high-resolution structural studies, it is very difficult and time consuming, to solve macromolecular complexes. Our goal is to combine X-ray crystallography and electron cryo-microscopy to solve the structure of membrane protein complexes. We will first solve the structure of each protein of a complex by using X-ray crystallography. Next, electron cryo-microscopy will give us the structure of the entire complex at lower resolution. The combination of these results will enable us to make a high-resolution model of the entire complex. Such a model will help us to understand the fundamental mechanisms by which membrane protein complexes perform their functions. This understanding will lead us to new testable hypotheses.

1. Staff

Researchers: Dr. Clive S. Barker (Started August 2007)
Dr. Hideyuki Matsunami (Started March 2008)
Dr. Vladimir Meshcheryakov (Started March 2008)
Technical staff: Ms. Tomomi Isono (Started April 2007; Left March 2008)
Ms. Irina Meshcheryakova (Started March 2008)
Research administrator/secretary: Ms. Saeko Hedo (Started November 2007)

2. Partner Organizations

Graduate School of Frontier Biosciences, Osaka University, Japan
Type of partnership: Joint Research
Name of principal researcher: Prof. Keiichi Namba
Research theme: Expression and purification of membrane protein complex

Institut Laue-Langevin, Grenoble, France
Type of partnership: Collaboration
Name of principal researcher: Dr. Giuseppe Zaccaï
Research theme: Molecular dynamic of the bacterial flagellum by neutron scattering

CNRS, Institute of Structural Biology and Microbiology (IBSM), Marseille, France
Type of partnership: Collaboration
Name of principal researcher: Dr. Jean-Romé Voulhoux
Research theme: Structural Study of the Type II secretion system of Pseudomonas aeruginosa

3. Activities and Findings

The Trans-Membrane Trafficking Unit was created in April 2007. The aim of the unit is the structural and functional study of membrane proteins. Ms. Tomomi Isono and I were the first members of the unit.

During the course of the first year, we spent time setting up the crystallization laboratory that is located at the Biocenter and the molecular biology laboratory that is located in the Annex of the Okinawa Industrial and Technology Center. Members of the unit, together with invaluable help from OIST administration, selected and ordered the equipment needed for our research. The equipment comprised one jar fermentor for medium volume (7 L) cell culture from “ABLE”, 2 protein purification systems from “GE Healthcare”, 2 centrifuges from “Beckman”, a nanodrop dispenser for protein crystallization from “TTP LabTech” and a complete X-ray diffraction system from “Rigaku”.

We are working on the structural study of membrane proteins of the flagellar Type III secretion system (T3SS) of Salmonella Typhimurium. During the construction of the bacterial flagellum, tens of thousands of proteins are exported across the membranes by the T3SS, which is homologous to the type III secretion system present in all Gram-negative bacteria. Gram-negative pathogenic bacteria use the T3SS to deliver bacterial toxin proteins into host cells. The consequences of these infections are very diverse.

While waiting for equipment to arrive, Dr. Clive S. Barker joined the unit. By early December we were able to start the first molecular biology experiments. We could successfully purify 3 out of 4 of our target proteins and we could start both the crystallization experiment and the functional assay of the system. For protein purification these proteins are over-expressed in E.coli.

In March 2008, Dr. Vladimir Meshcheryakov, Dr. Hideyuki Matsunami and Mrs. Irina Meshcheryakova joined the unit.

During the year, we were able to build a few collaborations with the CNRS in Marseille (France) for the study of membrane proteins of the Type II secretion system and with the Institute Laue Langevin (the world`s leading neutron research facility) in Grenoble (France) for the study of the dynamic of the bacterial flagellum by neutron scattering. We also established a joint research project with the Graduate School of Frontier Biosciences in Osaka University (Japan).

4. Publications

Oral presentations

Samatey, F.A., Namba K. Bacterial Flagella: Fibre X-ray Diffraction, The 9th International Biology and Synchrotron Radiation in Manchester, UK, August 12-17, 2007.

Samatey, F.A. Structure of the Bacterial Universal Joint, Annual Flagellar Meeting, Kyoto, Japan, March 3-5, 2008.

XX. Cellular and Molecular Synaptic Function Unit

Principal Investigator: Tomoyuki Takahashi

Research Theme: Regulatory mechanisms of neurotransmitter release

Abstract

In the central neuronal system, the synaptic strength changes dynamically, thereby playing critical roles in switching neuronal circuits. Despite a wealth of information accumulating on postsynaptic mechanisms regulating synaptic strength, much less is known for the presynaptic mechanism, primarily because of small nerve terminals preventing direct electrophysiological approaches. The calyx of Held is a giant glutamatergic nerve terminal, which is visible in auditory brainstem slices, and allows one to address questions on mechanisms regulating the efficacy of transmitter release. By applying molecular, and patch-clamp techniques to the calyx of Held synapse in brainstem slices of developing rodents, we aim at elucidating regulatory mechanisms of transmitter release.

1. Staff

Cellular and Molecular Synaptic Function Unit

Group leader: Tomoyuki Takahashi

Researchers: Takayuki Yamashita, Hiroyasu Watanabe, Kogaku Eguchi, Tetsuya Hori, Yukihiro Nakamura

Technical staff: Shoko Motohashi

Research administrator/secretary: Kaori Egashira

2. Partner Organizations

Nothing to be reported.

3. Activities and Findings

3.1

Developmental change in the regulatory mechanism of presynaptic Ca channels underlying short-term synaptic depression (Nakamura T et al, J Physiol 2008): Presynaptic Ca currents undergo inactivation upon repetitive activation, thereby causing synaptic depression. We found that this mechanism is robust at immature calyx synapse before hearing onset, but becomes weak during development. This is mainly because of a developmental reduction in residual bulk Ca in the nerve terminal, which makes it difficult to activate calmodulin in the nerve terminal for Ca/CaM-dependent Ca channel inactivation.

3.2

Involvement of AMPA receptor desensitization in short-term synaptic depression (Koike-Tani et al, J Physiol 2008): Paired-pulse facilitation (PPF) and depression (PPD) is generally thought to reflect transmitter release probability. However, we found that AMPA receptor desensitization is significantly involved in PPD at immature calyceal synapses, because of relatively high GluR1 subunit expression of postsynaptic AMPA receptors, and high transmitter release probability. As animals mature, PPD becomes less with decreased involvement of desensitization, thereby establishing high-fidelity high frequency synaptic transmission for sound localization at this auditory relay synapse.

4. Publications

4.1 Journals

Nakamura Y, Takahashi T. (2007) Developmental changes in potassium currents at the rat calyx of Held presynaptic terminal. J Physiol. 581, 1101-1112.

Suzuki D, Hori T, Saitoh N, Takahashi T. (2007) 4-chloro-*m*-cresol, an activator of ryanodine receptors, inhibits voltage-gated K^+ channels at the rat calyx of Held. Eur J Neurosci 26, 1530-1536.

Nakamura T, Yamashita T. (equal) Saitoh N, Takahashi T. (2008) Developmental changes in calcium/calmodulin inactivation of calcium currents at the calyx of Held. J Physiol 586, 2253-2261.

Koike-Tani M., Kanda T., Saitoh N., Yamashita T., Takahashi T. (2008) Involvement of AMPA receptor desensitization in short-term synaptic depression at the calyx of Held in developing rats. J Physiol 586, 2263-2275.

4.2 Book(s) and other one-time publications

none

4.3 Oral presentations

Takahashi T. Diverse roles of presynaptic calcium channels at the calyx of Held, Symposium (Heidelberg, Germany) , June 7-8, 2007.

Takahashi T. Looking into a rodent giant nerve terminal, Symposium (Rome, Italy), September 15-16, 2007.

Takahashi T. Regulatory mechanisms underlying chemical synaptic transmission in the mammalian CNS, Biomimetic Conference (Kyoto, Japan), December 8, 2007.

Yamashita, T., Nakamura, T., Saitoh, N., Takahashi, T. Developmental changes in calmodulin-dependent presynaptic calcium current inactivation at a calyx-type synapse, The 30th Annual Meeting of Japan Neuroscience Society, Yokohama, Japan, September 10-12, 2007.

4.4 Posters

none

XXI. Developmental Signalling Unit

Principal Investigator: Mary Ann Price
Research Theme: Genetic, biochemical, and cell biological studies on the mechanism of Hedgehog signalling

Abstract

The goal of our research is to understand the mechanism of the developmentally and medically important Hedgehog (Hh) signal transduction pathway, that is, how the extracellular Hh ligand brings about changes (primarily in gene expression) in its target cells. To this end, we use a variety of approaches, including Drosophila molecular genetics, biochemistry, cell biology, and functional genomics. Currently, our projects are focused on

・identifying novel binding partners for Hh signalling components, such as the transcription factor Cubitus interruptus (Ci), because many of these proteins are likely to be regulators of pathway activity

・understanding the subcellular localization of Hh signalling components, since this is known to be regulated on multiple levels by Hh signalling

・structure-function analysis of Ci repressor formation, an unusual incomplete proteasomal degradation reaction

・using functional genomic approaches (e.g., genome-wide RNAi screens in cultured cells) to identify novel Hh pathway components

1. Staff

Group leader: Mary Ann Price
Researchers: Alexander Soloviev, Chiemi Miyagi, Ingo Lehmann, Yawer Hussein
Technical staff: Jun Isoe
Research assistant/graduate student: Yifei Wang (University of Sheffield)
Research administrator/secretary: Chika Azama

2. Partner Organizations

Nothing to be reported.

3. Activities and Findings

The Hh family of extracellular signalling molecules regulates many important events during the development of animals from Drosophila to human. In Drosophila, where it was first discovered, Hh is involved in many processes including the anterior-posterior patterning of embryonic segments and larval imaginal discs (precursors of adult appendages) and the regulation of ovarian somatic stem cell self renewal. In vertebrates, the Hh family members pattern tissues such as the limb and neural tube and are involved in the development of many organs. Mutation of components of the Hh signalling pathway in humans leads to congenital developmental disorders, such as holoprosencephaly and polydactyly, while misregulation of the pathway later in life can lead to initiation and/or maintenance of many types of cancer.

Hedgehog signalling causes changes in target cells and tissues primarily by regulating gene transcription. In the absence of Hh signalling, the pathway transcription factor (Cubitus interruptus (Ci) in Drosophila) is processed by limited proteolysis to a shorter protein (Ci-75) that represses transcription. Hh signalling blocks this processing and converts full-length Ci (Ci-155) to a more

potent transcriptional activator, in part by stimulating nuclear localization of Ci-155. My lab has been focused on the regulation of Ci, primarily its processing to Ci-75.

My laboratory moved from the University of Sheffield (UK) to OIST in May 2007. One post-doc (Dr. Alexander Soloviev) moved with me to Okinawa (for 4 months in total) and one PhD student (Yifei Wang) remained in Sheffield, since he was in the final year of his PhD research. Over FY2007 I hired three additional post-docs (Dr. Chiemi Miyagi, Dr. Ingo Lehmann, and Dr. Yawer Hussein) and one technician (Dr. Jun Isoe), who started in the lab between September 2007 and February 2008.

Project 1: Identification and characterization of Ci binding partners. This project was initiated in Sheffield by Dr. Soloviev, and is being continued in OIST by Dr. Miyagi and Dr. Lehmann. We (and others) have previously shown that Kc cultured Drosophila cells recaptitulate the in vivo regulation of Ci. We have created a stable Kc cell line expressing tandem affinity purification (TAP)-tagged Ci (Fig. 1A) in order to purify Ci complexes and to identify their components by mass spectrometry (MS). Tandem affinity purification leads to a very clean preparation of Ci complexes (Fig. 1B), in which we consistently identify known Ci binding partners (Hh signalling components Cos2 and Su(fu)), known indirect interactors (Hh signalling component Fu), and several novel proteins, including a kinase, a protease, and a vesicular trafficking protein.

Dr. Lehmann will continue experiments identifying Ci binding partners and he will extend these studies to other Hh signalling components, such as Su(fu) and Smo. Importantly, he will also use the iTRAQ labeling method (developed by Applied Biosystems for the mass spectrometer we are using) to determine changes in complexes under different conditions. He will also use MS to determine post-translational modifications of Ci.

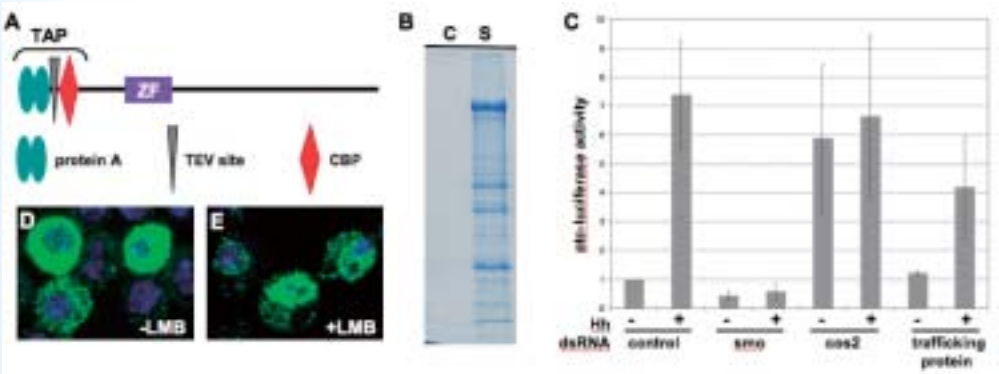


Figure 1. A.Schematic of TAP-Ci B. Coomassie-stained gel showing TAP complexes from TAP-Ci expressing cells or parental cells C. Transcriptional activity in response to Hh with the indicated dsRNA treatments (conclusion: knocking down expression of the Ci-interacting trafficking protein reduces the transcriptional response to Hh) **D-E.** An example of the assay we will use to study Ci localization. HA_3Ci is shown in green (nuclei are stained blue) in Kc cells treated without (D) or with (E) nuclear export inhibitor leptomycin B. We will use this assay in conjunction with RNAi to determine whether novel Ci binding proteins affect Ci localization.

Importantly, Dr. Miyagi is continuing with Dr. Soloviev's experiments to validate the novel Ci binding partners as true regulators of Ci and/or Hh signalling. First, we use double-stranded RNA interference (RNAi) to knock-down expression of the novel Ci interactors, and determine if this has an effect on the Hh transcriptional response using a Ci/Hh-responsive luciferase reporter construct (ptc-luciferase). As an example of our data, she has shown that the previously mentioned trafficking protein is involved in the Hh transcriptional response (Fig. 1C). Because effects on Ci repressor formation and/or localization will indirectly affect the transcriptional response, we will also determine whether these processes are altered by RNAi of novel Ci partners (Fig. 1D-E)

Project 2: Subcellular localization of Hh signalling components. Dr. Yawer Hussein is studying the subcellular locallization of Hh signalling components and how this changes when the pathway is activated. Since he only joined the laboratory in February, we do not yet have any results to report.

Project 3: Mechanism of Ci partial proteolysis by the proteasome. The proteasome usually completely degrades its polyubiquitinated substrate proteins. Ci is one of only a few known exceptions, where the C-terminus is degraded but the N-terminus (Ci-75) is left intact. My PhD student, Yifei Wang, has done a structure function analysis of Ci to determine which parts of Ci are required for Ci-75 formation(Fig 2A, B) and has classified these regions as to whether they are required

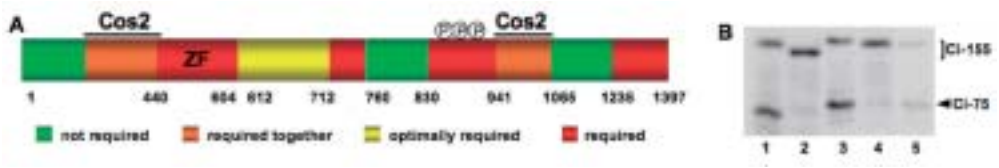


Figure 2. A. Schematic of regions required for Ci-75 formation. ZF indicates the five zinc finger DNA binding domain; P indicates the phosphorylated region required for Slimb binding (and presumably ubiquitination). **B.** Example of assay for Ci-75 formation: Western blot showing full-length and processed Ci for wild-type Ci (lane 1), CiD1235C (lane 2), CiK1253,1296,1304R (lane 3), CiD1319C (lane 4), CiD1235-1319 (lane 5). CiD1235C and CiD1319C form very little Ci-75, suggesting that the C-terminus of Ci is required for Ci repressor formation.

(I) for initiation of proteasomal degradation or (II) for protection of the N-terminus from complete degradation (Fig. 3A). In addition to regions of Ci already proposed to be required for phosphorylataion and ubiquitination of Ci, we find that the zinc finger DNA binding domain and a 163 amino acid C-terminal domain are required for initiation of proteasomal degradation. The regions of Ci required for protection of the N-terminus from complete degradation are the zinc finger region and a single lysine residue, K750, showing that Ci has a unique protection signal, distinct from that of other targets of partial proteolysis by the proteasome.

We show that the zinc fingers are required for protection of Ci-75 because they are a tightly-folded domain: substitution of the zinc fingers by wild-type barnase leads to a small amount of Ci-75 formation, while substitution by increasingly stable barnase mutants leads to increasing Ci-75 formation (Fig. 3B). These results fit with a model suggesting that a well-folded domain is required

for the protection of the N-terminus of partially degraded proteasome substrates. Substituting the zinc fingers with the GAL4 dimerization domain, however, does not lead to Ci-75 formation (not shown), suggesting that a model proposing that dimerization is critical for formation of the partially proteolyzed product is not generally applicable.

We also attempted to determine the function of the second part of the protection signal, K750. Lysine is the residue to which ubiquitin is covalently attached to proteins, thus we thought K750 may be a site of polyubiquitination. We were unable to show this directly, however, since even small fragments of Ci (containing only the Slimb binding site and the region around K750) appear to be ubiquitinated on multiple sites. Therefore, we substituted amino acids 712-760 with two or four in-frame copies of ubiquitin. Work from other labs showed previously that four in-frame copies of ubiquitin become polyubiquitinated, but that two copies do not. Our Ci4xUb construct is processed to a Ci-75 like product in cultured cells, but Ci2xUb is not (Fig. 3C), suggesting that polyubiquitination in the region of K750 is required for protection of the Ci N-terminus from complete proteasomal degradation. This is a novel (and counterintuitive) role for polyubiqitionation.

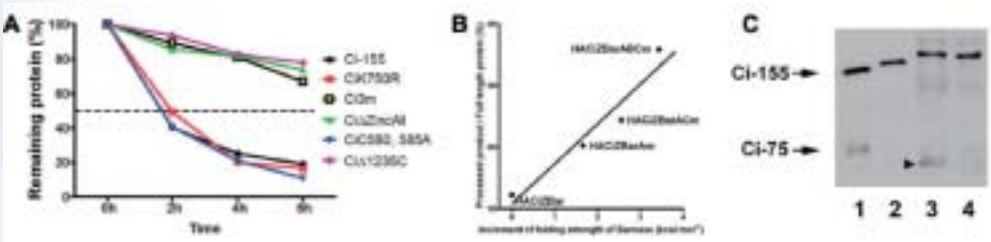


Figure 3. A. Pulse-chase assay determination of Ci (wild-type and mutant) half-lives. A half-life similar to wild-type Ci, but where no Ci-75 is formed (CiK750R and CiC580,585A, a previously characterized destabilizing mutation in the fifth zinc finger), indicates a mutation in the protection signal (class II); a half-life similar to the PKA site mutant Ci3m accompanied by no Ci-75 formation (CiDZincAll and CiD1235C), indicates that proteasomal degradation is not initiated normally in the Ci mutant (class I). **B.** The stability of barnase mutants substituted for the ZF region vs. amount of Ci-75 formed. **C.** Western blot showing Ci-75-like formation for Ci4xUb (lane 3) but not Ci2xUb (lane 2). Wild-type Ci is shown in lane 1, and a deletion of zinc fingers 1 and 2, a mutation that prevents Ci-75 formation in otherwise wild-type Ci, combined with the 4xUb insertion, is shown in lane 4.

Project 4: Genome wide RNAi screen to identify novel regulators of Ci proteolysis. We developed a luciferase reporter construct to use as a cell-based assay for Ci-75 formation. The reporter encodes a Ci fusion protein with Firefly luciferase at the N-terminal end and Renilla luciferase at the C-terminal end. When the full-length reporter is processed to Ci-75, the Renilla luciferase is degraded along with the Ci C-terminus, thus, the Renilla/firefly luciferase ratio is indicitive of the amount of Ci-75 formation. After optimizing the use of this reporter in Kc cell assays in 384-well format with control double-stranded RNAs (dsRNAs), Yifei conducted a screen of the full collection of dsRNAs at the Drosophila RNAi Screening Center at Harvard Medical School. Out of ~21,000 dsRNAs, Yifei found that ~240 had changes in Renilla/firefly luciferase ratios that indicated they were involved in Ci processing (Fig. 4), including Cos2, Cul1, Slimb, CKIα, and sgg/GSK3, all known to be involved in Ci-75 formation. We are currently testing these 240 genes in secondary screens, and will test a subset of these in in vivo assays for a role in Hh signalling.

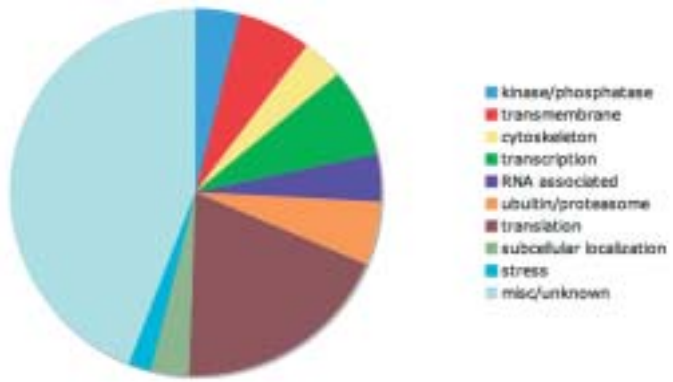


Figure 4. Pie chart showing the annotated functions of the ~240 genes identified in our RNAi screen.

Given our seeming success with this screen, we plan to do further cell-based genome wide RNAi screens in the future.

4. Publications

4.1 Journals

Price, M. A. (2007) Meeting Report: JDRC 8 Cell Biology and Development, Fly, 1(5), 294-296.

Wang, Y., Price, M. A. (2008) A unique protection signal in Cubitus interruptus prevents its complete proteasomal degradation, submitted.

4.2 Book(s) and other one-time publications

4.3 Oral presentations

Price, M. A. Mechanism of Ci Repressor Formation, 2nd KNU-OIST Workshoop, Daegu, Korea, March 13, 2008.

Price, M. A. Why I became a scientist, Afuso Junior High School, February 29, 2008.

4.4 Posters

Wang, Y., Strutt, H., Soloviev, A., Price, M. A. The Mechanism of Hedgehog Signalling in Drosophila, JDRC8: the 8th Japanese Drosophila Research Conference, Awaji Island, Japan, July 2-4, 2007.

Wang, Y., Soloviev, A., Strutt, H. Sidapra, B., Price, M. A. Ci Proteolysis and Hh Signalling, EDRC 2007: 20th European Drosophila Research Conference, Vienna, Austria, September 12-14, 2007.

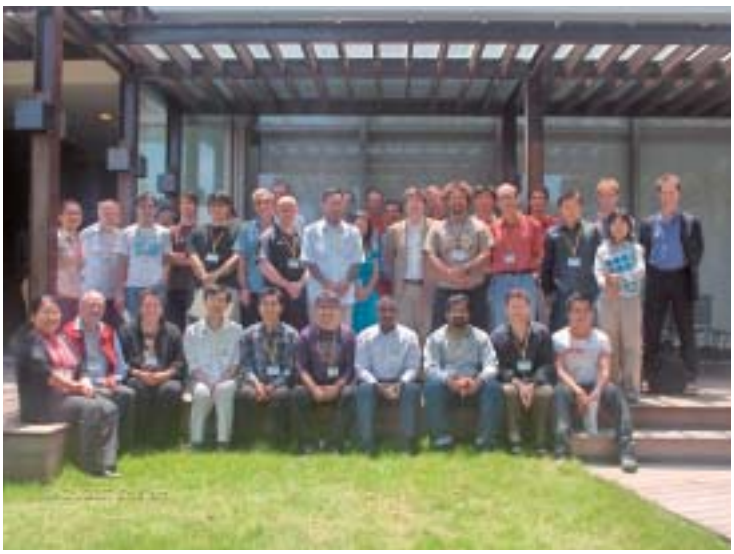
XXII. Education and Training Activities

During fiscal year 2007, 11 workshops either hosted or co-sponsored by OIST took place, brining as many as 250 lecturers and participants together for vigorous scientific discussions.

Inverse Problems and Biology

Outline: The aim of this workshop was to bring together distinguished biologists and mathematicians in the realistic expectation that new points of contact between biology and mathematics would be explored. It was a meeting of cutting edges, ones which are usually considered to be on opposing sides of that single blade one calls research.

Date: April 20 - 22, 2007
Organizers: Robert Sinclair, OIST
Klaus Stiefel, OIST
Venue: OIST Seaside House
Participants: Lecturers 9, Participants 22



Group Photo

Okinawa Computational Neuroscience Course (OCNC) 2007

Outline: The aim of OCNC 2007 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 11, 2007
*The original schedule (ending July 12) was shortened due to a typhoon.

Organizers: Erik De Schutter, OIST
Kenji Doya ,OIST
Klaus Stiefel, OIST
Jeff Wickens, OIST
Venue: OIST Seaside House
Participants: Lecturers 18, Tutors 10, Participants 30

Group Photo

Salk-OIST Joint Neuroscience Meeting

Outline: Two of the main features of the nervous systems of animals are their plasticity and their capability for processing time-critical information. Neural plasticity is often regulated in a time-critical manner, like in the case of spike-time dependent plasticity. On the other hand, neural plasticity can fine-tune the time-critical processes in nervous systems. This co-dependence between plasticity and time-critical processing necessitates an exchange between the research approaches addressing these two phenomena. The first aim of this workshop was to bring researchers together who are interested in either or both of them.

Date: November 8-9, 2007
Organizers: Terrence J. Sejnowski, Salk Institute
Klaus Stiefel, OIST
Venue: San Diego
Participants: Lecturers 12, Participants 26

Salk Institute for Biological Studies

Hardware and Software for Large-Scale Biological Computing in the Next Decade

Outline: The goal of this workshop was to support the planning for the HPC facility. OIST has quite a bit of expertise in scientific computing and data mining, but we lack hardware specialists who can help us to formulate a HPC plan. There were 13 speakers at the workshop, who covered a wide range of HPC issues.

Date: December 11-14, 2007
Organizers: Kenji Doya, OIST
Klaus Stiefel, OIST
Erik De Schutter, OIST
Venue: OIST Seaside House
Participants: Lecturers 13, Participants 16

HFSP International Workshop on Neural Control of Attention, Perception and Learning

Outline: The aim of the workshop was to bring together researchers working on the integration of bottom-up and top-down dynamic processes in visual attention and action selection. This joint workshop by the HFSP research team (Munoz, Isa, Itti and Theeuwes; http://www.hfsp.org/awardees/abstractRG.php?id=39&t=P&y=2005) and OIST promoted the recognition of OIST in neuroscience and gave opportunities for researchers to visit OIST and know its research activities.

Date: January 6-11, 2008

Organizers: Kenji Doya, OIST
Tadashi Isa, National Institute of Physiological Sciences
Laurent Itti, University of Southern California
Douglas Munoz, Queens University
Jan Theeuwes, Amsterdam Free University

Venue: OIST Seaside House

Participants: Lecturers 13, Participants 16

SBGN Super-Hackathon Workshop

Outline: The Okinawa super hackathon aimed to gather developers of the various standard formats and ontologies used in the field of Systems Biology, in order to improve their interfacing. It combined both the fifth SBGN meeting and the 3rd annual BioModels.net meeting.

The fifth SBGN meeting was to be a “Spec-a-thon” devoted to finalise SBGN Level 1, and a hackathon, where software support for the language was developed. The third annual BioModels.net meeting should feature presentations about progresses of the core projects and related efforts. We also worked on the interactions of SBGN and BioModels.net efforts with other formats such as BioPAX and SBML.

Date: January 28- February 2, 2008

Organizers: Hiroaki Kitano, OIST, SBI & Sony CSL
Nicolas Le Novere, EMBL-EBI
Mike Hucka, Caltech CDS
Akira Funahashi, SBI & Keio University

Venue: OIST Seaside House

Participants: Participants 31

Systems Biology of MAPK Pathways Workshop

Outline: Based on the success of the first workshop in February 2007, the second workshop on yeast signaling systems biology was organized with more depth and moderately increased size of participants. The goal of the workshop was to have intensive discussions on how to create mathematical model of cells, especially on signaling transduction of budding yeast. Participants were all focused on budding yeast signaling system. By sharply focusing the topic to specific signaling system with limited participants who are seriously working in the area, in depth discussions took place.

During the 2007 workshop, participants agreed on joint papers and the idea of global hub centers for yeast signaling where OIST is considered as one of the hubs. The workshop was a part of plan to attract international researchers in the area of systems biology to OIST so that OIST can emerge as global hub of the area.

Date: March 8-11, 2008

Organizers: Hiroaki Kitano, OIST
Stefan Hohmann, University Goteberg
Roger Brent, Institute of Molecular Sciences, USA

Venue: OIST Seaside House

Participants: Lecturers 16, Participants 19

Co-sponsored Workshop

"Mechanisms of Brain and Mind" on August 23-24 in Sapporo

"Mechanisms of Brain and Mind" on January 9-11 at Rusutsu Resort in Hokkaido

The 2nd OIST-Korea Workshop at Kyungpook National University in South Korea

The 9th Neuropeptide Y International Meeting on March 16-20 at Okinawa Kariyushi Beach Resort Ocean Spa

Published by Okinawa Institute of Science and Technology Promotion Corporation

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