OIST
Annual Report 2009

Okinawa Institute of Science and Technology Promotion Corporation

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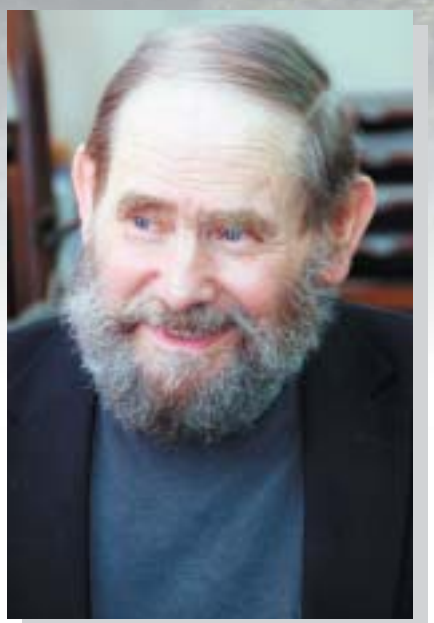
Preface

A major event this year was the enactment of the OIST School Corporation Act on July 10, which came following unanimous passage by the Japanese House of Representatives and House of Councillors. The Act stipulates the establishment of the Graduate University as an independent school corporation to preserve autonomy and management flexibility. The Act enables the OIST Graduate University to apply for accreditation by the Ministry of Education, Culture, Sports, Science and Technology to open in fiscal year 2012.

We added two new Principal Investigators and three Independent New Investigators. The Independent New Investigator appointments are for young scientists, which are central to our commitment to enable the best young scientists from Japan and around the world to initiate their own independent research projects. We have greatly expanded our capacity in genomic analysis and are now undertaking projects on diversity and evolution in marine organisms.

Another major activity this year was the relocation of research units from Uruma City to the new campus in Onna Village, following the completion of construction of Laboratory 1 and the Center Building. The design is striking and blends well with the beautiful natural environment of the campus. The interiors have been designed to encourage multidisciplinary research and interactions among the scientists. On March 28, we held the New Research Laboratory Opening to commemorate the start of the use of these buildings. Approximately 300 guests attended the event, including officials from the central government, Okinawa Prefecture, and local municipal governments.

We enthusiastically continue our progress toward the successful opening of the Graduate University, which will play an important role in the future of Okinawa.



Sydney Brenner
President
Okinawa Institute of Science and Technology Promotion Corporation

During FY2009, five research units were additionally established. The new research units are:

Evolutionary Systems Biology Unit
Independent New Investigator: Dr. Holger Jenke-Kodama
Establishment date: May 2009

Open Biology Unit
Principal Investigator: Dr. Hiroaki Kitano
Establishment date: June 2009

Ecology and Evolution Unit
Independent New Investigator: Dr. Alexander Mikheyev
Establishment date: August 2009

Marine Biophysics Unit
Independent New Investigator: Dr. Satoshi Mitarai
Establishment date: September 2009

Structural Cellular Biology Unit
Principal Investigator: Dr. Ulf Skoglund
Establishment date: January 2010

General Report

I. OIST P.C. 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 to prepare for the opening of a world-class graduate university of science and technology in Okinawa.

The main events in FY2009 were as follows:

June 11, 2009	The House of Representatives unanimously passed the Okinawa Institute of Science and Technology School Corporation Bill.
July 3, 2009	The House of Councillors unanimously passed the Okinawa Institute of Science and Technology School Corporation Bill.
July 10, 2009	The Okinawa Institute of Science and Technology School Corporation Act was enacted.
October 8-9, 2009	The eighth BOG meeting and the first meeting of the Establishing Members for OIST took place in Tokyo.
November 15, 2009	The Open House 2009 for the Okinawa community was held.
March 27-28, 2010	The ninth BOG meeting and the second meeting of the Establishing Members for OIST took place in Tokyo.
March 28, 2010	New Research Laboratory Opening was held.

II. BOG Meeting Summary

The Board of Governors (BOG) was established under the Corporation Act in 2005. The tasks of BOG are to oversee the Corporation and advise the President. BOG operates under Articles 12 and 13 of the Corporation Act. As of March 31, 2009, there were 10 Board members, with Dr. Arima and Dr. Wiesel serving as co-chairs of the Board since December 2006.

<Members of the Board of Governors>

Dr. Akito Arima*	Chairman, Japan Science Foundation Chancellor, Musashi Gakuen President and Chair of the Board of Trustees, Human Frontier Science Program
Dr. Jerome Friedman	Professor, Massachusetts Institute of Technology Nobel Laureate (Physics, 1990)
Dr. Tim Hunt	Cancer Research UK Nobel Laureate (Physiology or Medicine, 2001)
Dr. Ichiro Kanazawa	President, Science Council of Japan
Dr. Kiyoshi Kurokawa	Professor, National Graduate Institute for Policy Studies Former Special Cabinet Adviser in charge of science, technology and innovation Former President, Science Council of Japan
Dr. Yuan-Tseh Lee	President Emeritus, Academia Sinica Nobel Laureate (Chemistry, 1986)
Lord Martin Rees	President, The Royal Society
Dr. Hiroko Sho	Professor Emeritus, The University of the Ryukyus Former Vice Governor, Okinawa Prefecture
Dr. Susumu Tonegawa	Professor, Massachusetts Institute of Technology Director, Riken Brain Science Institute Nobel Laureate (Physiology or Medicine, 1987)
Dr. Torsten Wiesel*	President Emeritus Rockefeller University Former Secretary General, Human Frontier Science Program Organization Nobel Laureate (Physiology or Medicine, 1981)

* Co-chairs

The 8th BOG meeting

Date: October 8, 2009

Venue: Hotel New Otani, Tokyo

Participants: (Board of Governors) Drs. Akito Arima, Jerome Friedman, Kiyoshi Kurokawa, Yuan Tseh Lee, Hiroko Sho and Torsten Wiesel
(OIST P.C.) President Sydney Brenner and Vice President Robert Baughman
(CAO) Mr. Osamu Shimizu

Summary

- CAO reported on the establishment of OIST Act.
- CAO reported on the appointment and role of Establishing Members of OIST.
- CAO reported on the retraction of supplementary budget for FY 2009.
- OIST P.C. reported on research and academic progress.
- OIST P.C. reported on Principal Investigator hiring.
- OIST P.C. reported on campus construction.

The 9th BOG meeting

Date: March 28, 2010

Venue: OIST P.C. Seaside House

Participants: (Board of Governors) Drs. Akito Arima, Jerome Friedman, Timothy Hunt, Kiyoshi Kurokawa, Yuan Tseh Lee, Hiroko Sho, Susumu Tonegawa and Torsten Wiesel
(OIST P.C.) President Sydney Brenner and Vice President Robert Baughman
(CAO) Mr. Osamu Shimizu

Summary

- OIST P.C. reported on the accreditation progress, and the BOG decided that a document explaining the progress should be announced to the press.
  - A single unified interdisciplinary graduate program.
  - A single five-year integrated doctoral program.
  - About 50 faculty members with about 20 students accepted every year.
  - Awarded degree is Doctor of Philosophy.
  - Generous financial support for students to remain competitive as a leading international graduate school.
  - Graduate school aims to receive students in the early fall of 2012.
- OIST P.C. reported on research progress including publications and joint agreements.
- OIST P.C. reported on Principal Investigator recruitment, confirming that the recruitment is on schedule.
- OIST P.C. reported on campus construction.
- OIST P.C. reported on financial status and changes to its management. BOG passed a resolution confirming that the measures proposed are sufficient to improve the situation.

III. Campus Master Plan Status

Civil Works

Bridge No. 1, which is one of the two un-constructed bridges, was completed by February 2010 and an internal road that runs between the Village Zone and the Laboratory Zone has also been completed. Construction of the remaining Bridge No. 2 was contracted out in October 2009 to connect the hillside housing area and the Laboratory Zone.

Basic infrastructure works were completed by February 2010, including electric power lines, water supply pipes, and sewage pipes, as well as road pavement.

The landscaping of the pond in front of the Tunnel Gallery was completed by March 2010.

The site development work for the hillside housing, which commenced in FY2008, was completed by June 2009.

Environment Impact Assessment

Throughout the construction, the environmental impact was monitored carefully. This campus project has been subject to an Environmental Impact Assessment (EIA) process. The contractors were required to follow the environmental-management instructions, which were established in the course of the EIA process. Additionally, a third-party consultant was hired to monitor and investigate the environmental impact and also to instruct the contractors how to minimize the impact on the campus site environment during their construction. The construction progressed in compliance with Okinawa Prefectural Ordinance for Prevention of Red Clay Outflow, and careful coordination and planning were required to prevent the discharge of turbid storm water from running off to the local rivers. This was achieved by implementing turbid water treatment facilities and inspection patrols on rainy days. In FY 2009, OIST P.C. submitted a report of its environmental impact survey to Okinawa Prefecture and made the report open to the public.

Facility Construction

The core facility and fit-out work, such as interior, mechanical, electrical, and plumbing works for Laboratory 1 and the Center Building, virtually completed by January 2010. Following this, laboratory and general furniture, audio visual equipment and IT infrastructure were installed. The relocation of research laboratories from the site in Uruma City was successfully carried out and 15 research units started their research activities on the new campus in March 2010.

Construction of the energy center, which provides electricity and city water to the laboratory buildings, was completed in FY2009 and started to receive 66-kilovolt electricity power for the buildings.

IV. Finance

Budget

The total budget of FY2009 was 11,229 million yen which was approximately 5% increase from the previous Fiscal Year's original budget, 10,740 million yen. While the total amount of Subsidy for Facilities decreased mainly due to the funding of supplementary budgets in FY2008, Subsidy for Operations increased by approximately 28%. The increment was mainly attributed to the budget for the occupancy of the new buildings in Onna Village.

Million Yen

	FY2008		FY2009
Subsidy for Facilities	(6,286)	14,942	5,511
1) Land Development	(80)	80	0
2) Construction	(4,763)	10,905	3,182
3) Infrastructure Improvement	(1,300)	3,814	1,426
4) Land Acquisition	(143)	143	0
5) Large Equipment	(0)	0	903
Subsidy for Equipment	(0)	170	0
Equipment	(0)	170	0
Subsidy for Operations	(4,454)	4,454	5,718
1) Research Expenses, Equipment, Workshops etc.	(3,753)	3,753	3,918
2) General Administrative Expenses	(701)	701	944
3) New campus related expense	(0)	0	856
Grand Total	(10,740)	19,566	11,229

Note: ( ) FY2008 Original Budget

FY2008 Original Budget Total	10,740	million yen
1st Supplementary Budget	4,568	million yen
2nd Supplementary Budget	4,258	million yen

V. Human Resources

Recruitment

As of April 1, 2010, the total number of full-time employees was 216, up 19% from the previous year. Of the 216 employees, 148 were in research positions, including 57 from overseas, representing 20 countries. Approximately 40% of the administrative staff are from Okinawa.

Staff Training

For both research and administrative employees, training opportunities have been provided for further development. These included intellectual property management, employee evaluation and feedback, document management and language courses.

VI. Research Support Activities

Competitive Research Fund

The status of awarded grants is as follows:

KAKENHI (Grants-in-aid for Scientific Research): 3,870,725 yen for 9 research projects

Other grants: 3,399,100 yen for 1 research project

Patent Application

To increase awareness about intellectual property and patent applications of both OIST researchers and administrative staff, an intellectual property seminar was held in January 2010. The total number of patent applications submitted so far is 8, and one of them has been granted.

Joint Research

The number of joint research projects is as follows:

	Partner Organization		Total
	Domestic institution	Foreign institution	
Joint research	18	4	22
Externally sponsored research	3	0	3

Scientific Committees and Meetings

In FY2009, five scientific committees and meetings took place.

Date	Title	Main Topics
January 27, 2010	The 6th meeting of the Genetic Recombination Experiment Committee	-Review of seventeen applications for genetic recombination experiment plan -Review of amendments of OIST gene recombination experiment regulations
January 27, 2010	The 3rd meeting of the Biosafety Committee	-Review of six applications for the handling of pathogens and toxins -Review of amendments of OIST biosafety management regulations
February 19, 2010	The 1st Presentations on management of waste, chemicals and radioisotopes	-Waste disposal procedures at IRP, SH and Lab1 -Management of chemicals (including poisonous and deleterious substances) at IRP, SH, and Lab1 -Radioisotope utilization procedures at Lab1
February 25, 2010	The 2nd Presentation on management of waste, chemicals and radioisotopes	-Waste disposal procedures at IRP, SH and Lab1 -Management of chemicals (including poisonous and deleterious substances) at IRP, SH, and Lab1 -Radioisotope utilization procedures at Lab1
March 26, 2010	The 2nd meeting of the Human Subjects Research Review Committee	-Review of three applications for human subjects research -Review of two progress reports of human subjects research. -Review of amendments of OIST biosafety management regulations

Chemical and Wastes Management

Regulations for the Management of Chemical Materials, Detailed Stipulations for Poisonous and Deleterious Substances Management and Detailed Stipulations for the Management of Wastes were newly established.

Manuals for the management of chemicals and wastes were also compiled and distributed to OIST researchers.

RI Facility

Preparation for the establishment of the Radioisotope (RI) Facility in Lab1, an application for RI usage was submitted to the Minister of Education, Culture, Science and Technology (approved on April 13, 2010), and Regulations for Prevention of Radiation Hazards, Detailed Stipulations for Prevention of Radiation Hazards, as well as Detailed Stipulations for Radiation Safety Committee were established. Furthermore, RI monitoring system and common equipment for RI experiments, including ultra centrifuge, biosafety cabinets, etc., were installed at the RI facility.

DNA Sequencing Center

The DNA Sequencing Center has been equipped with five “next-generation” DNA sequencing machines and two conventional high-throughput DNA sequencing machines. The DNA sequencing facility has been moved and concentrated in the Okinawa Health Biotechnology Research and Development Center, located next to Okinawa Science and Technology Research Exchange Center.

Types and numbers of the DNA sequencers

Type		Number
Next-generation systems	Genome Sequencer FLX Titanium (Roche)	3
	Genome Analyzer IIx (Illumina)	2
Conventional systems	3730 DNA Analyzer (Applied Biosystems)	2

Relocation of Laboratories

The relocation of fifteen Research Units from Uruma City to the New Campus started on January 23, 2010, which was about one week after the hand-over of the buildings from the constructor. The relocation of most of the Units was completed by March 19. The relocation schedule was set in consideration of accomplishing two different requirements: 1) Minimize research down time: and 2) Finish within FY2009 in order to start preparing for the return of Uruma facilities in early FY2010 to Okinawa Prefecture. All of the Research Units were relocated within a three-week period of relocation down time. The vacant Uruma facilities are expected to be returned in April and May of 2010, following refurbishing to their former condition.

New Animal Facilities

Operations of new animal facilities in Laboratory 1 started on March 1, 2010 after the President’s approval of the application of the new facility utilization, which had been investigated by the Animal Care and Use Committee.

VII. IT Infrastructure

The network infrastructure on the new campus has been built with high-bandwidth cables and network equipment, enabling the establishment of 10Gbps (Giga bits per second) connectivity between any two points in the campus network, as well as 1Gbps connectivity to the external network. The campus server room has been built with chilled water cooling systems which are efficient and lead to power reduction. An IP telephone system has also been installed on the campus. In order to save cost, we have stopped using commercial Video Streaming Service and VPN Authentication Service, and instead built alternative systems internally by using Open Source Software with no additional cost.

VIII. Outreach Activities

Community and Public Relations

OIST P.C. provided various opportunities for local citizens, including school children, teachers, and lay people, to learn about our research and other activities through lectures, school visits, exhibitions, etc.

OIST P.C. held the “Open House” on November 15, 2009, providing an opportunity for local citizens to see OIST activities. Approximately 470 people came to see the event, which included lectures by the PIs, scientific exhibitions, demonstrations, and lab tours.

Throughout the whole year, OIST P.C. received many visitors, including those from abroad as well as officials from the central and local governments.

Two issues of newsletters and an annual report were published and distributed to the central and local governments, academic and research institutions at home and abroad, as well as to visitors to OIST P.C., including participants of OIST P.C.-sponsored workshops and events. In addition, we have revised our corporate brochure to reflect our current and future activities.

Information about each activity of the corporation was updated on the OIST P.C. Website.

	Types of activities	Number of activities
1	Lectures by PIs	8
2	Lectures by OIST P.C. officials / staff	3
3	Open House	1
4	Visitors to OIST P.C.	60
5	Exhibitions	6

Lecture by Dr. Robert Sinclair at Onna Junior High School

OIST P.C. Open House 2009 (Entrance)

OIST P.C. Open House 2009 (Demonstration)

OIST P.C. exhibition at Okinawa Prefecture's festival

Collaboration with Industry

One of the key concepts of OIST P.C. is collaboration with the industry. As part of our effort to form an intellectual cluster and an industrial cluster through collaboration with corporate laboratories and venture businesses, OIST P.C. took part in the following two events. On February 4, 2010, the Biotech Cluster Conference, sponsored by the University of the Ryukyus and the Japan Bio Industry Association, took place in Naha City. OIST was introduced to the conference participants, who had gathered from all over Japan. OIST P.C. collaborated with the project of creation of the industry and academia collaboration center for promotion and development of Okinawa health/bio industry Integrated Innovation Center for Community, University of the Ryukyus.

IX. Graduate University Preparation

OIST P.C. has renewed memorandums of understanding (MOUs) for research and graduate training with the Nara Institute of Science and Technology and the University of the Ryukyus, and newly signed MOUs with Doshisha University and Kyoto University.
In order to accept an even wider range of domestic and international graduate students, OIST P.C. newly formulated and implemented “Regulations for Receiving Students” and “Regulations for Short-Term Research Assistants”, and amended the existing “Employment Regulations for Graduate Student Research Assistants.” These information, all of which are now available on OIST P.C. website, will enable OIST P.C. to accept students under both short-term and long-term appointments in fiscal year 2010. http://www.oist.jp/en/research/partner.html

The newly established "Regulations for Short-Term Research Assistants" will not only allow acceptance of graduate students in doctoral programs on a short term, but also acceptance of a wider range of non-doctoral graduate students as short-term interns or trainees. This is important for enhancing an ability of OIST P.C. to recruit new students in a future graduate training program.

In FY2009, OIST P.C. studied appointment system for faculty members, including information about tenure, remuneration, benefits, retirement, as well as service obligations for performance evaluation, and later compiled documents which included the past case study survey report. The documents were distributed for deliberation at the 1st Meeting of the Establishing Members for OIST in October. With feedback from the Establishing Members, OIST P.C. examined the items in the appointment system internally. OIST P.C. formulated a draft student recruitment strategy so as to recruit talented graduate students in science majors. OIST P.C. also started a survey of graduate schools and universities in Asia concerning information about their recruitment of outstanding faculty and doctoral students.

The Graduate University Preparation Working Group and the Graduate Committees developed a draft document for graduate programs of the new university. This included consideration of characteristics of graduate schools and majors, types of graduate programs, curriculum design policies, educational and research guidance methods, course selection models, thesis examination systems, etc. They reflected the results of a survey on prestigious universities and graduate schools both at home and abroad. OIST P.C. formulated drafts of other major items required for accreditation applications, such as Bylaws, University Rules, and school corporation administrative structure.

Scientific Report

Brain Mechanisms for Behaviour Unit

Principal Investigator:
Gordon Arbuthnott
Research Theme:
The corticostriatal system in culture

Abstract

This year saw both the expansion of the Unit and several new sets of results. We presented a poster at the Society for Experimental Biology meeting, four at the Society for Neuroscience meeting, and saw the publication of six papers from our work so far.

Until March this year, people in the unit have worked under rather difficult circumstances since there were only two laboratories for three postdoctoral fellows and two short-term visitors. Nonetheless, we made important progress this past year. Dr. Takuya Hikima produced the first quantification of vesicular release from cortical cell axons in culture. Moreover, he successfully used the 2-photon microscope to measure membrane voltage using second harmonic imaging. Thanks to the persistence and care of Dr Marianela Garcia-Munoz we now have a type of corticostriatal culture in which the cortical and the striatal cells are plated in different compartments separated by 500 µm. This has allowed us: 1- to visualize the extension of the cortical connections into the striatal cell area and 2- to make controlled manipulations of the two populations of cells. Using these cultures Dr. Luis Carrillo-Reid has been studying the patterns of neuronal network activity using calcium imaging. Drs. Carrillo-Reid and Garcia-Munoz using multielectrode recording arrays and calcium imaging have studied the patterns of the corticostriatal neuronal activity under different pharmacological manipulations. Finally, thanks to the diligence of Dr Fiona Randall we now know that striatal cells synapse with cortical cells in the mixed cultures - a circumstance which never happens *in vivo*. One outcome of separating the two cell types is that such contacts may be less likely - but that remains to be determined.

Our collaborations have been fruitful as well. We may have an entirely new way to image the distribution of the calcium channel (Cav1.3) if we can repeat a very exciting early result from New Zealand. With our Canadian collaborators we have a manuscript with a detailed description of the



interneurons in our striatal cultures in final revision and the first set of results suggesting that the morphological development of striatal cells in our cultures is changed by cortical input, and that the changes depend on action at glutamate receptors.

1. Staff

Researchers: Dr. Marianela Garcia Munoz,
Dr. Fiona Randall,
Dr. Takuya Hikima (from April 2009),
Dr. Luis Carrillo-Reid (from July 2009)
Research Administrator / Secretary: Ms. Hiroko Chinone

2. Partner Organizations

University of Ottawa, Canada.
Type of partnership: Joint research
Name of researcher: Professor W.A. Staines,
Professor A. Krantis,
Professor Geoff Mealling,
Dr. Sarah Schock,
Kheira Jolin-Dahel, M.Sc.
Research theme: Isolating genetically marked neurons in culture

University of Otago, Dunedin, New Zealand
Type of partnership: Joint research
Name of researchers: Dr Beulah Leitch
Olga Shevtsova, M.Sc.
Research theme: Determining the location of L-type Calcium channels (CaV1.3) on striatal membranes

3. Activities and Findings

3.1 Synaptic release from individual cortical boutons.

We have developed a method that allows us to transfect cortical cells in culture and to image the axons arising from them by their content of synaptopHluorin. We have also managed to transfect cortical cells with the newer synpHluorin and hope to take advantage of the increased signal from that construct. Dr. Takuya Hikima was recognised by the Physiological Society of Japan for one of the publications from his Ph.D this year, and the many problems en route to this new beginning have been overcome thanks to his determination and skill. These results will allow us to look at presynaptic physiology in a new way in this system.

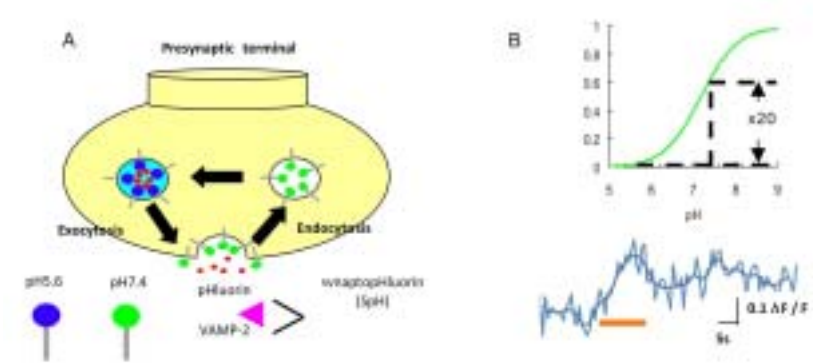


Figure 1

A. Diagrammatic representation of the visualization of synaptic vesicle cycling. SynaptopHluorin is non-fluorescent at pH 5.6 - the pH within vesicles in the cytoplasm. However, once released the pH rises to 7.4, and the protein fluoresces. B. Top, the pH sensitivity of synaptopHluorin fluorescence. Bottom, the response from a single varicosity on a cortical axon to field stimulation of 10 Hz for 10 sec (orange bar)

3.2 Measuring membrane voltage with light.

Dr. Hikima has also taken the lead in developing second harmonic generation as a method to study the voltage changes in cell membranes. In cortical cells he has been able to show that we can image neuronal membranes and detect voltage changes impressed upon them via a patch pipette.

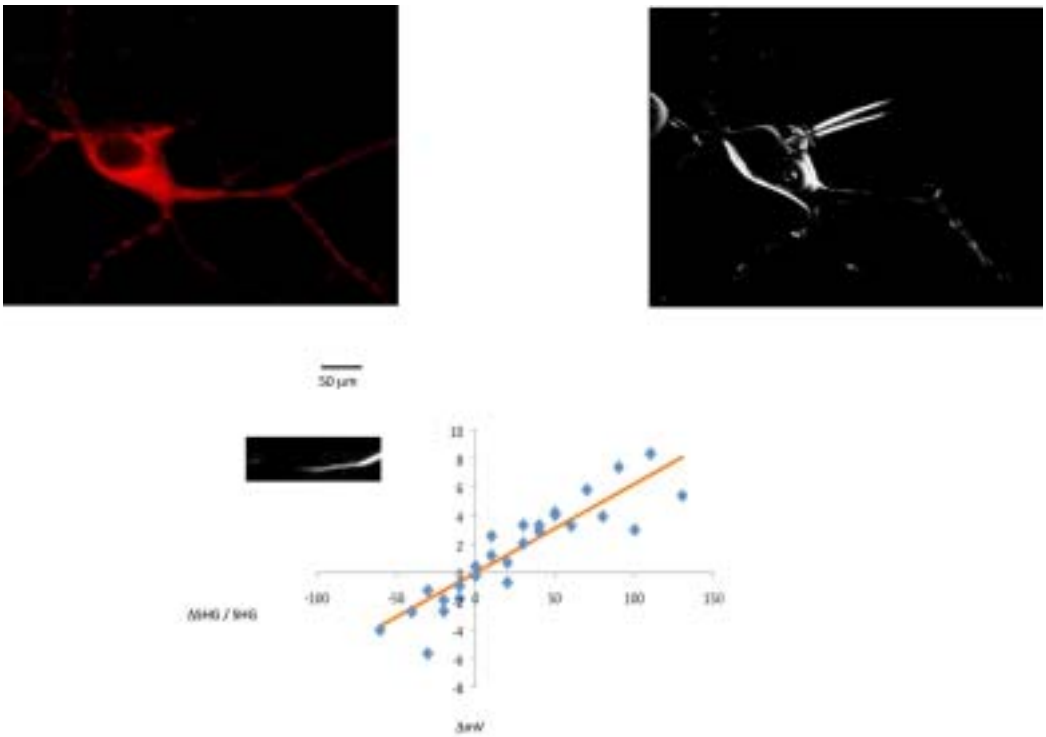


Figure 2: Second harmonic generation. The red image is of the dye (FM4-64) in a single neuron detected by conventional 2-photon methods in the reflected light path. The black and white images are from the photomultiplier under the condenser detecting transmitted light at the second harmonic of the laser frequency. The graph shows the change of transmitted light when the cell body is voltage clamped and the membrane voltages changed as shown.

Dr. Hikima managed both the synaptopHluorin and second harmonic generation projects in spite of having to reconfigure the microscope for each type of experiment. We now have a microscope that can both accommodate the 25x 'maximum transmittance' lens for the second harmonic generation and also adjust for the smaller 60x lens required for visualizing the fluorescent indicator.

3.3 Development of corticostriatal cultures.

In mixed cultures of cortex and striatum we had to use mouse cortical neurons expressing green fluorescent protein in order to tell the cells apart. If we could develop a way to grow cortical and striatal neurons which then remained separate - but connected - cultures, this would be much more like the situation *in vivo*. We have achieved this using small double well chambers called "campenots" after B.B. Campenot in 1977.

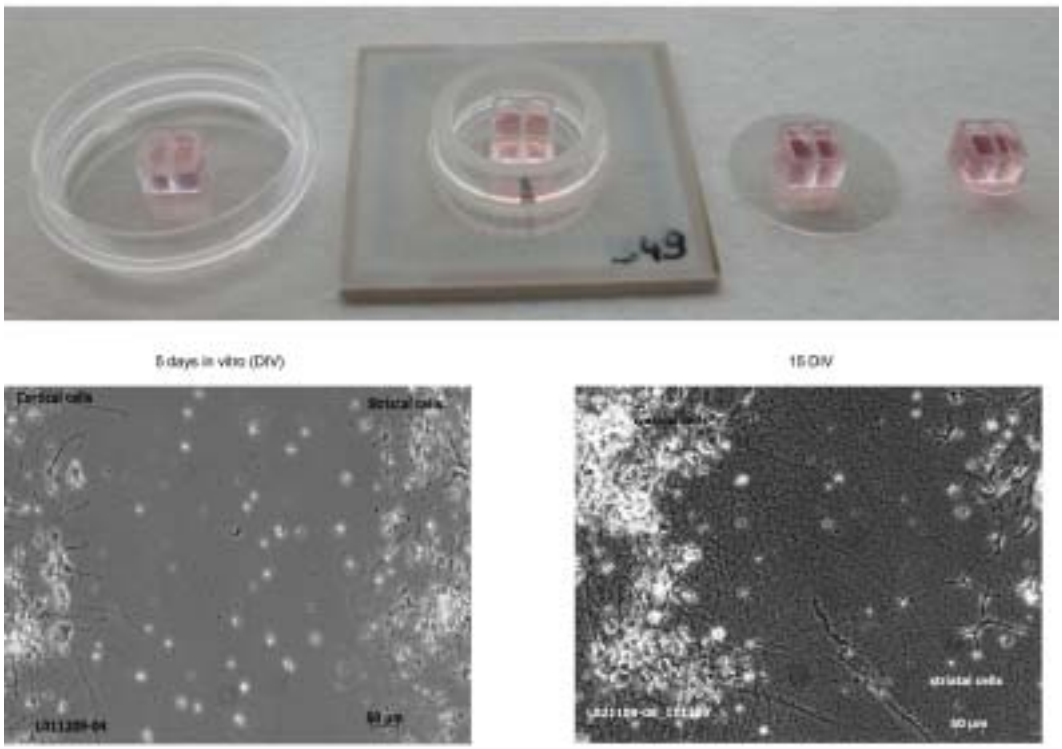


Figure 3. Top: Campenots, small compartments in which cells can be limited, allow in our hands, the development of cortical and striatal neurons. For the different experiments our cultures grow in 2.5mm plates, multielectrode recording arrays (MEA), 25mm and 12 mm coverslips (top picture from left to right). **Below:** Microscopic images of the gap between the two cell groups at 5 and 15 days *in vitro*. Initially completely separate these co-cultures make synaptic connections. Our data has shown that these cultures establish effective synaptic connections, display synchronous discharges which increase in frequency under pharmacological manipulation (e.g., $GABA_A$ receptor blockade) and that the cortical neurons seem to drive activity in striatal cells.

3.4 Dynamics of cell activity.

We have developed two methods with which to image the activity of groups of cells in culture. One of them involves the use of multi-electrode arrays on which cells are cultured while the second depends on the property of neurons to accumulate calcium during periods of activity.

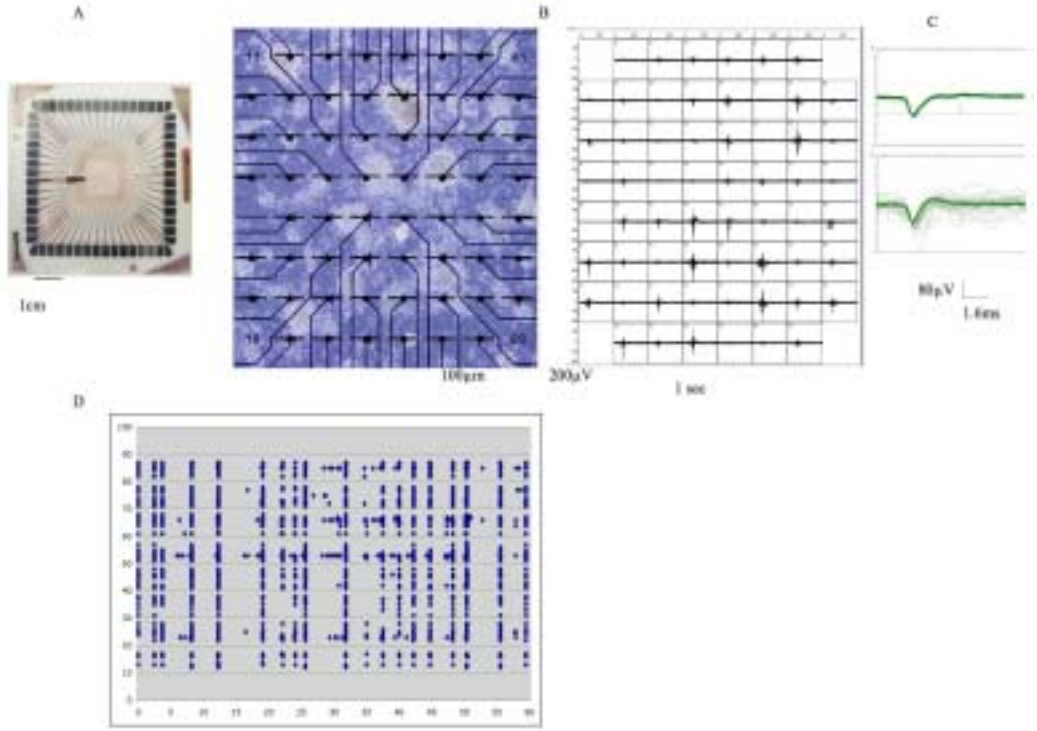


Figure 4: Corticostriatal network dynamics. **A**: A mix culture of cortical and striatal neurons were plated over a multielectrode array. **B**: Electrical activity from each electrode can be seen in the two middle pictures (a 1 sec record is displayed from each electrode). **C**: Single cell or multiunit activity are recorded as seen in the green traces. **D**: Raster plot derived from 1 min records show synchronous activity separated by silent intervals of several seconds.

The MEA system as shown above, was working by the last report, but the calcium imaging system has been developed since the arrival of Dr. Luis Carrillo-Reid in July. His analysis method, which uses local linear embedding in place of the more conventional principal component analysis, has the advantage that it conserves the physical structure projected onto fewer dimensions. With this analysis we can illustrate which cells belong to which groups and quantitatively describe the patterns of activation within and between groups of active neurons.

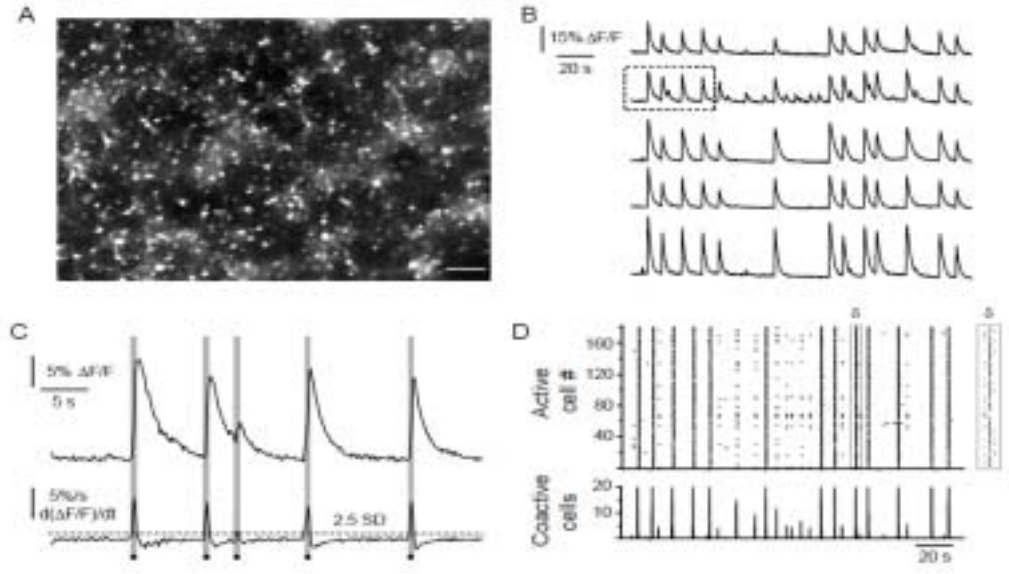


Figure 5: Similar cultures as those seen above were imaged by loading the cells with Fluo5AM. **A**: A low light level camera shows bright "spots" of light which at close inspection are revealed to be neurons. **B**. The calcium transients of five cells were taken at random from different points in the entire optical field. **C**: To identify the onset of bursting activity the first time derivative of the calcium transients was determined. The suprathreshold onset of the differentiated calcium signal generates a binary row vector that represents the neuronal activity of each cell. **D**: The row vectors of all active neurons were used to reconstruct the overall activity of the network. Each row in the raster plot illustrates an active neuron. The time histogram of the network activity (bottom) confirms that after 3 weeks *in vitro*, cultures of cortical cells display spontaneous activity characterized by periods of synchronous firing.

Dr. Carrillo-Reid had successfully developed the method for slices and has now expanded it to the cultures. Such an analysis reveals properties of the burst activity that we had seen in the MEAs, but gives us the single cell resolution, that cannot yet be determined in the microelectrode arrays. With this analysis we have confirmed that the activity of the network is anatomically widespread and that after 3 weeks *in vitro*, cultures of cortical cells display spontaneous activity characterized by periods of synchronous firing. We are working on a manuscript to describe these data and the properties of the networks. We are now ready to apply this methodology to the twin cultures that we have developed using campenots, where, at least in theory, we can cut the connections between cortex and striatum and study the differences in activity in the two sections that results.

3.5 Connections between pairs of cells in culture

This project was just beginning when we made the last report. We now have a reasonable database of pairs of cells and have seen contacts between cortical cells and striatal cells that are both excitatory and inhibitory in nature. The cells giving rise to the inhibitions are expected to be cortical interneurons which we now have good evidence are present in the cultures (see Ottawa collaboration).

Unexpectedly there are inhibitory connections also between the striatal cells and cortical ones in the cultures. They are more common than contacts between cortical and striatal cells - but they never happen *in vivo*. Therefore, we are trying to test the possibility that simple proximity leads to these connections. *In vivo* cortical and striatal neurons are separated by the corpus callosum. Our recently developed cultures in separate compartments may let us test that idea - at least as far as separation is concerned - of course we will not have a myelinated bundle in the way, but neither would early developmental brain.

We can now describe the electrophysiology of the culture in detail and give quantitative descriptions of the connectivity we see. The same material has been used to try to get anatomical detail about the cells. The first few images are with us now (Figure 6) and we hope to be able to extend this part of the project in the near future.

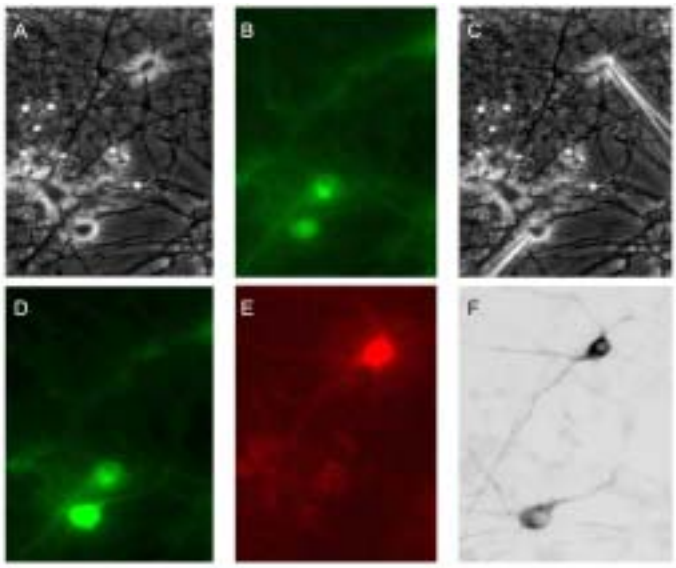


Figure 6: The development of an anatomical description of the connectivity between cells in the mixed cortex and striatal cultures. In **A-E** the pictures were taken when the cells were still alive. We first image them in phase contrast (A) and then in fluorescence (B). The cells are then patched (C) with red dye in the striatal electrode and green in the cortical one. At the end of the electrophysiological experiment the electrodes are slowly withdrawn and the cells are again imaged with the two filters so that the green dye in the cortical cell (D) and the red in the striatal cell (E) are visualized. Finally after fixing the culture and staining for the biocytin that was also in each of the electrodes we can see both cells with their processes (F). The final stage will be to get these to electron microscopy so as to study the synaptic connections between them

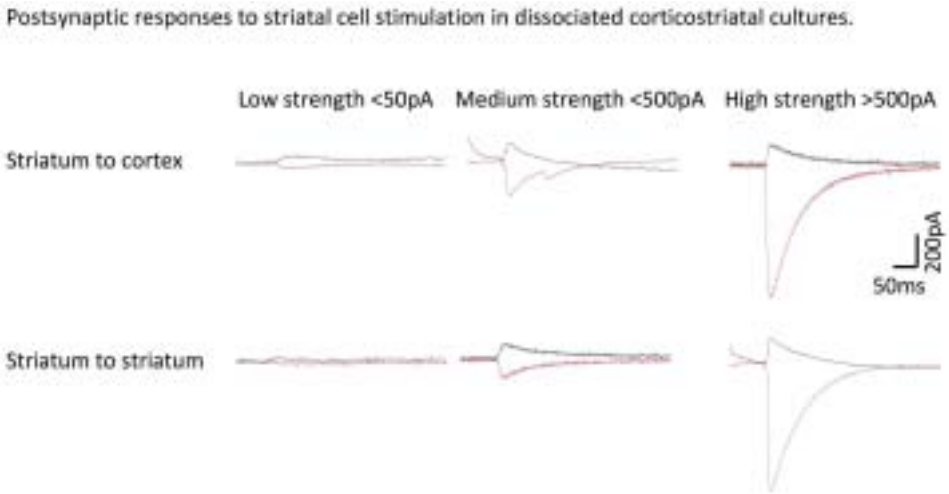


Figure 7: Three sets of records from different pairs of cells are shown. The records are averaged inhibitory post synaptic currents recorded in voltage clamp in cortical or striatal cells in response to striatal cell stimulation. A similar variety of size and time-course of responses is seen. The red traces are recorded at -30mV while the black ones are at -80 mV. The reversal of the direction of the currents as the membrane potential crossed the equilibrium potential for chloride ions demonstrates that these are likely IPSCs in response to GABA release from the striatal terminals.

3.6 Otago University Joint Research Projects

This collaboration has resulted in some spectacular images (e.g., Figure 8). Freeze fracture of the striatal tissue allowed the formation of a metal replica of the fractured surface. As the original tissue is cleaned away the proteins that adhere to the replica can be visualised by immunogold imaging. This is only one of a very few images that we have obtained so far but does suggest that the method works in this rather challenging project. Ms. Shevtsova will have to do other easier things in her Ph.D. project, but this early success bodes well for the future of the collaboration.

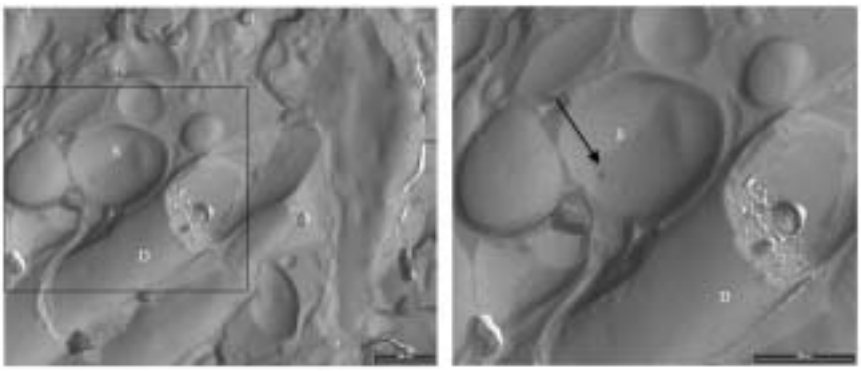


Figure 8: Replica images stained for C_{av} 1.3. The left lower power image shows a replica with some dendritic shafts and what looks like a spine head (S) which is shown at higher power on the right. The four gold particles at the arrow show the presence of an antibody raised against the C_{av} 1.3 calcium channel. The scale bars are 500µm. S-spine; D-dendrite.

3.7 Summer short working visits by Dr. Caroline Ingham and Dr. William Staines

Dr. Cali Ingham (University of Edinburgh, Scotland) developed a method with which to look at the cultured cells with electron microscopic resolution. The method was not at all straight forward and we managed it in spite of the loss of the electron microscope technician in OIST immediately after

Dr. Ingham arrived. The first images of synapses in the cultures are shown below Figure 9. The next challenge is to make such preparations on cells whose electrophysiology we have studied.
Dr. William Staines (University of Ottawa, Canada) helped us develop the light microscopy techniques used in filled cells that have been recorded from. This was also a nontrivial task in the cultures in spite of our expertise over many years at doing the same thing *in vivo* or in slices. Although the fluorescence images are spectacular we will not be able to see synaptic contacts at the light microscope level.
Therefore the development of a diaminobenzidine (DAB) method that allows us to image both the cells in detail was necessary, and we have the first beautiful fills to work on (Figure 6) in order to develop electron microscope resolution images of the same cells.

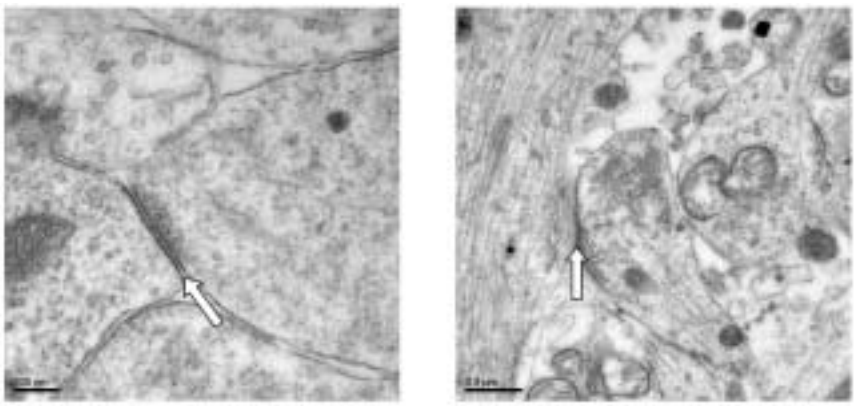


Figure 9: Photomicrographs developed from the neurons in culture. In both synaptic specializations are visible with the synaptic cleft across which neurotransmitters must diffuse marked by the arrows. The presynaptic profile with synaptic vesicles are on the right in both pictures. In these 'random' photographs we have not identified the cells which give rise to, or receive, the contacts.

3.8 Ottawa University Joint Research Project

We have a paper about the interneurons in the mixed age cultures in revision and we have two more manuscripts in development. In corticostriatal cultures cholinergic, calretinin and somatostatin interneurons showed increased dendritic branching and axon terminal outgrowth. The density of vesicular GABA transporter (vGAT) positive axons was much greater in the co-cultures than the vGAT innervation seen in cultures of striatum alone. The greatest difference seen between striatal cultures versus corticostriatal co-cultures was the very large increase in the vesicular acetylcholine transporter (vAChT) positive axon and terminal outgrowth seen in the cultures with cortical innervation. This finding is surprising given that cortical innervation of the cholinergic neuron *in vivo* is limited. Preliminary data suggest that at least part of this increased development engendered by the co-culture is dependent on glutamate action at NMDA receptors although there does also seem to be an action, particularly on calretinin expression by AMPA receptors.

This collaboration is going well though held up a little by the non-delivery of the transgenic animals. Our pilot experiment suggest that we will be able to isolate cholinergic interneurons by cell sorting and then grow them in culture, but so far we have not been able to receive the transgenic animals with cholinergic cells labeled.

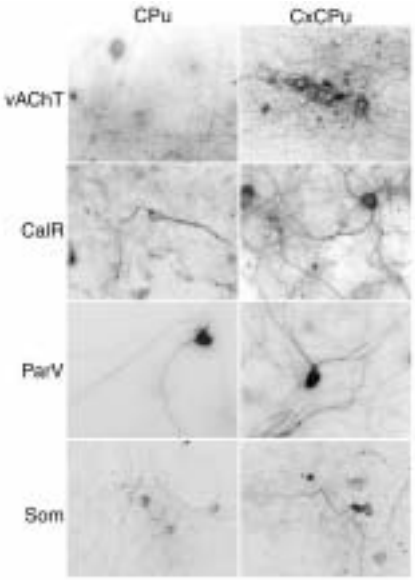


Figure 10: Axodendritic outgrowth of striatal interneurons. Inverted immuno-staining of fiber outgrowth shown with vesicular Acetylcholine Transporter (vAChT), calretinin (CalR), parvalbumin (ParV) and somatostatin (Som) in striatal cultures alone (CPu), or in corticostriatal cultures (CxCPu). Enhanced axodendritic outgrowth is observed for all types of interneurons in corticostriatal cultures compared to striatal cultures alone.

3.9 Future plans

During the move to the new building we have equipped a new patch clamp room so that Dr. Carrillo-Reid will be able to extend his work in slices from mouse brain. We have also upgraded the 2-photon microscope to allow both second harmonic imaging and caged glutamate release. This improvement will allow us to study the voltage effects of transmitter release perhaps even on a single spine in the striatum. We will also be able to use this microscope to study the details of vesicular release at terminals in slices as well as in transfected cultured cells.

Dr. Fiona Randall has so far been unable to use the separated cultures since her rig had no facility for moving the microscope field and we need to move between the two culture sets. In the new building we have also upgraded her system to allow this more complicated recording where the distance between the culture cells of interest can be larger than the field of view of the x20 objective.

The cultures in separate chambers will allow us to use rat cells and so to build striatal cells with and without acetylcholine interneurons as well as to study the formation of synaptic connections over time in the dish. Hopefully we will find that with this separation there will be fewer of the aberrant striatal to cortical connections too.

4. Publications

4.1 Journals

Arbuthnott, G., Garcia-Munoz, M. Dealing with the devil in the detail - some thoughts about the next model of the basal ganglia. *Parkinson & Related Disorders,* 15:S139-S142, (2009).

Carrillo-Reid, L., Tecuapetla, F., Vautrelle, N., Hernandez, A., Vergara, R., Galarraga, E. & Bargas, J. Muscarinic Enhancement of Persistent Sodium Current Synchronizes Striatal Medium Spiny Neurons. *J Neurophysiol* 102, 682-690, doi:DOI 10.1152/jn.00134.2009 (2009).

Dejean, C., Hyland, B. & Arbuthnott, G. Cortical Effects of Subthalamic Stimulation Correlate with Behavioral Recovery from Dopamine Antagonist Induced Akinesia. *Cereb Cortex* 19, 1055-1063, doi:DOI 10.1093/cercor/bhn149 (2009).

Hikima, T., Araki, R., Ishizuka, T. & Yawo, H. beta-Phorbol ester-induced enhancement of exocytosis in large mossy fiber boutons of mouse hippocampus. *J Physiol Sci* 59, 263-274, doi:DOI 10.1007/s12576-009-0031-0 (2009).

Wright, A. K., Garcia-Munoz, M. & Arbuthnott, G. W. Slowly Progressive Dopamine Cell Loss - A Model on which to Test Neuroprotective Strategies for Parkinson's Disease? *Rev Neuroscience* 20, 85-94 (2009).

Herrera-Marschitz, M., Arbuthnott, G. & Ungerstedt, U. The rotational model and microdialysis: Significance for dopamine signalling, clinical studies, and beyond. *Prog Neurobiol* 90, 176-189, doi:DOI 10.1016/j.pneurobio.2009.01.005 (2010).

4.2 Books and other one-time publications

Shindou, T., Arbuthnott,G.W., Wickens, J.R. N*euromodulation and neurodynamics of striatal inhibitory networks: implications for Parkinson's disease*. 233-243 (Humana Press, 2009).

Arbuthnott, G., Dejean, C., Hyland, B. *Antidromic cortical activity as the source of therapeutic actions of deep brain stimulation?* , 393-403 (Humana Press, 2009).

Wickens, J. R., Arbuthnott, G. W. *Gating of Cortical Input to the Striatum*. 341-352 (Academic Press, 2010).

Arbuthnott, G. W., Garcia-Munoz, M. *in Companion to Psychiatric Studies* (ed A.K. Zeally, Johnstone, E., Freeman, C.) Ch. 3, (Elsevier, 2009).

4.3 Oral presentations

Arbuthnott, G. *Some experiments that suggest a surprising source of the therapeutic effect of deep brain stimulation,* University of Dusseldorf, Dusseldorf, Germany, April 3, 2009

Arbuthnott, G. *Some experiments that suggest a surprising source of the therapeutic effect of deep brain stimulation*, Institute of Neuroscience, Newcastle University, Newcastle, U.K., April 6, 2009

Arbuthnott, G. S*ome experiments that suggest a surprising source of the therapeutic effect of deep brain stimulation*, Neuroscience Department, University of Aberdeen, Aberdeen, U.K., April 9, 2009

Arbuthnott, G. *Some experiments that suggest a surprising source of the therapeutic effect of deep brain stimulation*, Institute of Cell Physiology, Department of Biophysics and Neuroscience, National University of Mexico, Mexico City, Mexico, April 22, 2009
Arbuthnott, G. *What does cortex have to do with deep brain stimulation of the subthalamic nucleus*?, Northwestern University, October 22, 2009

Arbuthnott, G. *DBS in STN really changes cortex - is that the source of clinical benefit?*, Rosalind Franklin Univerisity, Chicago, IL, U.S.A., October 23, 2009

Arbuthnott, G. *Dealing with the devil in the details; some constraints on a new Basal Ganglia model*, World Ffederation of Neurology XVIII World Congress on Parkinson's Desease and Related Disorders, Miami Beach, FL, U.S.A., December 15, 2009

4.4 Posters

Arbuthnott, G., Hyland, B., Dejean, C. *Cortical effects correlate with the outcome of deep brain stimulation in the rat subthalamic nucleus*., World Federation of Neurology XVIII World Congress on Parkinson's Disease and Related Disorders, Miami, FL, U.S.A, December 13-16, 2009

Garcia-Munoz, M., Theiss, S., Staines, W., Arbuthnott, G. *Pharmacological resposes of corticostriatal neurons cultured on multielectrode arrays*, Society of Neuroscience, Chicago, IL, U.S.A., October 17-21, 2009

Randall, F. E., Arbuthnott, G., Staines, W., Garcia-Munoz, M. *Corticostriatal synapses in dissociated cell culture*, Society of Neuroscience, Chicago, IL, U.S.A., October 17-21, 2009

Schock, S. C., Jolin-Dahel, K., Staines, W., Arbuthnott, G., Garcia-Munoz, M. *Characterization of striatal interneurons in dissociated cultures.*, Experimental Biology Society, New Orleans, LA, U.S.A., April 18-22,2009

Schock, S. C., Jolin-Dahel, K., Schock, P., Staines, W., Arbuthnott, G., Garcia-Munoz, M. *Development of dissociated rat cortical neurons in vitro.*, Society of Neuroscience, Chicago, IL, U.S.A., October 17-21, 2009

Staines, W., Schock, S.C., Jolin-Dahel, K., Schock, P.,, Arbuthnott, G., Garcia-Munoz, M. *Influence of cortical innervaqtion on axodendritic maturation of striatal interneurons in culture*, Society of Neuroscience, Chicago, IL, U.S.A., October 17-21, 2009

5. Intellectual Property Rights and Other Specific Achievements

Nothing to report

6. Meetings and Events

6.1 Research Visit

Date: February 21, 2009 – March 05, 2009
Venue: IRP
Speaker: Stephan Theiss, Ph.D. University of Dusseldorf, Germany
Other remarks: Visit to introduce 'spanner' method to analyze MEA

6.2 Research Visit

Date: May 30, 2009- June 26, 2009
Venue: IRP
Speaker: Bill Staines Ph.D., Babben Tinner University of Ottawa, Canada
Other remarks: Visit established immunohistochemical methods for cell culture.

6.3 Research Visit

Date: June 14, 2009 – Sept. 16, 2009
Venue: IRP - Electron Microscope
Speaker: Cali Ingham Ph.D. University of Edinburgh, Scotland, UK
Other remarks: Developed methods to study EM of cultures on plastic dishes

6.4 Global Network for Brain Reconstruction

Date: February 17-19, 2010
Venue: Tokyo, Shiba, OIST
Co-organizers: Trade Commission (Science), Embassy of Canada,
Tokyo. SUMS Chiba, Japan
Co-sponsors: University of Ottawa, Canada
Speakers: Allan Rock President and Vice-Chancellor, University of Ottawa
Don Aldridge, MBA General Manager, Research & Life Sciences IBM Canada Ltd.
Danica Stanimirovic, MD. PhD Director, NRC-IBS Neurobiology Program National Research Council of Canada
Geoff Mealing Senior Research Officer Neurobiology Program, Institute for Biological Sciences National Research Council of Canada
Anthony Krantis, PhD Professor & Director, University of Ottawa, Centre for Research in Biopharmaceuticals & Biotechnology (CRBB)
Other remarks: Mission in support of Japan-Canada joint research.

6.5 Seminars

Title: 1 Neurochips – dissociated neuronal networks on microelectrode arrays: Applications in functional drug screening and clinical research.
Title: 2 Firing patterns of neuronal networks – which are important features?

Date: February 16 and 27, 2009
Venue: IRP Seminar Room
Speaker: Stephan Theiss, Ph.D. Neurochip Laboratory,
Department of Neurology University of Dusseldorf, Germany

6.6 Seminar

Title: Striatal synaptic plasticity – it's not just about dopamine.

Date: July 31, 2009

Venue: IRP Conference Room

Co-organizers: Neuroscience Graduate Committee

Speakers: James Surmeier, Ph.D. Nathan Smith Davis Professor and Chair, Department of Physiology Feinberg School of Medicine, Northwester University, U.S.A.

6.7 Seminar

Title: The role of phasic dopamine signalling in the determination of agency and the discovery of novel actions

Date: August 5, 2009

Venue: IRP Conference Room

Speaker: Peter Redgrave MSc., Ph.D. Department of Psychology, University of Sheffield, U.K.

6.8 Seminar

Title: Innervation of cholinergic interneurons in the rat striatum.

Date: September 14, 2009

Venue: IRP Conference Room

Speaker: Rachel J. Sizemore, Ph.D. Department of Anatomy and Structural Biology University of Otago, New Zealand

6.9 Seminar

Title: Plasticity in developing retinal networks in health and disease.

Date: November 13, 2009

Venue: IRP Conference Room

Speaker: Evelyne Sernagor Ph.D. Institute of Neuroscience, Faculty of Medical Sciences Newcastle University, U.K.

6.10 Seminar

Title: Recent advances in multi-site recording techniques.

Date: February 19, 2010.

Venue: IRP Conference Room

Speaker: Geoff Mealing. Senior Research Officer Neurobiology Program, Institute for Biological Sciences, National Research Council of Canada.

Other remarks: Part of symposium in conjunction with Global Network Visit

7. Others

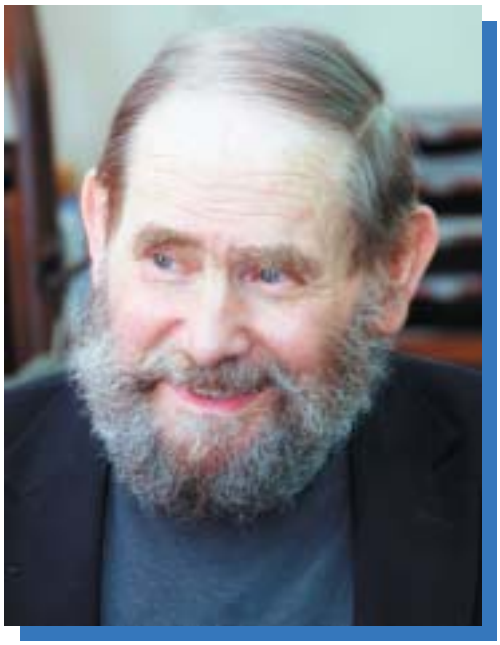
On August 19 2009 we made a presentation to the **Asian Youth Exchange Program**. Although the presentation had to be repeated the interest was still present at the final presentation with some of the students asking for - and using - email address to make contact later. The same day we took part in a photoshoot for "**Highlighting Japan through Images**" which resulted in two pages of press coverage of the Asian Youth event and OIST research activity.

Sunday, November 15, 2009

OIST Open House included a poster and a video presentation provided by the Unit and Dr. Takuya ably explained the video, that showed calcium activity in a large group of cultured neurons, to the visitors.

Molecular Genetics Unit

Principal Investigator:
Dr. Sydney Brenner
Research Theme:
Molecular genetics and genomic analysis



Abstract

Salamander project

Salamanders are thought to be a good material for neuroscience because their cells, including neurons, are large size. Due to the increased size of their genomes, the species *Ambystoma mexicanum* has 15 times the amount of DNA found in the human genome. Because of this we characterized the genes of Ambystoma by sequencing cDNAs for expressed genes. We previously constructed cDNA libraries of brain, retina, and spinal cord of Ambystoma. We carried out first-run sequencing of 5'-ends of about 200,000 clones from the libraries. After clustering, we have done full-length sequencing of about 10,000 representative clones. We are constructing a cDNA sequence database including these sequences.

Genome Analysis Center

Excellent facilities for DNA sequencing and genomic analysis were established that are provided to all Units as common resources in the Genome Analysis Center.

Technology development for whole-genome sequencing

Whole genome sequencing is rapidly evolving and many aspects can be improved. We are investigating approaches to make the techniques more efficient and less expensive.

1. DNA Sequencing Center Staff

Researchers: Dr. Yuya Yokoyama
Mr. Shinichi Yamasaki
Mr. Manabu Fujie

2. Partner Organizations

University of California, Santa Cruz Genome 10K Project

Type of partnership: Joint research

Name of researcher: Dr. Sydney Brenner, Dr. David Haussler

Research theme: Genome analysis of 10,000 species

3. Activities and Findings

3.1 Salamander Project

(In collaboration with Naito Unit – see the Naito Unit report)

3.2 Genome Analysis Center

Last FY Dr. Brenner transferred the resources of his unit to the Genome Analysis Center, which is being developed to serve the many laboratories in the Institute that require large scale sequencing of DNA using “next generation” approaches. This FY the Center reached full operational capacity and in addition to the Brenner unit, several other units have sequencing projects under way. The Center has five “next generation” sequencing machines, three Roche 454s and two Illumina sequencers. Three centrally supported staff have been assigned to operate the Center as a common resource.

3.3 Technology development for whole-genome sequencing

Massively parallel “next generation” sequencing is now used in whole-genome analysis, targeted sequencing/resequencing, gene expression analysis, de novo sequencing, RNA and small RNA sequencing and ChIP-sequencing. Unfortunately, however, sample preparation and sequencing library construction is currently a bottleneck. We therefore are investigating improvements in the technique in order to streamline and lower the cost of the procedure.

Computational Neuroscience Unit

Principal Investigator:
Erik De Schutter
Research Theme:
Modeling Cellular and Molecular Mechanisms of Neural Information Processing

Abstract

We use computational methods to study how neurons and microcircuits in the brain operate. We are interested in how fundamental properties like neuron morphology or homeostatic regulation affect information processing and learning. Most of our models concern the cerebellum because this brain structure has a relatively simple anatomy and the physiology of its main neurons has been studied extensively, allowing for detailed modeling at many different levels of complexity.

In FY2009 significant progress has been made on many research projects. Highlights include the release of the STEPS program for stochastic reaction-diffusion modeling, stochastic modeling of cerebellar LTD as a binary switch at single synapses, the development of growth models that explain the local flatness of dendritic branch points, the experimental verification of our modeling prediction that correlation of spiking activity between neurons depends on their excitability properties, and the demonstration that ethanol changes the excitability of cerebellar Golgi cells through its partial block of the Na^+/K^+ pump.

1. Staff

General services and neuroinformatics

Technical Staff: Ivan Raikov

Research Administrator / Secretary: Tsuyuki Nakabayashi

Molecular modeling

Researchers: Gabriela Antunes, Weiliang Chen (from June 2009)

Technical Staff: Iain Hepburn

Cellular modeling
Researchers: Sungho Hong, Yihwa Kim
Technical Staff: Haroon Anwar, Werner Van Geit

Network modeling
Researchers: Fabio M.S. de Souza (from July 2009), Mario Negrello (from January 2010), Rodrigo Publio, Thomas Sangrey (till June 2009)

2. Partner Organizations

University of Antwerp, Belgium
Type of partnership: Scientific collaboration and graduate program
Name of principal researchers: M. Giugliano, D. Snyders
Name of researchers: Q. Robberecht, K. Tahon, K. Veys
Research theme: Cerebellar physiology, multiple themes

University of Ghent, Belgium
Type of partnership: Scientific collaboration
Name of principal researcher: W. Fias
Name of researcher: F. Gheysen
Research theme: Cerebellar activation in sequence learning

PRESTO, University of Tokyo, Japan
Type of partnership: Scientific collaboration
Name of principal researcher: K. Tanaka
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 IP_3 receptors

University of Tübingen, Germany
Type of partnership: Scientific collaboration
Name of principal researcher: H.P. Thier
Name of researcher: A. Ignashchenkova
Research theme: Spiking activity of monkey cerebellar neurons

Duke University, United States of America
Type of partnership: Scientific collaboration
Name of principal researcher: G.J. Augustine
Research theme: Diffusion in dendrites and cerebellar optogenetics

University Texas, San Antonio, United States of America
Type of partnership: Scientific collaboration
Name of principal researcher: F. Santamaria


Research theme: Molecular modeling of diffusion in dendrites

University California, San Diego, United States of America
Type of partnership: Scientific collaboration
Name of principal researchers: M. Ellisman and M. Martone
Name of researcher: L. Fong
Research theme: Ultrastructural morphology of Purkinje cells

University of New Mexico, United States of America
Type of partnership: Scientific collaboration
Name of principal researcher: C.F. Valenzuela
Research theme: Modeling of effects of ethanol on the cerebellum

University of Pittsburgh, United States of America
Type of partnership: Scientific collaboration
Name of principal researcher: S.A. Prescott
Name of researcher: S. Ratté
Research theme: Correlation of neurons linked to their excitability

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

3. Activities and Findings

3.1 Neuroinformatics standards

3.1.1 Layer-oriented approach to neuroscience modeling language design

The challenge of interoperability between neuroscience modeling software require that biological modeling languages be developed in accordance with well thought out semantic principles. We have proposed a layer-oriented approach as a methodology to develop these semantic principles and have developed a prototype language for describing models of ion channels. The elements of the prototype language are divided in two semantic layers, the descriptive layer and the abstraction layer. The descriptive layer consists of elements that correspond to neuroscience modeling concepts, such as gates and ionic conductances. The abstraction layer consists of elements that correspond to general mathematical concepts, such as rate equations and functions. All elements in the descriptive layer are defined in terms of a combination of elements from the abstraction layer.

This two layer approach has become an important guiding principle in the work of the INCF Task Force on Multiscale Modeling (3.1.2).

3.1.2 Language for spiking network modeling

Neural network modeling has become so complex that it is difficult to reproduce scientific results

(Brette et al., 2007). For that reason, the International Neuroinformatics Coordinating Facility (INCF) has initiated a program on establishing standards for describing computational models of large-scale networks of spiking neurons, chaired by Dr. De Schutter. Work in the unit has made important contributions to the INCF efforts.

As large networks of biophysically accurate models of neurons are computationally expensive to simulate, integrate-and-fire neuron models are a convenient simplification in the study of neuronal dynamics. Consequently, the initial focus of the INCF task force on multiscale modeling has been on large-scale networks of integrate-and-fire neurons. In such models, neuronal spikes are reduced to discrete events with associated firing times. The dynamics of integrate-and-fire models are by definition discontinuous at the points where spikes occur, and conventional time-stepping integration methods cannot be used to obtain precise firing times. The proposed abstraction layer includes a flexible block diagram notation for describing spiking dynamics. The notation represents continuous and discrete variables, their evolution according to a set of rules such as a system of ordinary differential equations, and the conditions that induce a change of operating regime, such as the transition from subthreshold mode to spiking and refractory modes.

3.2 Molecular mechanisms of synaptic plasticity

3.2.1 Software development of STEPS

STEPS is a software system for simulation of biological reaction- diffusion systems in well-mixed systems or complex 3D morphologies, such as the signaling pathways involved in synaptic plasticity.

It is important to validate scientific software by comparing the output of the simulator to analytical solutions for a number of systems over a large but feasible range of simulation conditions. Unfortunately no validation standard exists for reaction-diffusion software, so we designed a testing suite ourselves to validate the implementation of our core algorithm, which is an extension of Gillespie's Direct Method to include diffusive fluxes between elements in a tetrahedral mesh (Figure 1), as well as our implementations of the Direct Method for well-mixed systems and the Runge-Kutta fourth-order method.

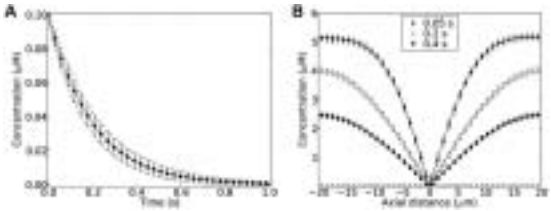


Figure 1: STEPS validation. A: Example of a validation of reactions: first-order irreversible reaction. Mean for 1000 iterations of STEPS (points with error bars showing the sd) matches the analytical solution (full line, broken lines are the predicted sd) perfectly. B: Example of a validation of reaction-diffusion: two separated reactants diffuse towards each other in the center of a cylinder and annihilate. Comparison of STEPS simulation at different times (symbols) and the analytical solution for clamped diffusion.

With successful validation results, the next step was to ensure that detailed documentation was available for users. A STEPS user manual was written, including chapters introducing the available features for building a model in STEPS and a description of every function in the STEPS Python interface. With well-validated code and extensive documentation, we released STEPS Version 1.0.0 under the GNU General Public License in January 2010 (http://steps.sourceforge.net/). Meanwhile the release has already been upgraded to 1.1.1, with a

better compilation and distribution system that meets the standard for Python applications and a visual toolkit (Figure 2). A new, generic interface was designed to support importing of tetrahedral meshes generated from 3rd party mesh generators such as CUBIT and TetGen. Checkpointing was implemented to ensure that long running simulations can be interrupted or restarted after unexpected crashes.

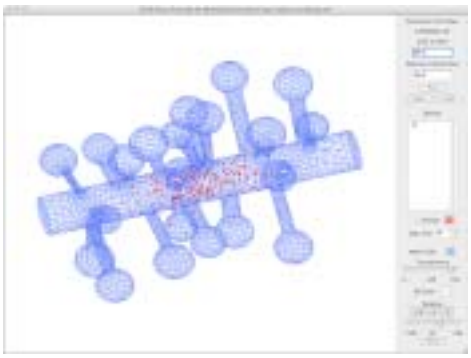


Figure 2: New STEPS visual tool showing diffusion of particles in a dendrite.
Simulations like these are used to study anomalous diffusion in spiny dendrites (Santamaria et al., 2006).

3.2.2 Membrane potential computations in STEPS

STEPS currently only computes the reactions between and the diffusion of molecules, which enables it to simulate a broad set of signaling pathways. 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 will therefore further increase the power of STEPS.

We implement an approximate method that computes the effect of membrane channel currents on membrane potential in the same 3D mesh used for the reaction-diffusion simulation. Voltage-dependent transitions, ligand binding and ionic flux are all extensions to the Direct Method and thus are solved with the reaction-diffusion simulation in one framework that drives the overall simulation clock. We now have stable code that was tested by comparing action potential shape and propagation in the deterministic limit to a standard ODE solver (Figure 3) and comparison of spontaneous spike rate to a stochastic ion channel simulator.

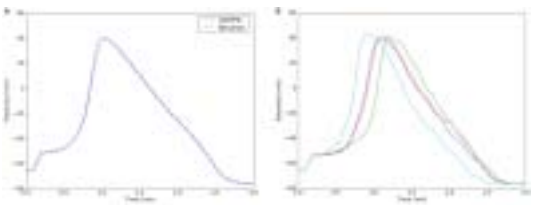


Figure 3: Action potential simulation in STEPS. **A**: Comparison of simulation in STEPS (close to deterministic behavior because of large number of channels) with ODE solution in NEURON. **B**: Stochastic behavior when the number of channels is lowered in STEPS.

3.2.3 Stochastic modeling of induction of cerebellar long-term depression

Long-term depression in granule-Purkinje cell synapses is a persistent decrease in the efficacy of synaptic transmission caused by the removal of AMPA receptors from the postsynaptic membrane. Long-term depression can be induced by synaptic activity, or by increasing the

postsynaptic calcium concentration ([Ca^{2+}]) using flash photolysis of caged Ca^{2+}. However, the biochemical processes activated during each form of induction remain poorly understood.

To gain insights on these processes, we have developed a molecular model of cerebellar long-term depression in STEPS. It consists of a well-mixed biochemical network composed by cGMP-dependent protein kinase pathway, and a positive feedback loop involving conventional protein kinase C pathway, protein phosphatase 2A and the mitogen-activated protein kinase pathway. We also included in this model mechanisms of Ca^{2+} dynamics. To model the removal of AMPA receptors from the postsynaptic membrane during long-term depression, we simulated the processes related with AMPA receptors trafficking according to experimental data.

Simulation of the long-term depression induced by Ca^{2+} uncaging showed that there is a sigmoidal relationship between the mean magnitude of the depression, measured as a persistent removal of AMPA receptors from the postsynaptic membrane, and the amplitude of the [Ca^{2+}] change, as has been demonstrated previously (Tanaka et al., 2007). However, although the mean responses of our stochastic simulations confirm that there is a graded relationship between magnitude and [Ca^{2+}], single runs of our model show that the induction and expression of long-term depression always happens in an all-or-none manner (Figure 4). Furthermore, our results point out that the bistable behavior of our model is caused by the positive feedback loop in the biochemical network. Blocking the feedback loop prevents the persistent removal of AMPA receptors from the postsynaptic membrane, and completely inhibits bistable responses in the model.

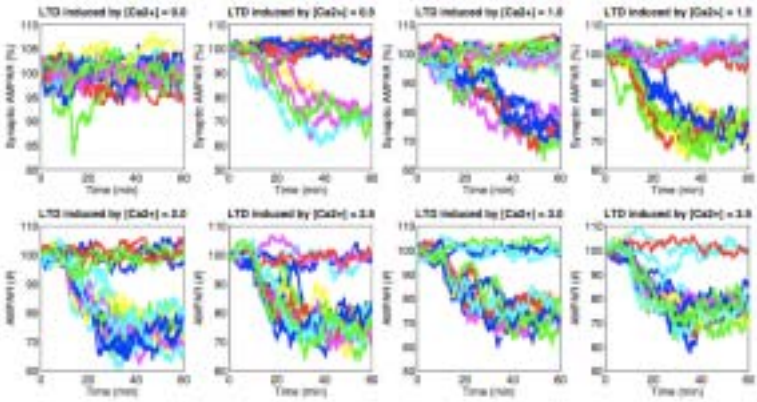


Figure 4. All-or-none LTD induction in binary synapses. Examples of stochastic simulations (20 runs per panel) of the change in synaptic AMPA receptors caused by a 1 second Ca^{2+} pulse to the concentration indicated. Note that as the amplitude of the Ca^{2+} pulse increases, the number of simulations where the model switches from a high conductance (~100% AMPA receptors) to a stable low conductance (~70%) increases and that the jitter on the timing of the transition decreases.

Recent experimental data shows that the nitric oxide nitric oxide (NO)-cGMP-dependent protein kinase (PKG) pathway acts by modulating Ca^{2+}-induced long-term depression, decreasing the half-maximum [Ca^{2+}] necessary to induce long-term depression. Therefore, we used our model to verify the biochemical process that are responsible for this modulation. Our results indicated that NO-PKG inhibits the protein phosphate 2A, facilitating the induction of long-term depression and, consequently, decreasing the requirement for [Ca^{2+}]. We also studied the action of NO-PKG on long-term depression induced by synaptic activity. This required a great expansion of the model to include other biochemical pathways. With this model, we could demonstrate that NO-PKG is not fundamental to the occurrence of long-term depression, but it can modulate the induction by its effect on protein phosphate 2A activity and on IP3 receptors.

3.3 Cellular mechanisms regulating firing and synaptic properties of neurons

3.3.1 Software development of Neurofitter

Neurofitter (http://neurofitter.sourceforge.net/) is a tool to automatically optimize neuron model parameters. The phase plane trajectory density (PPTD) method used to compare the experimental data with data generated by the model was improved in two ways. We implemented “regions of interest” that give extra weight to specific areas of the PPTD histogram. This allows specific detection of spikes by defining a region of interest at positive potentials. We also introduced an option to handle simulation voltage traces with a variable time step. Neuron models can be run much faster with variable time steps in the NEURON simulator, resulting in a faster overall parameter search.

3.3.2 Development of a new Purkinje neuron model

We are using Neurofitter to generate an active multi-compartmental model that replicates in vitro recordings (provided by E. Rancz and M. Haüsser, London) of the spiking response of a Purkinje neuron to current injection steps in detail. In the previous years we used a single compartment model because that allows much faster Neurofitter runs. As reported previously, we discovered that it was not sufficient to search for the maximal conductances of the ion channels but that their kinetics also needed to be included in the search.

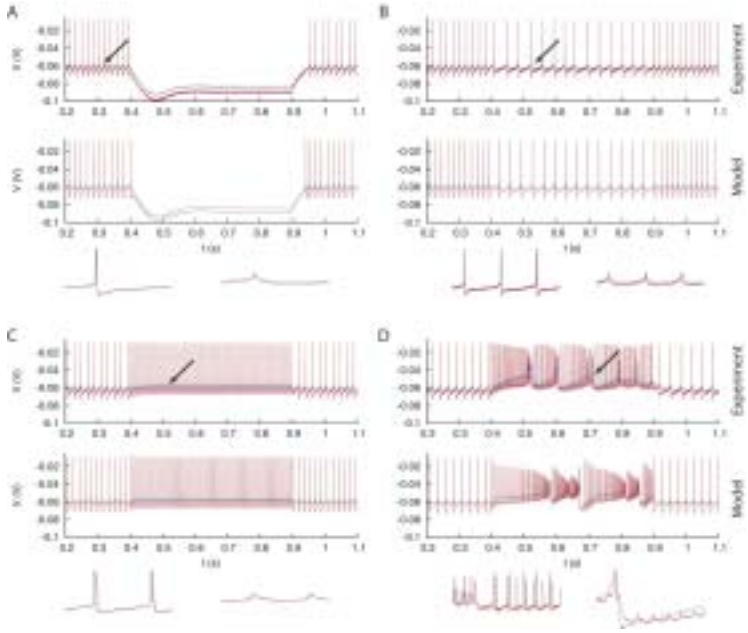


Figure 5: Comparison of experimental data with best Purkinje cell model. The cell was held at a level were it spikes and a 500 ms current step was applied, first hyperpolarizing (A), followed by steps of increasing amplitudes (only a subset is shown). Each panel shows a somatic trace (red) and a dendritic trace (blue) from the experimental data (upper drawing) and from the model simulation (mid drawing). Note that the model does not try to match the actual timing of spikes, the aim is to reproduce spike shape and frequency accurately. The lower drawings in each panel show this match for a selection of somatic and dendritic spikes aligned on a spike (red: experimental data, broken black: model). The model matches the data very well for the first three current steps (A – C) where no calcium spikes were evoked. The model generates calcium spikes (D) at the correct current injection level and with a good frequency, but the calcium spikes do not have a correct shape.

In this period we have made further progress mainly by using more complex models. To faithfully replicate action potential shape we included several compartments to model the initial segment and axon. This is based on observations by Yu (2008) that action potentials generated in the axon have a different shape when they are recorded in the soma. This explained why a PC model without the axon was never able to replicate the rising phase of action potentials, even while the kinetic parameters of all the ion channels were fitted also. With the axon included we found models that simulate the experimental traces in detail as long as no dendritic calcium spikes are present in the model.

Fitting also dendritic spiking required more changes to the model. We first tried using a two compartment dendrite, representing the proximal and the distal dendrites. Despite success in generating dendritic spiking in such a model, as reported last year, we never could tune this model to replicate the detailed shapes of both somatic and dendritic spikes. We therefore have started using a reduced dendritic morphology with around 40 compartments (reported in FY2007). We can now produce fairly realistic dendritic spiking combined with accurate somatic spiking, though the dendritic spikes are still too wide (Figure 5). Based on results reported in section 3.3.3, this may be caused by the simple model for calcium dynamics used, so we are now testing more complex calcium dynamics.

A constant observation in this work has been that it is not possible to achieve accurate modeling of Purkinje cell spiking with simplified models. Most advances in the automated parameter fitting occurred when the model was made more complex.

3.3.3 Modeling detailed calcium dynamics in the Purkinje cell dendrite

Intracellular Ca^{2+} concentration plays a crucial role in the physiological interaction between Ca^{2+} channels and Ca^{2+}-activated K^+ channels. We have investigated the role of different Ca^{2+} buffering mechanisms in simulating this physiological interplay (Anwar et al., 2010). The exponential decaying pool used in most multi-compartmental neuron models could not simulate characteristic Purkinje cell Ca^{2+} spikes. The reason is its short relaxation time which can approximate fast transients and therefore activate a BK-type channel, but fails for the small conductance (SK) channel that activates slowly and requires different Ca^{2+} dynamics. A simple and effective solution may be to use two Ca^{2+} pools respectively for the fast and slow transient. Alternatively one could use a detailed model of Ca^{2+} dynamics (Schmidt et al., 2003) including Ca^{2+} diffusion, diffusible parvalbumin and calbindin buffers and pumps.

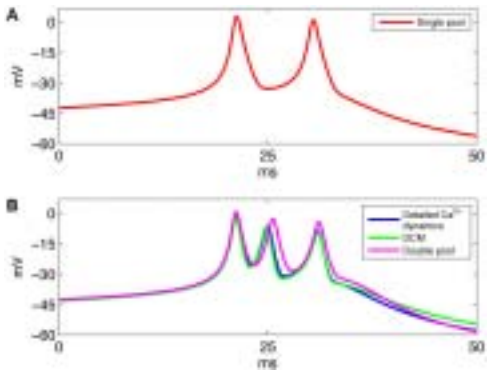


Figure 6: Comparison of simulated dendritic spikes using different models for calcium dynamics. (adapted from Anwar et al., 2010) A: Single pool model cannot produce a triple spike. B: The diffusion compensated model (DCM) approximates the spikes produced by the full model much better than the double pool model.

Unfortunately simulating diffusion is very computation intensive and would make simulations of a multi-compartmental model prohibitively slow. Therefore we developed a Diffusion Compensation Method (DCM) that replaces diffusion of Ca^{2+} and buffers with a DCM buffer. We could fit diameter-dependent curves for the four parameters controlling DCM, reliably reproducing calcium dynamics for a range of diameters (0.8 – 20 µm) and calcium concentrations (0.5 – 8 µM).
Next we compared the ability of four buffering methods (single pool, double pool, DCM and complete Ca^{2+} dynamics) to generate realistic dendritic calcium spikes in a single compartment model (Figure 6). Each of these models was tuned independently using Neurofitter to approximate a trace containing three spikes as much as possible. The single pool model failed to do so. The double pool model performed better but could not fully match the amplitude or width of the Ca^{2+} spikes. The DCM model, however, produced a close match to the detailed model including diffusion.
We conclude that detailed Ca^{2+} dynamics models including buffers have a significantly better control over Ca^{2+} activated K^+ channels and lead to physiologically more realistic simulations of Ca^{2+} spikes than pool based models. Furthermore, the new compensating mechanism deals with the effects of removing diffusion from the model quite well.

3.3.4 Local planar structure of dendritic trees

The morphology of dendrites is a key attribute in distinguishing different neuron types in the brain and changes of morphology affect both signal processing and intrinsic firing properties of neurons. Dendritic growth is governed by only partially understood interactions between intrinsic and extrinsic factors. While it can be described by simple mechanisms like branching, elongation and pruning, the interplay of these creates complex developmental patterns, specific to each neuron type. Good measurements of dendritic morphological features and quantification of how they change during neural development is important to understand how such processes are controlled. Uylings and Smit (1975) introduced the cone angle as a measurement of the planarity of dendritic branch bifurcations. They showed that most bifurcations in rabbit visual cortex neurons have cone angles in the range of 150 to 180°, indicating that bifurcations tend to be flat even in neurons that do not have flat dendritic trees. Last year we reported that we could reproduce their findings in 8 different neuron types and could fit these distributions with a simple Monte Carlo model assuming minimal distance lengths.
We have developed in collaboration with Dr. R. Sinclair (OIST) an analytical approximation that predicts the cone angle (α) distribution with its peak at 180° well:

$$P_3(\alpha) = \frac{3}{4}\sin^3\left(\frac{\alpha}{2}\right) cc P_1^3(\alpha)$$

In performing control measurements on the data we discovered a strong dependence of the cone angle distribution on the distance from the branch point over which it is measured (Figure 7). Previously we measured the angle between the first experimentally recorded data points from the branch point, while in Figure 7 consecutive data points equidistant from the branch point are used (up till the next bifurcation point along any branch). Surprisingly, cone angles become flatter as the processes over which they are measured become longer.

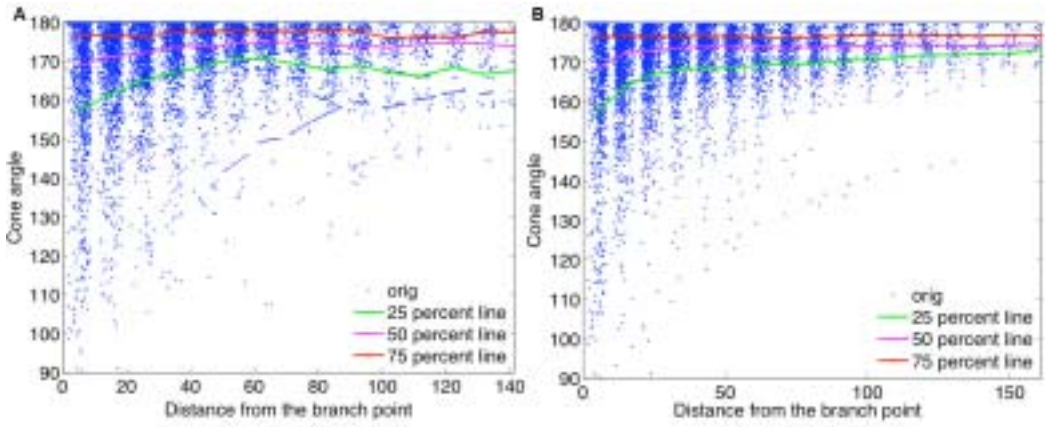


Figure 7: Distribution of cone angles measured at different distances from the branch point. **A**: Experimental data from dentate gyrus granule neurons (source: NeuroMorpho.org). **B**: Stochastic growth model replicates the experimental distribution well. Notice that as the measurement is taken over a larger distance the cone angle becomes less variable and flatter.

We have developed growth models of a single bifurcation (parent and two daughter branches) that capture these properties. These models are governed by four parameters: repulsion between the two daughter branches, repulsion between the parent and the daughters, tendency to follow the previous growth direction and a noise level. We were successful in fitting the cone angle distributions for 6 of the 8 neuron types (one example shown in Figure 7), but found that to obtain faithful models we also had to constrain them for the distribution of the 2D angle between the two daughter branches. These growth models demonstrate that the increasingly flat cone angle can be explained by internal growth mechanisms for most neurons, exceptions being the flat Purkinje cell dendrite and bitufted neuron dendrites, and predict interesting differences in the importance of specific parameters for the dendritic growth of disparate cell types.
Though highly simplified and simulating only a part of a neuron's morphology, we believe that these growth models are much more tightly constrained by data than previous ones.

3.3.5 Single neuron firing properties impact correlation-based population coding

We study how the computational properties of individual neurons is linked to the coding scheme of a neural population. Even though correlations within population firing are commonly observed in experiments, their role in coding has been under debate. In particular, a recent paper by de la Rocha et al. (2007) claimed that, if a common input is the main cause of the correlated activity, the correlation is completely determined by the average gain of the individual neurons. Therefore the correlation would not transmit more information than the population mean firing rate. Last year we reported the observation that one type of biophysically defined neuron model, the HHLS neuron with type 3 excitability (Lundstrom et al., 2008), shows significant deviations from the predictions of de la Rocha et al. We have extended this work by obtaining experimental verification and investigating why the prediction does not work well with type 3 neurons.

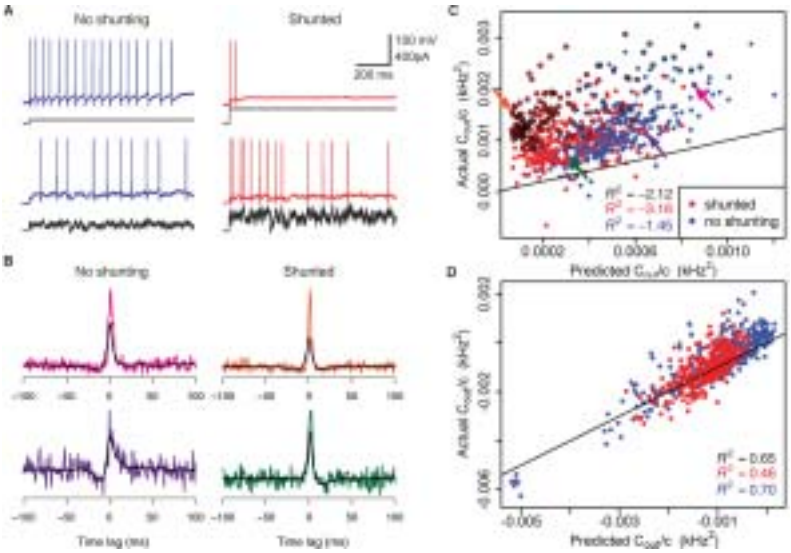


Figure 8: Figure 8: Firing properties and spike train correlations in pyramidal neurons. A: Sample traces from a typical CA1 pyramidal neuron (data from S. Prescott). Sustained repetitive spiking was elicited by constant input in the low-conductance state (*no shunting*) but not in the high-conductance state (*shunted*). The neuron was responsive to stimulus fluctuations, even when shunted. B: Sample cross-correlograms measured from spike trains are shown in color and corresponding predictions based on the de la Rocha et al.'s prediction are shown in black. C: Output covariance normalized by input correlation measured from experimental data (a*ctual* C_{out}/c) plotted against de la Rocha et al.'s prediction *(predicted* C_{out}/c). Each dot represents a pair of recordings with various input means and variances for no shunting (blue) and shunted (red) conditions. Line shows where *actual* C_{out}/c equals *predicted* C_{out}/c. Arrows identify data points corresponding, based on color, to cross-correlograms in B. Circled points deviate significantly from prediction ($p < 0.05$; t-test). D: Same data as in c but with central region of cross-correlograms (±3 ms of peak) excluded from calculation of C_{out}.

First, we collaborated with Dr. Steven Prescott of the University of Pittsburgh, who carried out experiments on hippocampal CA1 pyramidal neurons. He used two different recording conditions: one is the normal slice condition, and, in the other, a constant shunting conductance is injected via dynamic clamp. This operation is designed to simulate the so-called high conductance state mimicking type 3 excitability state (Prescott et al., 2008). Indeed, the recording data and analysis (Figure 8) revealed that pyramidal neurons under the normal/shunting condition show qualitatively the same patterns as the HH/HHLS neuron. The shunted neuron pairs tend to show much higher correlations, mostly due to very sharp synchrony (< 6 ms), which cannot be explained by the prediction of de la Rocha et al.

Further analysis, based on computing spike-triggered covariances, revealed that these deviations mostly originate from the second order corrections to the de la Rocha et al.'s first order approximation. These second order corrections are related to encoding of the stimulus variance at single neuron level. This is consistent with the previous observations that the type 3 neurons behave as coincidence detectors (Lundstrom et al., 2008; Prescott et al., 2008) and therefore have higher sensitivity to the stimulus variance whereas the neurons studied by de la Rocha et al., or neurons in the normal slice condition, behave more like integrators, primarily sensitive to the stimulus mean.

Our findings contribute to understanding how population codes depend on the diversity in the computational/dynamical properties of individual neurons, a relation that is often ignored in recent discussions on population coding and neural networks.

3.4 Information processing in the olivocerebellar system

3.4.1 Intrinsic mechanisms underlying ethanol induced excitability of Golgi cells

Ethanol increases the resting membrane potential of Golgi cells and raises their firing frequency in a concentration dependent manner (Botta et al., 2010). We used a compartmental Golgi cell model containing kinetic descriptions of 11 different voltage and calcium-gated channels (Solinas et al., 2007) to predict which channels are involved in the increase of membrane potential and excitability caused by ethanol. An extensive parameter sweep, mimicking either partial blockage or enhancement of specific channels by ethanol, showed that none of the ion channels expressed would cause the excitability changes without also extensively changing the action potential shape, which was not observed.

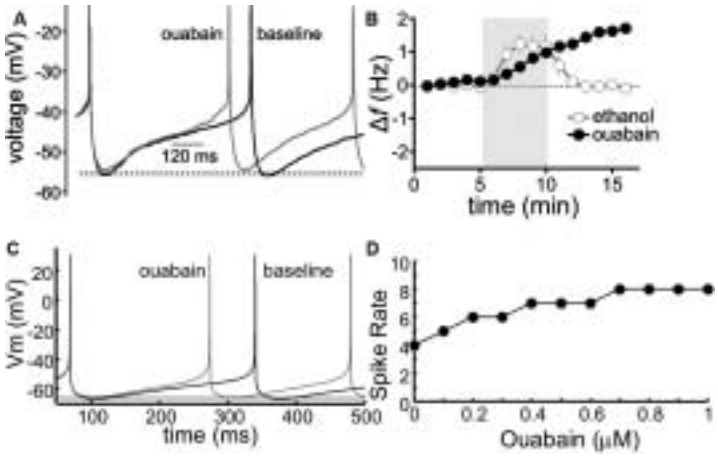


Figure 9: Na^+/K^+ ATPase block mimics effects of ethanol on Golgi cell excitability (modified from Botta et al, 2010). Comparison of experimental (A, B) to modeling (C, D) data. A: Effect of 0.1 μM ouabain on Golgi cell firing. B: Change in Golgi cell firing rate over time for 40 mM ethanol and 0.1 μM ouabain (which has an irreversible effect). C: Simulation of the data presented in panel A. D: Dose effect of the ouabain on firing rate of the Golgi cell model.

Having excluded a direct contribution by ion channels we next turned to ion pumps as a potential target of ethanol. To investigate the effect of partial block of the Na^+/K^+ ATPase we added to the Golgi cell model a standard model of this pump, used in cardiac simulations and incorporating an ouabain binding site (Takeuchi et al., 2006). Without any further changes to the model we could now replicate all the experimental observations by assuming that 40 mM ethanol has the same effect as 0.1 μM ouabain (Figure 9). The fact that such a close match between simulation and experiment was possible without extensive model tuning strengthens the model predictions. We have also successfully reproduced the effects of ethanol on granule cell IPSC amplitude and frequency.

3.4.2 Golgi cell oscillations

Previous one-dimensional network modeling of the interaction between cerebellar Golgi and granule cells has been successfully linked with a range of in vivo oscillation data from the

cerebellar cortex (Maex and De Schutter, 1998, 2005). However, recently discovered gap junctions between Golgi cells may cause oscillations by themselves (Dugué et al., 2009). This raised the question of how gap junction coupling would affect granular layer oscillations. We investigated this in a novel two-dimensional computational model comprising 9,225 units of the granular layer circuit with and without gap junctions between Golgi cells (Figure 10).

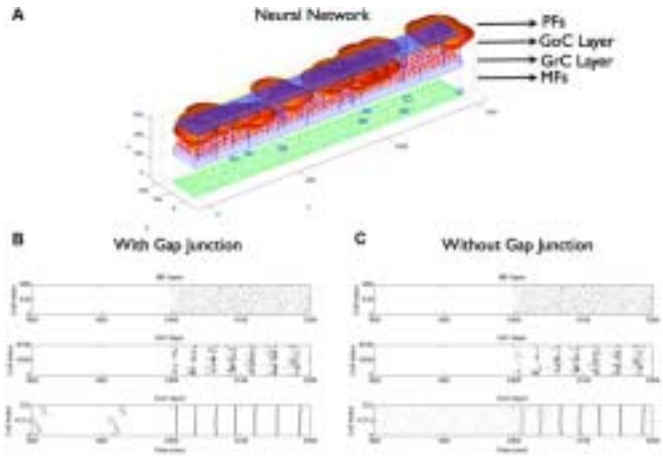


Figure 10: 2D model of granular layer oscillations. A: Structure of the network model with parallel fiber excitation of Golgi cells (GoC); the receptive fields of the neurons are indicated. B, C: Simulations of the change in firing caused by an increase of the mean firing rate of afferent mossy fibers to 100 Hz (at time 1000 ms) in a network with weak parallel fiber synapses. In both cases the network starts to oscillate with tighter synchronization of Golgi cells than granule cells (GrC). In presence of gap junctions (B) Golgi cells also show loose synchronization before the mossy fiber activation and during stimulation the activity is more synchronized than in their absence (C). In a network with normal parallel fiber synaptic strengths no difference between the two conditions is observed (not shown).

Isolated Golgi cells coupled by gap junctions had a strong tendency to generate spontaneous oscillations without affecting their mean firing frequencies in response to distributed mossy fiber input. Conversely, when Golgi cells were synaptically connected in the granular layer, gap junctions increased the power of the oscillations, which were primarily driven by the synaptic feedback loop between Golgi and granule neurons, without changing the oscillation frequency or the mean firing rate of Golgi and granule neurons. Our modeling results suggest that gap junctions between Golgi cells increase the robustness of the cerebellar cortex oscillations, which are primarily driven by the synaptic feedback loop between Golgi and granule neurons as described previously (Maex and De Schutter, 1998, 2005).

3.4.3 3D model of cerebellar cortex

We are constructing a detailed 3D network model of cerebellar cortex. The goal is to represent the real connectivity in detail with realistic neural excitability and synaptic physiology, but without morphologically realistic neurons. The network model will be a general tool that can be used to address many questions of cerebellar function and therefore flexibility is part of the design. For example, dimensions and neuron density can be scaled easily and in situations where the biology is unclear different scenarios can be modeled, like whether a relation exists between granule position in the granular layer and position of the corresponding parallel fiber in the molecular layer.

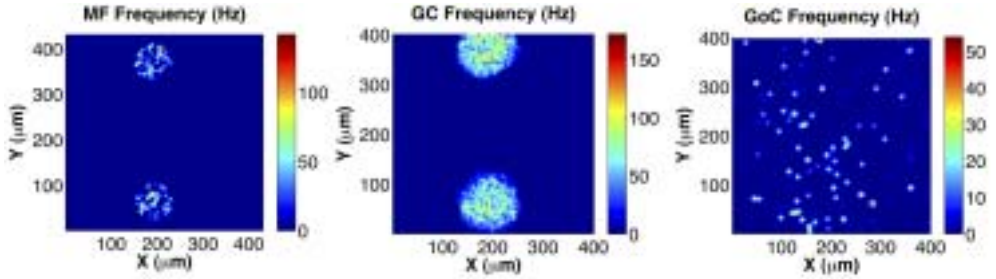


Figure 11: Simulation of activation of two mossy fiber bundles in the 3D network.
Each panel shows a top view (parallel fibers run vertically) of the color coded firing frequency of one population, from left to right: mossy fibers, granule cells and Golgi cells in a simulation with 63,500 units. Note that because of their parallel fiber excitation Golgi cells are activated along the parallel fiber beam.

The structure of the model is close to complete and the granular layer is fully functional. It is implemented in parallel NEURON. The attention given to connectivity in the model allows us for the first time to simulate the effect of complex input patterns on spiking activity (Figure 11), compared to the uniform excitation used previously (e.g. Figure 10).

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4.1 Journals

Anwar, H., Hong, S. & De Schutter, E. Controlling Ca^{2+}-activated K^+ channels with models of Ca^{2+} buffering in Purkinje cells. *Cerebellum*, in press (2010).

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4.2 Books and other one-time publications

De Schutter, E. (Editor): *Computational Modeling Methods for Neuroscientists*. MIT Press, Cambridge, MA, USA (2009) ISBN-13:978-0-262-01327-7

4.3 Oral presentations

De Schutter, E. *Model studies of Purkinje cell excitability and plasticity*, Kyoto University, Kyoto, Japan, April 9, 2009

De Schutter, E. *Large-scale modeling in neuroscience: from signaling networks to neural networks*, Seeds and Needs for Large-Scale Computing Workshop, Onna, Okinawa, Japan, June 5, 2009

De Schutter, E. *What can Computational Neuroscience learn from Systems Biology and vice versa?*, Computational Systems Biology, WCSB 2009, Århus, Denmark, June 10-12, 2009

De Schutter, E. *What should be the goal of automated parameter fitting?*, Computational Neuroscience Workshops, Berlin, Germany, July 22-23, 2009

De Schutter, E. *Theoretical and functional importance of the shapes of dendrites and spines*, 6th PRESTO Meeting of JST Systems Biology Research Area, Zampa, Okinawa, Japan, September 24, 2009

De Schutter, E. *Theoretical and functional importance of the shapes of dendrites and spines*, Meeting of the Brain and Neural Systems Team in the Next Generation Supercomputer Project, RIKEN, Wako City, Japan, October 7, 2009

De Schutter, E. T*heoretical and functional importance of the shapes of dendrites and spines*, National Centre for Biological Sciences, TIFR, Bangalore Bangalore, India, November 19, 2009

De Schutter, E. *STEPS: Reaction-diffusion simulation in complex 3D geometries*, Brain Mind Institute, École Polytechnique Fédérale de Lausanne, Switzerland, Feburary 4, 2009

De Schutter, E. T*heoretical and functional importance of the shapes of dendrites and spines*, National University Singapore, Singapore, March 15, 2009

Hepburn, I. *STEPS - Reaction-diffusion and membrane potential simulation in complex 3D geometries*, Computational Cell Biology Meeting, Hinxton, UK, February 10-14, 2010

Hong, S., De Schutter, E. *Rich single neuron computation implies a rich structure in noise correlation and population coding*, Computational Neuroscience Meeting, Berlin, Germany, July 18-21, 2009

Hong, S. *Rich single neuron computation implies a rich structure in noise correlation and population coding*, Kyoto University, Japan, October, 2009

Hong, S. *Rich single neuron computation implies a rich structure in noise correlation and population coding*, Hiroshima University, Japan, October, 2009

Kim, Y. *A geometric explanation of dendritic local planarity*, University College London, UK, September 22, 2009

Raikov, I. *Entities and Abstraction Layer in the Neural Network Model Description Language*, 3rd Meeting of INCF Task Force for Multiscale Modeling, Antwerp, Belgium, November 5-6, 2009

Raikov, I. *NineML Abstraction Layer: A Proposal*, 4th Meeting of INCF Task Force for Multiscale Modeling, Stockhom, Sweden, February 8-10, 2010

Van Geit, W., De Schutter, E. *Fitting models to specific experimental data traces using the phase-plane method*, Computational Neuroscience Workshops, Berlin, Germany, July 22-23, 2009

4.4 Posters

Antunes, G., De Schutter, E. *The regulatory role of NO-PKG in cerebellar long-term depression*, Computational Neuroscience Meeting, Berlin, Germany, July 18-21, 2009

Antunes, G., De Schutter, E. *The role of NO-PKG in the induction of cerebellar LTD*, Computational Cell Biology Meeting, Hinxton, UK, February 10-14, 2010

Anwar, H., Hong, S., De Schutter, E. *Modeling the excitability of the cerebellar Purkinje cell with detailed calcium dynamics*, Computational Neuroscience Meeting, Berlin, Germany, July 18-21, 2009

Fong, L. L., Busong, E.A., De Schutter, E., *Martone, M.M. Purkinje cell dendritic spine density from correlated three-dimensional light and electron microscopy*, Society for Neuroscience Meeting, Chicago, USA, October 17-21, 2009

Hepburn, I., Wils, S., De Schutter, E. *STEPS: reaction-diffusion simulation in complex 3D geometries*, Computational Neuroscience Meeting, Berlin, Germany, July 18-21, 2009

Hituri, K., Linne, M.-L., De Schutter, E. *Comparison and validation of two stochastic models for the cerebellar inositoltrisphosphate receptor*, Society for Neuroscience Meeting, Chicago, USA, October 17-21, 2009

Kim, Y., Sinclair, R., De Schutter, E. *Local planar dendritic structure: a uniquely biological phenomenon?*, Computational Neuroscience Meeting, Berlin, Germany, July 18-21, 2009
Kim, Y., Sinclair, R., De Schutter, E. *A geometric explanation of local dendritic planarity*, Society for Neuroscience Meeting, Chicago, USA, October 17-21, 2009

Publio, R., De Schutter, E. *Links between complex spikes and multiple synaptic plasticity mechanisms in the cerebellar cortex*, Computational Neuroscience Meeting, Berlin, Germany, July 18-21, 2009

Raikov, I., De Schutter, E. *The Layer Oriented Approach to Modeling Language Design*, Computational Neuroscience Meeting, Berlin, Germany, July 18-21, 2009

Raikov, I., De Schutter, E. *Design of hybrid neuroscience modeling languages, Society for Neuroscience Meeting*, Chicago, USA, October 17-21, 2009

Van Geit, W., De Schutter, E. *Fitting conductances and ion channel kinetics of a Purkinje cell model to experimental data using the Neurofitter software*, Neuroinformatics 2009, Pilsen, Czech Republic, September 6-8, 2009

5. Intellectual Property Rights and Other Specific Achievements

Nothing to report

6. Meetings and Events

6.1 Okinawa Computational Neuroscience Course 2009

Date: June 15 – July 2, 2009

Venue: Seaside House, OIST

Co-organizers: K. Doya, K. Stiefel, J. Wickens

Co-sponsors: Nara Institute of Science and Technology
Japanese Neural Network Society

Speakers: Bartos, Marlene, University Aberdeen, UK
Câteau, Hideyuki, RIKEN BSI, Japan
De Schutter, Erik, OIST, Japan
Doya, Kenji, OIST, Japan
Fukai, Tomoki, RIKEN BSI, Japan
Gewaltig, Marc-Oliver, Honda RIE, Germany
Häusser, Michael, University College London, UK
Knöpfel, Thomas, RIKEN BSI, Japan
Mainen, Zachary, Inst. Gulbenkian Ciência, Portugal
Paninski, Liam, Columbia University, USA
Prescott, Steven, University Pittsburgh, USA
Redish, David, University of Minnesota, USA
Sinclair, Robert, OIST, Japan
Stevens, Charles, Salk Institute, USA
Stiefel, Klaus, OIST, Japan
Stuart, Gregory, Australian National University
Wickens, Jeff, OIST, Japan
Wolpert, Daniel, Cambridge University, USA

6.2 Seminar: The role of cytoarchitecture in Purkinje cell synaptic plasticity and homeostasis: Experiments and simulations

Date: November 30, 2008

Venue: IRP

Speaker: Dr. T. Launey (RIKEN BSI)

Neural Computation Unit

Principal Investigator:
Kenji Doya

Research Theme:
Neural Computation for Flexible Learning

Abstract

The Neural Computation Unit pursues the dual goals of developing robust and flexible learning algorithms and elucidating the brain's mechanisms for robust and flexible learning. Our specific focus is on how the brain realizes reinforcement learning, in which an agent, biological or artificial, learns novel behaviors in uncertain environments by exploration and reward feedback. We combine top-down, computational approaches and bottom-up, neurobiological approaches to achieve these goals. The major achievements of the three subgroups in this fiscal year 2009 are the following.

a) The Dynamical Systems Group started to join the software development project for the next-generation super computer being built by RIKEN in Kobe. We continued to improve the Bayesian estimation engine and the user interface of "LetItB," a parameter estimation software tool for systems biology models. To understand the cellular and molecular mechanisms behind the synaptic plasticity of cortico-striatal synapses, we combined our cellular electric compartment model and synaptic molecular cascade model and found out that the dopaminergic modulation of calcium entry to synaptic spines can have a major effect on the spike timing dependent plasticity. We also developed a new reinforcement learning framework to cope with hidden states behind high-dimensional sensory inputs.

b) The Systems Neurobiology Group started a new project of recording the neural activities of three different parts of the striatum while rats performed a decision making task and revealed hierarchical roles of the ventral, dorso-medial, and dorso-lateral striatum. Through local drug injection and chemical measurement using micro dialysis, we found out that the blockade of the forebrain serotonin release impairs the ability of rats to wait for delayed rewards. We started neural recording from rats' lateral habenula to clarify the control mechanism of dorsal raphe serotonin neuron activity. We also continued functional brain imaging experiments and analysis to clarify the brain mechanisms for valuation of stochastic and delayed rewards and for model-free

and model-based action strategies.
c) The Adaptive Systems Group worked on evolution of learning mechanisms in a colony of robots called “Cyber Rodents.” We developed a mechanism to allow robots to acquire their own “intrinsic reward,” or curiosity, to facilitate effective exploration. We also observed emergence of different mating strategies within a colony and investigating its relevance to the origin of genders. We also developed new reinforcement learning algorithms to best utilize sensory-motor experiences and examined the role of sensory feedback in the control of locomotion under uncertain environments.

Under the partnership with Nara Institute of Science and Technology, two Ph.D. students and a master’s student finished theirs degrees in March 2010 from their research at OIST. These add up to five Ph.D. and nine master’s graduates from our research unit since 2004.

1. Staff

Dynamical Systems Group
Researcher: Junichiro Yoshimoto
Technical Staff: Aurelien Cassagnes, 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, Eiji Uchibe
Research Assistant / Graduate Student: Mayumi Haga, Ken Kinjo, Mikihiro Kobayashi, Viktor Zhumatiy
Research Administrator / Secretary: Emiko Asato, Chikako Uehara

2. Partner Organizations

ATR Computational Neuroscience Laboratories
Type of partnership: Joint Research
Name of researchers: Kenji Doya, Makoto Ito, Mitsuo Kawato, Erhan Oztop, Alan Rodrigues
Research theme: Functional brain imaging study on the brain mechanisms of behavioral learning

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

Honda Research Institute Japan Co., Ltd.
Type of partnership: Joint Research
Name of researchers: Kenji Doya, Makoto Ito, Katsuhiko Miyazaki, Kayoko Miyazaki,



Osamu Shono, Yohane Takeuchi, Hiroshi Tsujino, Eiji Uchibe
Research theme: Study on a biological learning model for behavior learning strategy acquision

Kyoto University
Type of partnership: Joint Research
Name of researchers: Aurelien Cassagnes, Kenji Doya, Shin Ishii, Jun-ichiro Yoshimoto
Research theme: Development of multi-scale models of visual-oculomotor system

3. Activities and Findings

3.1 Dynamical Systems Group

Application of Bayesian inference to biological networks [Yoshimoto, Inoue]

Bayesian inference provides 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. So far we have applied it to the parameter estimation and model selection issues of intracellular signaling cascade models, and developed a software, LetItB (Let It be found by Bayes) by combining the estimation engine and a graphical user interface for easy operation by biologists.

In this fiscal year, we aimed at extending the principle to the connectivity estimation of neural networks. Assuming that the morphological data and channel kinetics of the target network are known, estimating the synaptic efficacy and post-synaptic current from time-series of membrane potential is regarded as a class of constrained linear regression problems. However, naive algorithms do not work in many cases, because the number of parameters to be estimated, such as the synaptic connection weights, can be larger than the number of available data points. To solve the difficulty, we assumed the prior knowledge of spatio-temporal sparseness in cellular interactions and derived a Bayesian regression algorithm. We are now implementing the algorithm and testing the applicability to the real problems.

Figure 1: Overview of “LetItB” software

** The software can be downloaded from http://www.nc.irp.oist.jp/software/.*

Integration of visuo-motor network models by MUSIC [Cassagnes, Yoshimoto]

A challenging goal in computational neuroscience is to realize a “whole-brain” simulation linking sensory inputs to motor outputs. The visuo-motor system is a good instance for approaching the goal, because the neural networks in its pathway have been well studied and mathematical models of each part have been proposed. The aim of our present project is to connect such models and close the visual sensor-motor loop.

In connecting multiple pre-existing models, a major difficulty is communicating the events among heterogeneous processes developed independently and implemented in different simulation platforms. The MUSIC (Multi-Simulation Coordinator) was developed recently by the initiative of INCF to overcome this difficulty. In this fiscal year, we started implementing the oculomotor control system by connecting three models (the superior colliculus, brainstem integrator network, and the eyeball plan) with MUSIC and confirmed its efficient execution. We are now scaling up the implementation to run on highly parallelized computers.

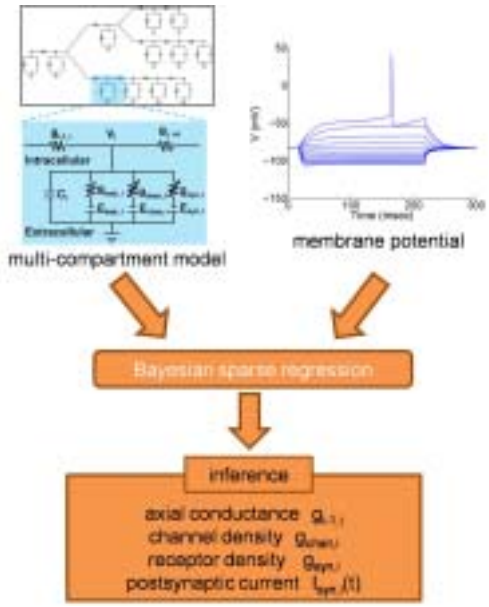


Figure 2: Basic concept of Bayesian estimation of neural connectivity.

Signaling cascade and electric compartment models for the synaptic plasticity in the striatum [Nakano, Yoshimoto]

The striatum is the input nucleus of the basal ganglia, and receives glutamate input from the cortex and dopamine input from the substantia nigra. These control the plasticity of corticostriatal synapse. To understand the molecular and electrophysiological mechanisms of the synaptic plasticity, we constructed models at molecular and intracellular levels. Our electric multi-compartment model predicted that the calcium response in post-synaptic neurons would depend on the timing of both the dopamine and glutamine inputs from their pre-synaptic neurons, suggesting that the timing of dopamine inputs would be involved in the regulation of corticostriatal spike-timing dependent synaptic plasticity (STDP). This results were presented in an international conference (Nakano et al., *ICANN*, 2009). The simulation results from our intracellular signaling cascade model predicted that the direction of synaptic change would be determined by calcium input strength, and that a positive feedback loop including dopamine- and cAMP-regulated phosphoprotein of molecular weight 32 kDa (DARPP-32) would have bistability, inducing dopamine dependent long-term potentiation (LTP). These findings were published in a journal paper (Nakano et al, *PLoS Comput. Biol., 2010*). Furthermore, we are now trying to integrate these models in different scales to make the mechanism of corticostriatal STDP much clearer. To test the validity of our striatum neuron model, we are setting up an electrophysiological recording platform in collaboration with Neurobiology Research Unit.

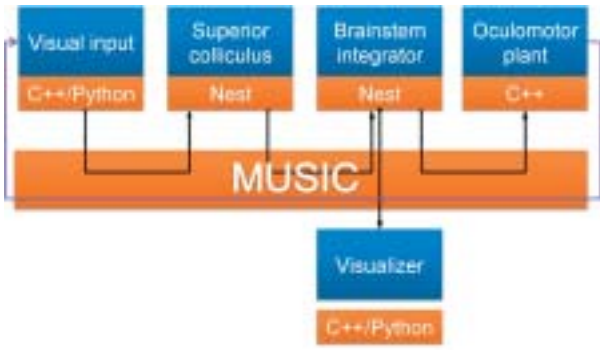


Figure 3: Overall architecture of the visual-oculomotor model.

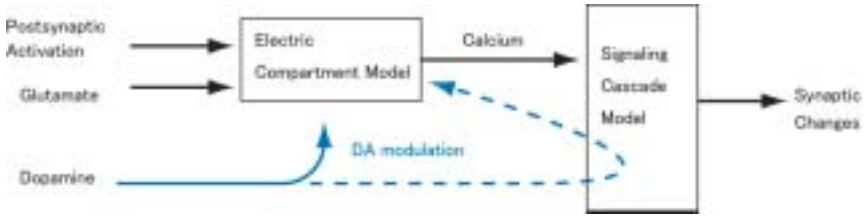


Figure 4: The electric compartment model predicts calcium response to glutamate, dopamine, and activation of medium spiny neurons. The signaling cascade model predicts changes in synaptic efficacy induced by dopamine and calcium input. Combining them can predict synaptic efficacy by physiological triggers. A simple connection is that dopamine directly modulates the calcium prediction from the electric compartment model (solid arrow). In an actual neuron, dopamine modulation of calcium is through intracellular chemicals (dashed arrow).

Efficient spatio-temporal representation in reinforcement learning tasks with high-dimensional stochastic observations [Otsuka, Yoshimoto]

Reinforcement learning (RL) is an attractive framework to build decision making function into autonomous agents, and many methods for solving RL problems (e.g. Q-learning, SARSA, Actor-Critic, policy gradient, etc.) have been proposed so far. These methods have potential to solve any problem formulated as 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 the limitation of computational resources in the agents. The key for enhancing the performance of the RL methods in such problems, which is called a partially observable Markov decision process (POMDP), is to extract spatio-temporal features lying behind the environment. In this fiscal year, we proposed a novel architecture for solving POMDP with high-dimensional stochastic observations.

We adopted the restricted Boltzmann machine (RBM) as a basic architecture of the reinforcement learning system. In order to handle the temporal component, we incorporated a recurrent neural network that learns a memory representation sufficient for predicting future observations and rewards. We demonstrated that the proposed method successfully solved POMDPs with high-dimensional stochastic observations without any prior knowledge of the environmental hidden states and dynamics. After learning, task structures were implicitly represented in the distributed activation patterns of hidden nodes of the RBM (Otsuka, Ph.D. Thesis, 2010).

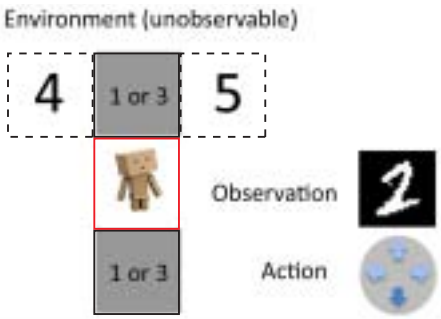


Figure 5: Matching T-maze task: A example of POMDP environment. An agent selects an action upon receiving noisy high-dimensional observations (images of handwritten digits). A terminal reward obtained at goal positions (dotted squares) depends on the digit at the start position and at the T-junction (gray squares). If digits at these two positions matches, the agent receives either a large positive reward (+5) at the goal with digit 5 or a large negative reward (-5) at the goal with digit 4. The reward conditions are reversed when the digits at these two positions do not match. In

order to solve this task, an agent needs to combine current observations with memory of past observations on the fly.

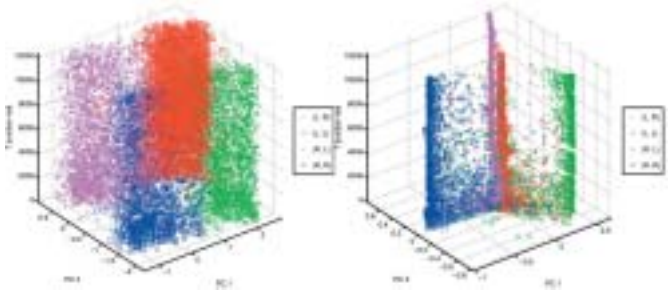


Figure 6. Functionally differentiated activation patterns of nodes in the RBM-based actor. Activations of node groups are projected onto the first two principal component spaces for visualization. (Left panel) Activations of state nodes. (Right panel) Activations of hidden nodes.

3.2 Systems Neurobiology Group

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).

In order to 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.

So far, we found that the level of serotonin release was significantly elevated when rats performed a task working for delayed rewards compared with for immediate reward. We also found many serotonin neurons in the dorsal raphe nucleus increased firing rate while the rat stayed at the food or water dispenser in expectation of reward delivery.

To examine the causal relationship between waiting behavior for delayed rewards and serotonin neural activity, 5-HT_{1A} agonist, 8-OH-DPAT was directly injected into the dorsal raphe nucleus to reduce serotonin neural activity by reverse dialysis method. We found that rats made significantly more errors in waiting for long delayed reward under 8-OH-DPAT treatment. These findings show the causality between activity of serotonin and effortful waiting for delayed reward.

Furthermore, to examine which neural inputs are important to drive serotonin neural activity for delayed rewards, we focused on the lateral habenula which sends strong projection to the dorsal raphe nucleus. We found that some lateral habenula neurons increased firing for delayed rewards, but in a different way compared to the serotonin neurons. Simultaneous recording of serotonin and lateral habenula neurons will be helpful to elucidate functional relationship between the lateral habenula and dorsal raphe nucleus.

Hierarchical information coding in the striatum during decision making tasks [Ito]

The striatum, which is involved in movement control and reinforcement learning, has a dorsal-ventral gradient in input information: the more dorsolateral striatum receives sensorimotor-related information and the more ventral part receives associative and motivational information. This organization is suitable for hierarchical control of behaviors.

In this study, we recorded neuronal activities from the dorsolateral striatum (DLS), the dorsomedial

striatum (DMS) and the ventral striatum (VS) of rats performing two types of choice tasks; a fixed-reward task (FR) and a varied-reward task (VR). In FR, when a rat poked into a center hole, either tone A or B was presented and then the rat should select the left or right poking hole. A food pellet was delivered probabilistically depending on the presented tone and the selected choice. VR was the same as FR except that tone C was always presented and the reward probability was varied in a block-wise manner after the choices of the rat reached to 80% optimal.

We isolated 204, 112 and 118 phasically-active neurons (PANs; putative medium spiny projection neurons) from DLS, DMS and VS, respectively. During the start phase, namely, during rats approached toward the center hole, the proportion of activated PANs was significantly larger in VS than in DLS and DMS. Furthermore, the information on types of tasks was most strongly represented in VS. During the choice phase, namely, from the exit of the center hole to the entry in the left or right hole, the proportion of activated PANs was significantly larger in DMS than in DLS and VS, and DMS neurons coded the information of the chosen action most strongly. The temporal distribution of the activity of PANs was sharpest in DLS, and widest in VS.

These results support the idea of a hierarchical control that VS represents the type of the tasks as the top module, DMS, the middle module, contributes to action selection, and DLS, the lower module, serves for detailed movement.

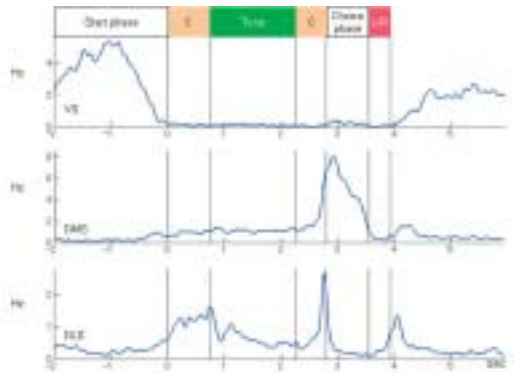


Figure 7: Representative Examples of activities of phasically-active neurons in the ventral striatum (VS), the dorsomedial striatum (DMS), and the dorsolateral striatum (DLS).

Brain mechanisms for evaluating probabilistic and delayed rewards [Yoshida, Rodrigues, Ito, Yoshimoto]

Humans prefer sooner, more probable rewards over delayed, less probable ones. To clarify such a reward-discounting mechanism, we investigated the brain activity in the presence of both probabilistic and delayed rewards using functional magnetic resonance imaging.

Ten human subjects performed a choice task in which two visual stimuli, each coding a pair of delay and probability of a juice reward, were presented in series and the subject selected one of them by pressing a button. While activity of right posterior cingulate gyrus, right superior parietal lobule and caudate correlated with the reward probability, activities of left anterior cingulate and right inferior parietal lobule correlated with the reward delay. The separate activity loci suggest distinct processing mechanisms for delay and probability discounting.

Activation of adjacent areas in the right parietal lobule for more probable and sooner rewards, respectively, suggests that the area integrates two sources of information on reward discounting.

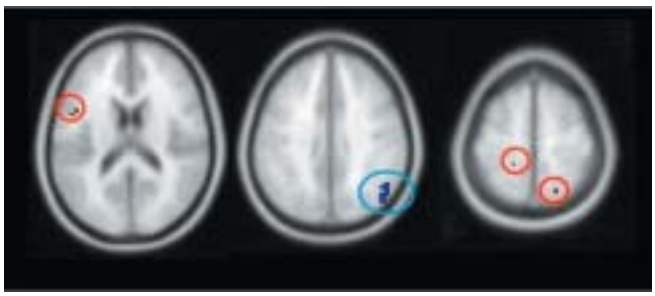


Figure 8: Brainregions related to evaluation of reward. Red: Brain regions showing correlation with the delay discounting. Blue: Brain regions correlating with the probabilistic discounting. (from left z=17, 37, 60)

Neural mechanisms for model-free and model-based reinforcement strategies in humans performing a multi-step navigation task [Rodrigues, Yoshida, Ito, Yoshimoto]

Humans learn to select actions from scratch, by trial and error, and by using knowledge from past experiences. As learning goes on, action selection may rely on predictive mechanisms such as retrieval of motor memories, or construction of internal models in newly encountered environments. Reinforcement Learning (RL), a computational theory of adaptive optimal control, suggests two methods that resemble real human behavior: Model-Free (MF) method uses action value functions to predict future rewards based on current states, and Model-Based (MB) method uses a forward model to predict the future states reached by hypothetical actions. We tested whether humans utilize MF and MB strategies by having subjects perform a grid-sailing task whose goal was to move a cursor from a start position to a target by sequentially pressing three keys which moved a cursor in three different directions. A response started immediately after a go signal or a delay period of 4~6 seconds. Subjects received visual feedback of cursor motion, and reward feedback (points) at trial end. After one day of training and one night sleep, subjects performed the experiment inside the fMRI scanner under three task conditions: (1) learning of new key-map and start-goal positions, (2) use of learned key-map for new start-goal positions, (3) well-learned action sequences. Behavior analysis revealed distinct performance profiles: condition 1 - slow and variable reward acquisition; condition 2 - boosted performance especially after a delay period; condition 3 - automatic and high level performance unaffected by the start time. The fMRI analysis of the delay period showed that task condition 2 required the activation of areas including the dorsolateral prefrontal cortex, ventral premotor cortex, anterior basal ganglia and posterior cerebellum, whereas task condition 1 activated the dorsal premotor cortex and areas related to visuo-spatial information processing including the superior parietal cortex and visual areas. These results were confirmed by analysis of the time course of signal intensity of activation in these areas, where task condition 2 required the strongest activation in this prefrontal-basal-ganglia-cerebellum network. Preliminary results of a computational model-based analysis using RL algorithms showed that we could successfully classify which action selection strategy was used by subjects, namely, MF for task condition 1 and MB for task condition 2.

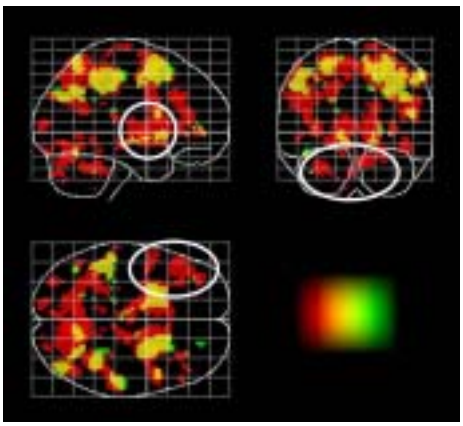


Figure 9: Brain areas related to action selection strategies during the delay period. Red color, areas that were more activated in condition 2 than in condition 3 and whose role we speculate to be involved in implementing model-based strategy; Green (yellow overlapping) *color, areas more activated in condition 1 than in condition 3.*

The computational principle of flexible learning of action sequences [Hoshino]

When we apply machine learning techniques to robotic intelligence, the major challenge is huge uncertainty in the environment. In order to solve these problems, we investigate how our brain flexibly selects learning algorithms and parameters. We have developed an original motor sequence task and showed that the rat could acquire the correct sequence of lever pressing in the octagonal operant chamber voluntarily using only an ICSS reward for a clue. The results of a behavior analysis of the rat and of the simulation with reinforcement learning model suggested that the rat used a multiple learning modules and a mechanism to change their parameters. We aim to develop a new modular learning framework for flexibly choosing suitable learning modules for given situations.

3.3 Adaptive Systems Group

Finding intrinsic rewards based on the embodied evolution [Uchibe]

Understanding the design principle of reward functions is a substantial challenge both in artificial intelligence and neuroscience. Successful acquisition of a task usually requires not only rewards for goals, but also for intermediate states to promote effective exploration. We have already proposed a method for designing `intrinsic' rewards of autonomous agents by combining constrained policy gradient reinforcement learning and embodied evolution. Our previous study showed promising results, but the intrinsic reward was limited to a simple network with three weight parameters. Therefore, we investigate more detailed analysis by using more complicated networks to represent a complex intrinsic reward function. To improve the optimizing capability, each robot contains a set of parameters, which can be regarded as virtual agents in the physical robot. To validate the method, we use Cyber Rodent robots (CR), in which collision avoidance, recharging from battery packs, and `mating' by software reproduction are three major `extrinsic' rewards. We found that the obtained reward function allowed CRs to acquire a variety of exploring behaviors. In order to investigate the obtained intrinsic reward during evolution in more detail, we select one intrinsic reward parameters at the 5th generation. To be able to visualize it, we divide the state space into nine distinct types. The top row of the left panel shows the intrinsic rewards when the battery of the robot is sufficiently charged. Despite a large battery of the robot, the

intrinsic reward produces a large positive reward, and its peak is regarded as the desired state when the CR catches the battery pack. The intrinsic reward at the 5th generation is not appropriate for learning mating behaviors, and it is mainly used for learning surviving behaviors. Then, we select one intrinsic reward parameters at the 10th generation shown in the right panel. For example, the top row shows the intrinsic reward when the battery is sufficiently charged. The intrinsic reward generates a small negative reward when the CR sees the battery pack and another CR is not observed. Experimental results suggest that the intrinsic reward at 10th generation generates a large positive reward for searching another CR and a small positive reward for searching the battery pack, respectively. The reason for this is that the CR had many chances to find one of the battery packs because the number of them is greater than that of the CRs. In addition, a large supplementary reward related to the nearest battery pack prevented the CR from approaching it. On the other hand, a successful mating behavior was not sensitive to the distance between two CRs because of the property of IR communication. In this case, watching another CR was regarded as an appropriate strategy for mating. Therefore, it is concluded that appropriate intrinsic rewards were obtained through embodied evolution.

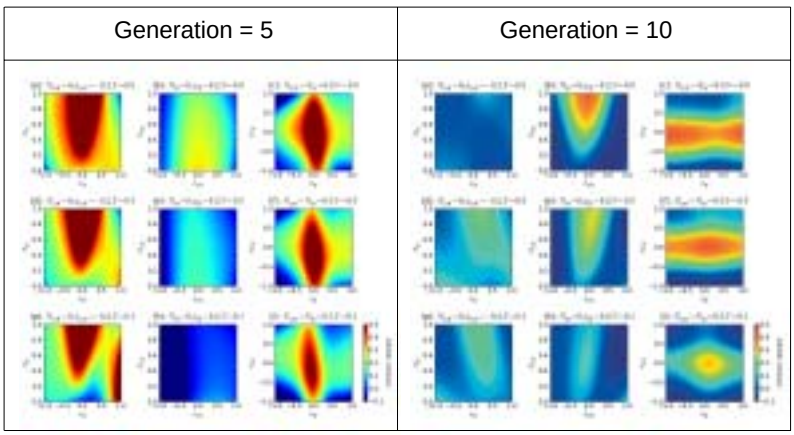


Figure 10: Intrinsic rewards at different stages of evolution.

Emergence of Different Mating Strategies in Embodied Evolution [Elfwing]

Embodied evolution is a methodology for evolutionary robotics that mimics the distributed, asynchronous, and autonomous properties of biological evolution. The evaluation, selection, and reproduction are carried out by cooperation and competition of the robots, without any need for human intervention. An embodied evolution framework is therefore well suited to study the adaptive learning mechanisms for artificial agents that share the same fundamental constraints as biological agents: self-preservation and self-reproduction. This study proposes a framework for performing embodied evolution with a limited number of robots, by utilizing time-sharing in subpopulations of virtual agents. Within this framework, we explore the combination of within-generation learning of basic survival behaviors by reinforcement learning, and evolutionary adaptations over the generations of the basic behavior selection policy, the reward functions, and meta-parameters for reinforcement learning. We apply a biologically inspired selection scheme, in which there is no explicit representation or communication of the individuals' fitness information. The individuals can only reproduce offspring by mating, a pair-wise exchange of genotypes, and the probability that an individual reproduces offspring in its own subpopulation is dependent on the individual's "health", i.e., energy level, at the mating occasion. In the experiment, we observed two individual mating strategies: 1) Roamer strategy, where the agents never wait for potential mating partners; and 2) Stayer strategy, where the agents wait for potential mating partners depending on

their internal energy level, the distance to the mating partner's tail-LED, and the distance to the closest battery. To analyze the three main obtained population mating strategies (roamers, stayers, and mixture of strategies), we investigated the relationship between genotype, phenotype, behavior, and performance. These relationships are illustrated in the following figures, where the data for all virtual agents that survived (their internal energy levels were positive for the full 1200 time steps lifetime) in the final generations are shown. For the mixture of strategies, the two clusters in the PCA-space (left panel) were used to identify two separate genetic traits in the population. The two subpopulation representing the two traits are color-coded according to their phenotype-distributions (right panel): red and blue colors for the roamer subpopulation and red and green colors for the stayer subpopulation.

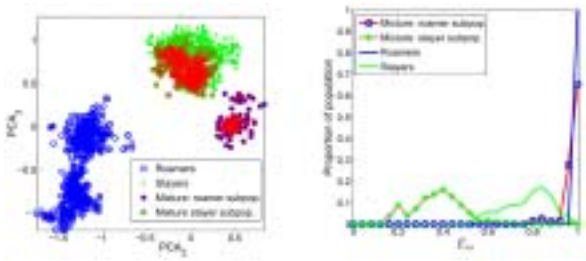


Figure 11: The Genotypes (left) and phenotypes (right) for different mating strategies.

Effect on observational uncertainty in locomotion control using CPGs with sensory feedback. [Kimura, Haga, Uchibe]

Central Pattern Generators (CPG) are becoming a popular model for the control of rhythmic locomotion in robots. CPG integrates sensory feedbacks which include touch sensors, joint angles and so on, and generates a variety of gait patterns for robots. However, sensory feedback is selected by experimenters and to our best knowledge, it has not been well investigated how environmental dynamics and observational uncertainty affect the locomotion control using CPGs with sensory feedback. So we investigate what kind of sensory information are selected by depending on sensory noise or environmental condition for CPG with sensory feedback. We use a four-legged robot with passive knee joints, which is implemented in the dynamic simulator. For simplicity, a coupled Hopf oscillators with linear sensory feedback is tested, in which touch sensors attached on the sole are selected for feedback information. The following figure shows the mean distance according the weight values, where w_f and w_h are the linear feedback weights for fore and hind legs, respectively. In these experiments, we changed the variance of noise (σ^2) added to the touch sensor values. These figure show that sensory feedback is not effective when sensory information is too noisy. We will continue to investigate more systematic analysis to find a relationship between observational uncertainty and feedback control.

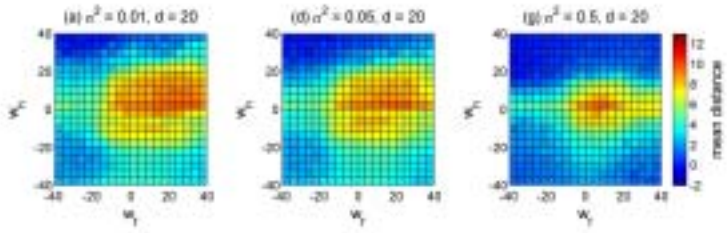


Figure 12: The effects of the sensory noise on the optimal sensory feedback gain.

Data-efficient trajectory-based Reinforcement Learning [Zhumatiy]

While some planning algorithms demonstrate that reuse of experienced trajectories is beneficial for robot control in the real world, there is little progress in trajectory-based RL that potentially

copes with dynamic environments requiring feedback control. The aim of this project is to establish a theoretical framework of trajectory-based RL that works in POMDP environments with highly-dimensional, multi-modal perception that are encountered in mobile robot control.
For the purpose, we have developed a basic algorithm, *PC-NSM* (Piecewise-Continuous Nearest Sequence Memory), which was shown to work well in a real-time robot control task. On the other hand, we found several limitations through standard benchmark tests in continuous RL. To overcome them, we refined the algorithm by devising the following modules: 1) model-free search for the nearest trajectory in continuous time domains; 2) value function approximation employing smooth kernel functions; 3) value-updating rule based on detection of reachable manifold underlying the entire state space. The efficiency and feasibility is being tested by applying them to automatic control problems of an inverted pendulum and cart-pole balancer.

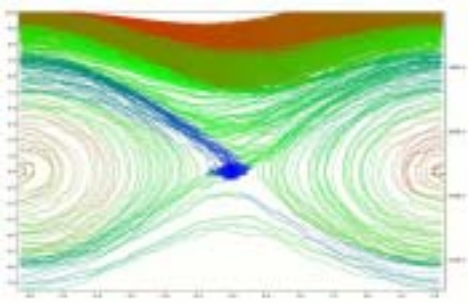


Figure 13: Typical example of experienced trajectories and their approximated value (utility) in an inverted pendulum control. Colder colors indicate higher values (corresponding to closer points to the goal state). Please note that adjacent states in terms of Euclidian distance are not always close in terms of the value function.

4. Publications

4.1 Journals

Doya, K. How can we learn efficiently to act optimally and flexibly. *Proceedings of the National Academy of Sciences, USA* 106, 11429-11430 (2009).

Elfwing, S., Uchibe, E., Doya, K. & Christensen, H. I. Darwinian embodied evolution of the learning ability for survival. *Adaptive Behavior* (In press).

Ito, M. & Doya, K. Validation of decision-making models and analysis of decision variables in the rat basal ganglia. *Journal of Neuroscience* 29, 9861-9874 (2009).

Ito, M., Shirao, T., Doya, K. & Sekino, Y. Three-dimensional distribution of Fos-positive neurons in the supramammillary nucleus induced by exploring behavior of rats. *Neuroscience research* 64, 397-402 (2009).

Klein, M., Kamp, H., Palm, G. & Doya, K. A computational neural model of goal-directed utterance selection. *Neural Networks* (23, 592-606) (2010).

Morimura, T., Uchibe, E., Yoshimoto, J., Peters, J. & Doya, K. Derivatives of logarithmic stationary distributions for policy gradient reinforcement learning. *Neural Computation* 22, 342-376 (2010).

Nakano, T., Doi, T., Yoshimoto, J. & Doya, K. A kinetic model of dopamine and calcium dependent striatal synaptic plasticity. *PLoS Computational Biology* 6, e1000670 (2010).

Otsuka, M., Yoshimoto, J. & Doya, K. Reward-dependent sensory coding in free-energy-based reinforcement learning. *Neural Network World* 19, 597-610 (2009).

Tanaka, S., Shishida, K., Schweighofer, N., Okamoto, Y., Yamawaki, S. & Doya, K. Serotonin affects association of aversive outcomes to past actions. *Journal of Neuroscience* 16, 15669-15674 (2009).

Yoshimoto, J., Sato, M.-A. & Ishii, S. Bayesian normalized Gaussian network and hierarchical model selection method. *Intelligent Automation and Soft Computing* (In press).

4.2 One-time Publication

Doya, K., Ito, M. & Samejima, K. Model-based analysis of decision variables. In M. Delgado, E. Phelps, & T. Robbins (eds.), *Decision Making: Attention and Performance XXIII,* Oxford University Press (In press).

4.3 Oral presentations

Doya, K. *Roles of disparate brain regions*, Satellite Meeting of the Society for the Neural Control of Movement. Waikoloa, Hawaii, May 1, 2009

Doya, K. *Prediction of future reward in the basal ganglia and regulation of patience by serotonin*, Seminar at National Institutes of Health. Bethesda, USA, May 4, 2009

Doya, K. *Computational models of basal ganglia function*, MIT McGovern Institute symposium: The Basal Ganglia in Health and Disease. Cambridge, USA, May 7, 2009

Doya, K. *Model-based and model-free strategies in reinforcement learning*, Second German-Japanese Workshop on Computational and Systems Neuroscience. Berlin, Germany, May 27, 2009

Doya, K. *Bayesian sampling methods in neuroscience: from synapses to behaviors*, IPAB Open Workshop on Seeds and Needs for Large-Scale Computing. Onna, Japan, June 5, 2009

Doya, K. *Multiple representations and algorithms for motor learning and control*., The Fifth Computational Motor Control Workshop at Ben-Gurion University of the Negev. Beer-Sheva, Israel, June 11, 2009

Doya, K. *Brain mechanisms of reinforcement learning (Japanese),* Special Lecture at Workshop on Innovative Nano-science of Supermolecular Motor Proteins Working in Biomembranes. Nago, Japan, June 17, 2009

Doya, K. *Introduction to decision making and Bayesian inference*, Okinawa Computational Neuroscience Course. Onna, Japan, June 19, 2009

Doya, K. *Reinforcement learning: a tool for cracking the neural codes of behavioral learning*., Bernstein Tutorial at 18th Annual Computational Neuroscience Meeting. Berlin, Germany, July 18, 2009

Doya, K. *Understanding the brain by creating it (Japanese)*, Open Seminar for 2009 Asian Youth Exchange Program in Okinawa, Japan, August 23, 2009

Doya, K. *Learning algorithms for modeling human behaviors and identifying molecular cascades*, Plenary Lecture at the 2nd INCF Congress of Neuroinformatics. Pilsen, Czeck Republic, September 8, 2009

Doya, K. *Temporal discounting and serotonin*, QBI Lecture, Brisbane, Australia, January 28-29, 2010

Doya, K. *Neural mechanisms of reinforcement learning*, Annual meating of Australian Newroscience and Physiological Societies. Sydney, Australia, January 31 - February 3, 2010

Doya, K. *Model-based and model-free strategies in sequential motor learning*, Reward and Decision Making in the Brain. Jerusalem, Israel, February 16-19, 2010

Doya, K. *Bayesian inference, reinforcement learning*, and decision making, Joint Tamagawa-Caltech Lecture Course. Tamagawa, Tokyo, March 3-5, 2010

Elfwing, S., Uchibe, E., Doya, K. *Emergence of different mating strategies in artificial embodied evolution*, 16th International Conference on Neural Information Processing. Bangkok, Thailand, December 2, 2009

Kimura, S., Uchibe, E., Yoshimoto, J., Doya, K. *Effect on environmental dynamics and observational uncertainty in locomotion control using CPGs with sensory feedback (Japanese)*, IEICE Neurocomputing Workshop. Tokyo, Japan, March 9-11, 2010

Kobayashi, M., Uchibe, E., Doya, K. *Reinforcement learning through the active low-dimensional sensory information (Japanese)*, IEICE Neurocomputing Workshop. Okinawa, Japan, May 25-26, 2009

Nakano, T., Yoshimoto, J., Wickens, J., Doya, K. *Calcium response model to timed inputs in the striatum (Japanese)*, 17th Bioinformatics workshop, Information Processing Society of Japan. Okinawa, Japan, May 25-26, 2009

Nakano, T., Yoshimoto, J., Wickens, J., Doya, K. *Calcium responses model in striatum dependent on timed input sources*, 19th International Conference on Artificial Neural Networks (ICANN), Part I: 249-58. Limassol, Cyprus, September 14-17, 2009

Yoshimoto, J. *Bayesian inference and system identification for biochemical reaction models (Japanese)*, Workshop on Stochasticity in Next-Generation Information Processing. Wako, Japan, March 3-4, 2010

4.4 Posters

Cassagnes, A., Moren, J., Shibata, T., Yoshimoto, J., Doya, K. *Integration of visuo-motor network models by MUSIC*, 10th Winter Workshop on the Mechanism of Brain and Mind. Rusutsu, Japan, January 12-14, 2010

Cassagnes, A., Moren, J., Shibata, T., Yoshimoto, J., Doya, K. *Integration of visuo-motor network models by MUSIC*, 2nd Biosupercomputing Symposium. Tokyo, Japan, March 18-19, 2010

Fermin, A., Yoshida, T., Tanaka, S., Ito, M., Yoshimoto, J., Doya, K. *Model-free and model-based reinforcement learning strategies in the acquisition and selection of sequential actions*, 19th Annual Meeting of the Society for the Neural Control of Movement. Waikoloa, Hawaii, May 1, 2009

Fermin, A., Yoshida, T., Tanaka, S., Ito, M., Yoshimoto, J., Doya, K. *Candidate neural networks for implementing model-free and model-based reinforcement learning strategies in the selection of sequential actions*, 10th Summer Workshop on the Mechanism of Brain and Mind. Sapporo, Japan, August 9, 2009

Fermin, A., Yoshida, T., Tanaka, S., Ito, M., Yoshimoto, J., Doya, K. *Reinforcement Learning Strategies for Sequential Action Learning*, 32nd Annual Meeting of the Japan Neuroscience Society. Nagoya, Japan, September 18, 2009

Funamizu, A., Ito, M., Doya, K. , Kanzaki, R., Takahashi, H. *Model-free and model-based strategies in rats' choice behaviors*., 32nd Annual Meeting of the Japan Neuroscience Society. Nagoya, Japan, September 16,2009

Ito, M., Doya, K. *Differential representation of actions in the dorsal and the ventral striatum*, 10th Summer Workshop on the Mechanism of Brain and Mind, Sapporo, Japan, August 9, 2009

Ito, M., Doya, K. *Different representation of action and reward in the dorsal and the ventral striatum*, 32nd Annual Meeting of the Japan Neuroscience Society. Nagoya, Japan, September 16, 2009

Ito, M., Doya, K. *Different representation of action command in the dorsal and ventral striatum*, 39th annual meeting of the Society for Neuroscience. Chicago, USA, October 17-21, 2009

Miyazaki, K.W., Miyazaki, K., Doya, K. *Serotonin facilitates waiting behavior for future reward*, 39th annual meeting of the Society for Neuroscience. Chicago, USA, October 17-21, 2009

Moren, J., Shibata, T., Doya, K. *Toward Multi-Scale Modeling of the Mammalian Visual-Oculomotor System: The Intermediate Superior Colliculus*, 10th Summer Workshop on the Mechanisms of Brain and Mind. Sapporo, Japan, August 8-9, 2009

Moren, J., Shibata, T., Doya, K. *Toward multi-scale modeling of the mammalian visual-oculomotor system: the intermediate superior Colliculus*, 19th Annual Conference of the Japanese Neural Network Society. Sendai, Japan, September 24-26, 2009

Moren, J., Shibata, T., Doya, K. *Toward a spiking neuron-level spatial model of the early visual system*, 10th Winter Workshop on the Mechanism of Brain and Mind. Rusutsu, Japan, January 12-14, 2010

Yoshida, T., Ito, M., Morimura, T., Samejima, K., Okuda, J., Yoshimoto, J., Doya, K. *Brain mechanisms for evaluating probabilistic and delayed rewards*, 32nd Annual Meeting of the Japan Neuroscience Society. Nagoya, Japan, September 18, 2009.

Yoshimoto, J., Inoue T., Doya, K. *System identification of intracellular molecular cascades based on Monte Carlo sampling*, The Next-Generation Supercomputing Symposium 2009. Tokyo, Japan, October 7-8, 2009

5. Intellectual Property Rights and Other Specific Achievements

Nothing to report

6. Meetings and Events

6.1 Joint Workshop of Neuro-Computing, Bioinformatics and Genomics

Date: May 25-26, 2009
Venue: OIST Seaside House
Co-organizers: IEICE Technical Committee on Neurocomputing
IPSJ Special Interest Group of Bioinformatics and Genomics (SIG-BIO)
Co-sponsors: Japanese Neural Network Society
IEEE Computational Intelligence Society Japan Chapter
Speakers: Special lecture by Dr. Jonathan Miller(OIST)
16 regular presentations

6.2 Seeds and Needs for Large-Scale Computing Workshop

Date: June 5, 2009
Venue: OIST Seaside House
Co-organizers: Initiative for Parallel Bioinformatics(IPAB)
Co-sponsors: OIST
IPSJ Special Interest Group of High Performance Computing (HPC)
Grid Consortium Japan
BESTSYSTEMS, Inc.
AXIOHELIX
Speakers: Erik De Schutter (OIST)
Kenji Doya (OIST)
Miron Livny (Univ of Wisconsin)
Satoshi Sekiguchi (AIST)
Osamu Tatebe (Univ of Tsukuba)

6.3 Seminar

Date: July 6, 2009
Venue: OIST IRP Conference Room
Speaker: Cengiz Gunay (Emory University)

6.4 Summer Workshop on the Mechanisms of Brain and Mind

Date: August 9, 2009
Venue: Sapporo
Co-organizers: Integrative Brain Research Project
Speakers: Kuniyoshi L. Sakai (University of Tokyo)
Asif A.Ghazanfar (Princeton University)
Kazuo Okanoya (RIKEN・JST/ERATO)

6.5 Seminar

Date: December 12, 2009
Venue: OIST IRP Conference Room
Speaker: Shin Nagayama (The University of Texas Medical School at Houston)

6.6 Winter Workshop on the Mechanisms of Brain and Mind

Date: January 12-14, 2010
Venue: Rusutsu Resort
Co-sponsors: Integrative Research Project for Brain Function
Speakers: Shinsuke Shimojo (California Institute of Technology)
Patrick Haggard (University College London)
Takeo Watanabe (Boston University)
Yuka Sasaki (Harvard Medical School)
Hironori Nakatani (RIKEN BSI)
Satoshi Hirata (Great Ape Research Institute)
Hidehiko Takahashi (NIRS・JST/PRESTO)
Thomas J. McHugh (RIKEN BSI)
Yumiko Yoshimura (Okazaki Institute for Integrative Bioscience)

Evolutionary Systems Biology

Independent New Investigator:
Holger Jenke-Kodama

Research Theme:
Evolution of secondary metabolism in bacteria

Abstract

The overall aim of the Evolutionary Systems Biology Unit is to elucidate the evolutionary processes shaping secondary metabolism in bacteria. Secondary metabolites are small compounds that belong to diverse chemical substance classes and show a taxonomically inhomogeneous distribution. Until recently, research on secondary metabolites had been dominated by classic natural product chemistry, which aims to find and characterise new compounds with pharmaceutically important bioactivities like antibiotic, cytotoxic, and antifungal activities. The last years, however, have seen an increasing interest in the biological functions of those compounds. From an evolutionary perspective, the key questions are: how is the plethora of substances created and how does natural selection influence secondary metabolism? The unit approaches these questions using a combination of experimental and computational methods. The concept is to analyse secondary metabolism at the systems level. Our research is focused on modular polyketide synthases, nonribosomal peptide synthetases, and hybrid enzyme systems thereof, which are frequently found in various bacterial phyla. In the initial project stage, the model organism is the cyanobacterium *Nostoc punctiforme*. This choice is based on the facts that (1) the genome of *N. punctiforme* has been completely sequenced, (2) it shows complex physiological and ecological features including nitrogen fixation, cell differentiation, and symbiotic interactions with plants and fungi, and (3) it has the genetic potential to synthesise several polyketides and nonribosomal peptides.

1. Staff

Researchers: Dr Kugako Sugimoto, Dr Maiko Tamura

Research Administrator / Secretary: Ms Midori Tanahara

2. Partner Organizations

The University of Tokyo, Graduate School of Agricultural and Life Sciences

Type of partnership: Collaborative research

Name of researchers: Prof. Shigeki Matsunaga, Dr Shigeru Okada

Research theme: Botryococcene biosynthesis in the green microalga *Botryococcus braunii*

3. Activities and Findings

3.1 Preparatory research activities

The Evolutionary Systems Biology Unit was created in May 2009. In the course of the first year, the laboratory space was planned and set up including equipment for transcriptome analysis. Furthermore, the software environment was established and we started phylogenomics analyses and reconstruction of the metabolic network of *N. punctiforme*. Two post-doctoral researchers, Dr Sugimoto and Dr Tamura, joined the unit in March 2010 and April 2010, respectively.

3.2 Collaborative research

The collaboration with the University of Tokyo, Graduate School of Agricultural and Life Sciences, has been continued. Several enzymes of the MEP (2-C-methylerythritol 4-phosphate) pathway of the green microalga *Botryococcus braunii* were identified and expressed heterologously in *Escherichia coli* in order to conduct their biochemical characterisation.

4. Publications

4.1 Journals

None.

4.2 Books and other one-time publications

None.

4.3 Oral presentations

Jenke-Kodama, H. *Evolution of bacterial secondary metabolism - New perspectives from systems biology-related approaches*, Saarland University, Germany, Jun 19, 2009

Jenke-Kodama, H. *Phylogenomics of bacterial secondary metabolism*, The University of Tokyo, Japan, July 23, 2009

Jenke-Kodama, H. *Network Reconstruction Approaches for the Cyanobacterial Symbiont Nostoc punctiforme*, The 7th Okazaki Biology Conference, “The Evolution of Symbiotic Systems”NIBB, Shizuoka, Japan, Jan 11-14, 2010

4.4 Posters

None.

5. Intellectual Property Rights and Other Specific Achievements

Nothing to report

6. Meetings and Events

6.1 OIST seminar

Date: November 16, 2009

Venue: OIST IRP conference room

Speakers: Prof. Rolf Müller, Saarland University, Germany

Open Biology Unit

Principal Investigator:
Hiroaki Kitano
Research Theme:
Computational research on systems biology

Abstract

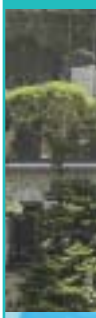
This project aims at development of novel software platform and computational forms of biological knowledge (models and active knowledge-bases) of budding yeast, cancer cells, and developmental/reprogramming pathways that takes full advantage of recent advances in information sciences. The platform developed will be a next generation information infrastructure for biological science.

Payao system has been developed as an OIST flagship software for systems biology network annotation based on Web 2.0 type of approach. A new initiative The Garuda Initiative was launched to further enhance our activity and to solve some of fundamental issues in computational platform in biology.

Concurrently, theoretical studies has been carried out one of which lead to discovery of a specific hierarchical architecture in yeast and human protein networks that is similar to internet router level topology. It has been said that biological networks are like internet as an intuitive analogy. However, this is the first time this analogy is not just an analogy but it is the fact. Statistically similarity discovered shed lights in properties in PPI as internet topology is one of the most investigated network currently exists. Multi-disciplinary research on this direction may uncover yet-to-be-discovered properties of biological networks. At the same time, universality of the topology is insightful for designing distributed engineering systems such as distributed energy networks.

1. Staff

Research Administrator / Secretary: Midori Tanahara



2. Partner Organizations

The Systems Biology Institute

Type of partnership: Research collaboration (no financial transaction)

Name of researchers: Dr. Samik Ghosh, Ms. Yukiko Matsuoka

Research theme: Software platform for systems biology

The University of Manchester

Type of partnership: Research collaboration (no financial transaction)

Name of researchers: Prof. Sophia Ananiadou

Research theme: Integration of Manchester text mining system to Payao

SRI International

Type of partnership: Research collaboration (no financial transaction)

Name of researchers: Dr. Huaiyu Mi

Research theme: Garuda Alliance

3. Activities and Findings

3.1 Standard formation in systems biology information platform

(a) SBGN (systems biology graphical notation)

Standardizing the visual representation is crucial for more efficient and accurate transmission of biological knowledge between different communities in research, education, publishing, and more. When biologists are as familiar with the notation as electronics engineers are familiar with the notation of circuit schematics, they can save the time and effort required to familiarize themselves with different notations, and instead spend more time thinking about the biology being depicted. A global alliance was formed to establish a community-agreed standard for graphical notation of biological interactions. This year, effort was focused on finalizing specification of SBGN Level 1.0. As a result, the first specification was agreed and announced. At the same time, a paper describing SBGN was published in Nature Biotechnology August 2009 issue.

SBGN PD Reference Card

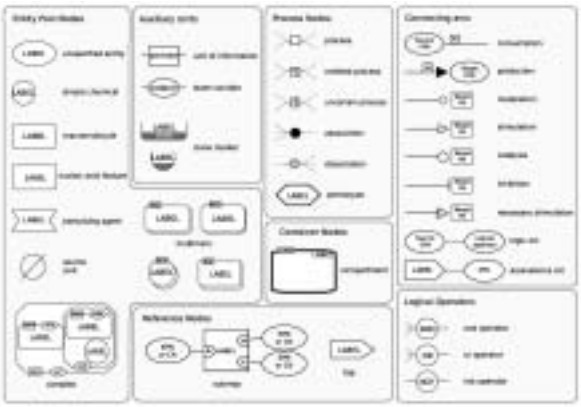
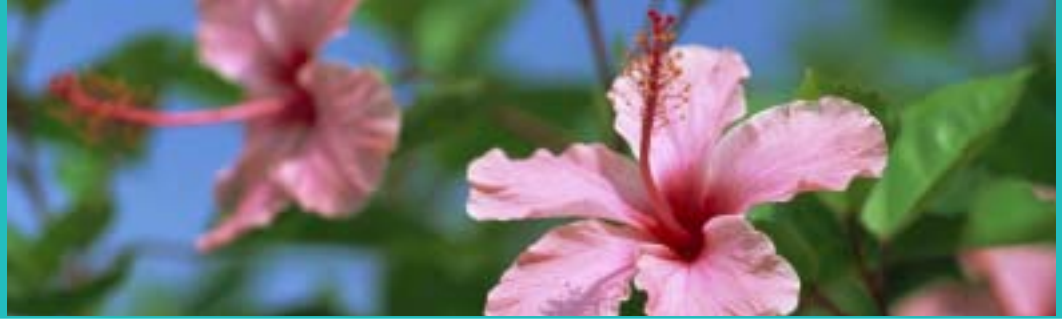


Figure 1: A set of graphical icons defined for SBGN Process Diagram

(b) The Garuda Alliance

Biology is increasingly turning into data-driven science. Numbers of tools and resources are being developed to foster systems biology and healthcare research. However, most tools are developed independent of each other. In most cases, there is no coordination with other groups. One of the



reasons computer graphics (CG) in general, Hollywood SFX studios in particular, made a rapid progress was existence of stable and open common software platforms and standards. Recent efforts on standard formation such as SBML and SBGN contributed to solve some of compatibility problems, yet no sufficient work has been initiated for interoperability and efficient data sharing. Without rectifying current problems of interoperability and sharing, development of solid platform for systems biology and healthcare research cannot be expected. Thus, a new project was launched. The Garuda project that aims at creating the standard and extensible platform for systems biology, healthcare research and service.

The project, hence the name of the platform is named Garuda taken from Hindu mythology that is the bird in which Vishnu rides. Vishnu is one of three Hindu Gods that governs maintenance of the universe and commanded the Churning of the Sea of Milk. (Churning of the *Sea of Milk*: *A Hindu* myth in which gods and demons cooperate to churn the primordial ocean, in order to produce amrita, the elixir of immortality.)

The ultimate goal of the Garuda project is to develop a common software and knowledge platform for healthcare research and services that can be used in both academic and industrial sectors. With the Grauda platform, academic community and industries do not have to re-invent similar tools with only minor variance. Third parties can invest their resources to develop plug-in and software tools without worrying about compatibility and sustainability of the platform. Domain specific applications such as for oncology, cardiovascular diseases, infectious diseases, metabolic syndromes will be developed using Garuda Common Platform, so that interoperability between different disease domains and re-use of expertise can be enhanced.

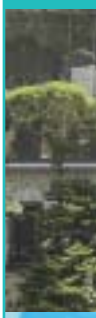
Figure 2: Garuda and Vishnu Projects

The project takes phased approach. Phase-I will focus on ensuring interoperability of currently existing tools, define API and other protocols for interoperability, development of Look&Feel and GUI guidelines, interfacing with genome databases, and development of some of key components missing at present. Milestones for Phase-II and beyond will be decided based on discussions in Phase-I.

In Feb. 2010, the Garuda One workshop was organized at OIST seaside house with participations of researchers around the globe. The second meeting will be held in July at the University of Manchester.

3.2 Open-flow model of knowledge aggregation

Research is making a progress to achieve the open-flow model of knowledge aggregation. In the open flow model, multiple knowledge sources are integrated and annotations and added knowledge from the community is fed back to original databases thereby independent database developer can benefit to join the scheme. At the same time, the community as a whole will benefit



by improved quality of the database due to cross checking and community-based feed back naturally taking place in the open flow model.
In order to achieve this model, a series of improvement were carried out for Payao a community annotation system.

A collaboration with The University of Tokyo and the University of Manchester enabled us to couple Payao and PathTEXT that is their text mining system (Figure 3). While Payao is already compatible to CellDesginer for its file format, visual rendering, and APIs, integrated operation for network reconstruction and annotation can be made systematic (Figure 4). This system has been used in Indian national project Open Source Drug Discovery (OSDD) to reconstruct a comprehensive metabolic network of TB.

Figure 3: Payao and PathTEXT coupling

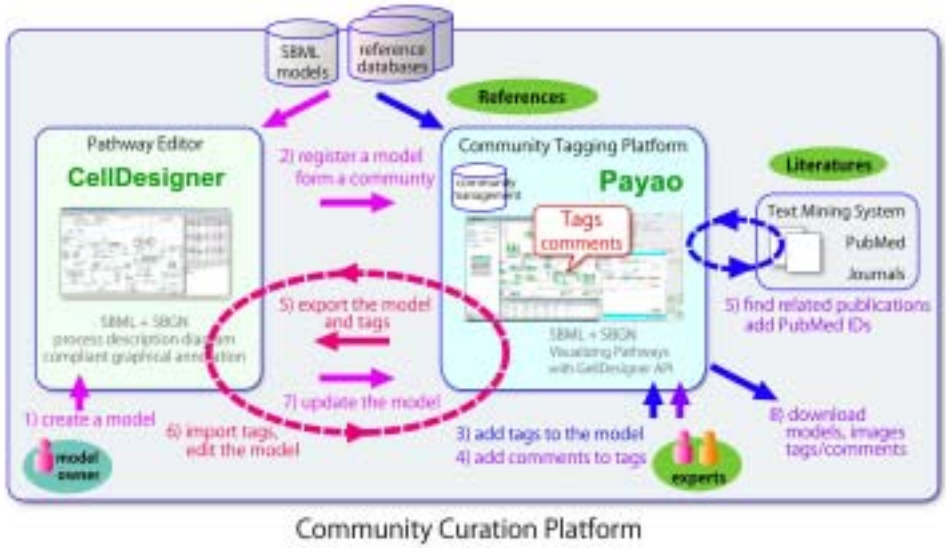


Figure 4: Integrated operation of CellDesigner and Payao

3.3 Biological Network Analysis

A study to uncover structural properties of biological network made a significant progress. Using protein-protein interaction data of budding yeast and human, it was revealed that there are specific hierarchical network topology highly conserved between yeast and human (Figure 5). Interestingly, statistically property of this network is almost identical to that of internet router

topology. Internet router topology is known to be very robust against component failure yet provides high band-width to maximum number of users.

At the same time, the study discovered that molecular targets of most marketed drugs are concentrated on molecular that has interactions between 6 – 35 (Figure 6) that is placed on backbone of the network. In addition, this distribution is different between cancer drugs and non-cancer drugs (Figure 7). Cancer drugs tend to target molecules with larger numbers of interactions that is consistent with the fact cancer drugs incur sever side effects than non-cancer drugs.

Universality of this topology needs to be noticed. If this network topology is one of the optimal structures for large-scale, robust, and high performance systems, further understanding of this network may uncover novel insights on how to design robust high performance systems. Applications are far reaching from wider communication networks to distributed energy systems. Implication of the findings on energy network systems with array of clean energy stations is significant. In the clean energy network, each power generating stations may be unstable due to weather, mechanical failures, and other disruptions. Nevertheless the system as a whole needs to adapt to the situation and maintain to provides stable energy. Studies on how biological systems achieve similar functions may explore a novel network architecture design principles beyond biological sciences.

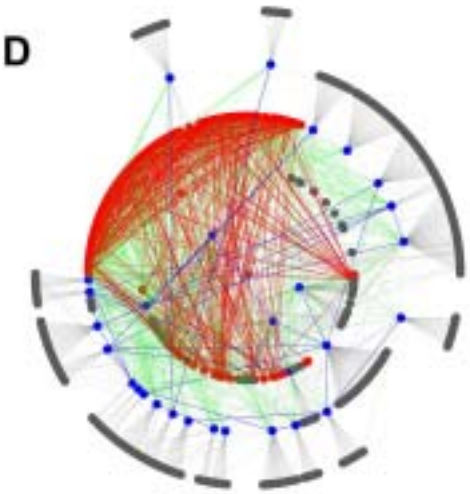


Figure 5: PPI topology of human

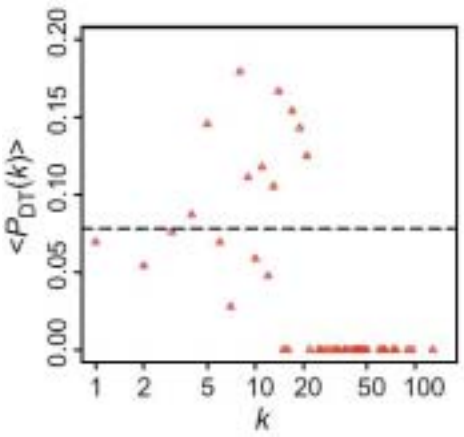
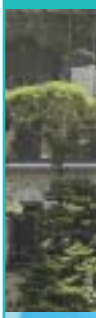


Figure 6: Distribution of molecular targets of FDA approved drugs
(K= number of interactions, Pdt(k)= probability of a molecule being a drug target)



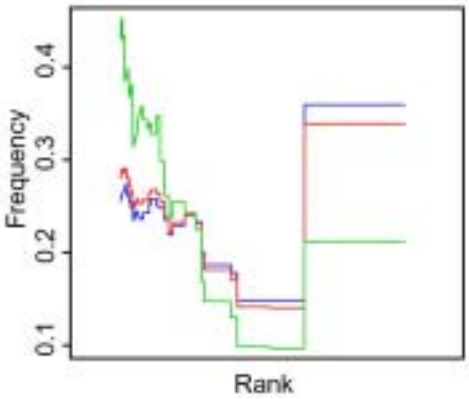


Figure 7: Probability of molecule being drug target
(blue = all non-cancer drugs, green= cancer drugs, red= all drugs)

4. Publications

4.1 Journals

Gehlenborg, N., O'Donoghue, S. I., Baliga, N. S., Goesmann, A., Hibbs, M. A., Hitano, H., Kohlbacher, O., Neuweger, H., Schnider, R., Tenebaum, D., Gavin, A. C. Visualization of omics data for systems biology. *Nature Methods Supplement* 7, S56-S68 (2010).

Hase, T., Tanaka, H., Suzuki, Y., Nakagawa, S. & Kitano, H. Structure of Protein Interaction Networks and Their Implications on Drug Design. *Plos Computational Biology* 5, -, doi:ARTN e1000550DOI 10.1371/journal.pcbi.1000550 (2009).

Kazunari Kaizu, H. M., Hiroaki Kitano. Fragilities Caused by Dosage Imbalance in Regulation of the Budding Yeast Cell Cycle. *PLoS Genetics* 6, e1000919 (2010).

Krantz, M., Ahmadpour, D., Ottosson, L. G., Warringer, J., Waltermann, C., Nordlander, B., Klipp, E., Blomberg, A., Hohmann, S. & Kitano, H. Robustness and fragility in the yeast high osmolarity glycerol (HOG) signal-transduction pathway. *Molecular Systems Biology* 5, -, doi:Artn 281Doi 10.1038/Msb.2009.36 (2009).

Le Novere, N., Hucka, M., Mi, H. Y., Moodie, S., Schreiber, F., Sorokin, A., Demir, E., Wegner, K., Aladjem, M. I., Wimalaratne, S. M., Bergman, F. T., Gauges, R., Ghazal, P., Kawaji, H., Li, L., Matsuoka, Y., Villeger, A., Boyd, S. E., Calzone, L., Courtot, M., Dogrusoz, U., Freeman, T. C., Funahashi, A., Ghosh, S., Jouraku, A., Kim, S., Kolpakov, F., Luna, A., Sahle, S., Schmidt, E., Watterson, S., Wu, G. M., Goryanin, I., Kell, D. B., Sander, C., Sauro, H., Snoep, J. L., Kohn, K. & Kitano, H. The Systems Biology Graphical Notation. *Nat Biotechnol* 27, 735-741, doi:Doi 10.1038/Nbt.1558 (2009).

Matsuoka, Y., Ghosh, S. & Kitano, H. Consistent design schematics for biological systems: standardization of representation in biological engineering. *Journal of the Royal Society Interface* 6, -, doi:DOI 10.1098/rsif.2009.0046.focus (2009).

4.2 Book(s) and other one-time publications

北野宏明. *生命システムの設計原理としてのロバストネス. 現代生物科学入門 第8巻* システムバイオロジー (岩波書店刊, 2010). Biological robustness as an organizational principle of life. *Intro to Modern Biology*, vol.8 Systems biology (pub by Iwanamishoten, 2010)

4.3 Oral presentations

Kitano, H. *A robust planet*, 14th European Congress on Biotechnology, Palau de Congressos de Catalunya, Barcelona, Spain, Sep. 15, 2009

Kitano, H. *Introduction and status on CellDesigner*, Protein-Protein Interaction Annotation Jamboree, Hallsnas Konferens & Affarsklubb, Gothenburg, Sweden, September 24, 2009

Kitano, H. *Computational models in biology*, Winter School on Computational Modeling in Biology -Data and model sharing by SBML and insilicoML-, OIST, Okinawa, Japan, December 15, 2009

Kitano, H. *Biological Robustness*, The International Forum on Ecosystem Adaptability. Robustness and stability of organisms and ecosystems. Sendai International Center, Sendai, Miyagai, Japan, Feb. 21, 2010

Kitano, H. *The Garuda Alliance*, Visions and Milestones. Garuda One Workshop, OIST, Okinawa, Japan, Feburary 26, 2010

Kitano, H. *Solving global issues*, Eisai E-elite Japan Program, Cross-wave Higashi Nakano, Tokyo, Japan, Feburary 26, 2010

Kitano, H. *Systems drug discovery and computational platform*, University Research Priority Program (URPP) Systems Biology / Functional Genomics Retreat, University of Zurich, Switzerland, Feburary 28, 2010-March 2, 2010

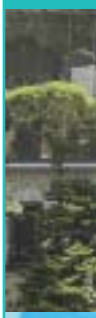
Kitano, H. *Biochemical Pathways*, EMBO Workshop - Visualizing Biological Data (VizBi), EMBL Heidelberg, Germany, March 4, 2010

Kitano, H. *Systems drug discovery*, robustness theory, and computational platform, The first China-Japan Workshop on Systems Biology - Systems Biology and Complex Diseases -, Shanghai, China, March 13-15, 2010

Kitano, H. *Biological robustness and drug discovery*, Systems Biology of Microorganisms Conference, Institut Pasteur in Paris, France, March 22, 2010

4.4 Posters

Matsuoka, Y., Ghosh, S., Kikuchi, N., Saere, R., Kemper, B., Okazaki, N., Ananiadou, S., Tsujii, J., Kitano, H. *Community Platform for Pathway Model Building*, The 10th International Conference on Systems Biology (ICSB2009), Stanford University, California, 2009



5. Intellectual Property Rights and Other Specific Achievements

Nothing to be reported

6. Meetings and Events

6.1 Co-sponsored Workshop: Winter School on Computational Modeling in Biology -Data and model sharing by SBML and insilicoML

Date: December 14-17, 2009

Venue: Seaside House, OIST

Co-organizer: Prof. Taishin Nomura (Osaka University)

Co-sponsors: Global COE program “an in silico Medicine-oriented Worldwide Open Platform”
The Center for Advanced Medical Engineering and Informatics,Osaka University
Systems Biology Institute

Speakers: Yoshiyuki Asai (Osaka University)
Akira Funahashi (Keio University)
Michael Hucka (CDS. California Institute of Technology)
Hiroaki Kitano (OIST & SBI)
Pradeep Kumar S (Gwangju Institute of Science and Technology Department of Life Sciences)
Yoshihisa Kurachi (Osaka University)
Yukiko Matsuoka (JST ERATO Infection-induced host responses)
Huaiyu Mi (SRI International Evolutionary Systems Biology Group. Artificial Intelligence Center)
Taishin Nomura (Osaka University)
Sven Sahle (University of Heidelberg BIOQUANT BQ0018)

6.2 Workshop: Garuda One

Date: February 22-24, 2010

Venue: Seaside House, OIST

Co-sponsor: Osaka University global COE

Speakers: Sophia Ananiadou (The University of Manchester)
Yoshiyuki Asai (Osaka University)
Sarah Boyd (La Trobe university)
Akira Funahashi (Keio University)
Samik Ghosh (Systems Biology Institute)
Brian Kemper (The University of Tokyo)
Hiroaki Kitano (OIST & SBI)
Yukiko Matsuoka (Systems Biology Institute & JST)
Huaiyu Mi (SRI International)
Taishin Nomura (Osaka University)
Natalia Polouliakh (Sony CSL)
Junichi Tsujii (The University of Tokyo)

Information Processing Biology Unit

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

Abstract

One of our unit interests is to understand how cells/neurons detect external information and transmit it to the inside of the cell. For the last 20 years, ligand-induced dimerization has widely been thought to be a property common to the transmembrane signaling mechanism of all known growth factor and cytokine receptors. In previous years, however, we found all the members of the EGF/ErbB receptor family have preformed, yet inactive, homo- and heterodimeric structures between the family members prior to ligand binding. This year we have further solidified the discovery by showing that the carboxy-terminal tails of the receptors play a pivotal role in the spontaneous formation of the receptors' dimeric structures through electrostatic interaction with the intracellular kinase domain. Moreover, we have determined that in living cells, cell-surface receptors for neurotrophic factors also exist as homo- and heterodimers prior to ligand binding.

We are also interested in an understanding of how the nervous system processes external information as cellular networks to regulate animal behaviors including learning and memory. Animals can survive in a narrow pH range by monitoring the pH in the environment and their body fluids. However, little is known about how animals monitor alkaline pH in the environment. In this study, we have employed the nematode *Caenorhabditis elegans (C. elegans)* as a model system. While *C. elegans* is attracted to low-alkaline pH, it is repelled from high-alkaline pH. We have found that GCY-14, a membrane receptor-type guanylyl cyclase, is expressed in the cilia of the ASEL amphid sensory neuron, and plays as a sensor molecule for the mild alkalinity of the environment. Furthermore, it has also been elucidated that OSM-9 (TRPV) channels on ASH sensory neurons play a crucial role in the sensation of strong alkalinity as a noxious stimulus. This year we have also studied learning and memory in *C. elegans,* and have found that the animal can learn and form long-term associative memory.

These results provide us insights into an understanding of a molecular mechanism underlying information transfer from the outside of cells/neurons 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 cancers and mental diseases.

1. Staff

Researchers: Aini Suzana Adenan, Hisayuki Amano, Takashi Murayama, Hideki Nagahama, Jianying Shen, Zacharie Taoufiq, and Qingsheng Yu (Until Nov. 10, 2009)

Technical Staff: Mayuki Fujiwara (Until April 30, 2009), Rashmi Katiyar (Until Oct. 31, 2009), Hiraku Miyagi, Saori Nishijima (From June 01, 2009), Shigeki Sanehisa, and Toshihiro Sassa (From April 01, 2009)

Research Administrator : Yoko Kudeken

2. Partner Organizations

Keio University School of Medicine

Type of partnership: Joint

Name of principal researchers: Takanori Moriki

Name of researchers: Atsuko Igari, Yasuo Ikeda, and Mitsuru Murata

Research theme: Application of lambda phage display

University of the Ryukyus School of Medicine

Type of partnership: Joint

Name of principal researcher: Ken-ichi Kariya

Research theme: Total internal reflectance fluorescence microscopic analysis of membrane proteins

Niigata University School of Medicine

Type of partnership: Joint

Name of principal researcher: Michihiro Igarashi

Research theme: Studies on roles of axonal growth cone-specific proteins in rat and *C. elegans* nervous systems

3. Activities and Findings

3.1. Project Aims

All forms of life are separated from non-life by cell membranes, and all cells/neurons have cell-surface receptor proteins that span the membranes in order to transmit 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 cells/neurons 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 sensory neurons, 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/neurons

3.2.1.1. Background. The bacterial cell-surface receptor Tar recognizes aspartate molecules in the environment, and brings bacterial cells toward the higher concentration of the attractant as a nutrient, or the lower concentration 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 its ligands, which bind to the extracellular domain of the receptor and lock/freeze the rotational/twist movement of the receptor's transmembrane domains (Maruyama *et al.,* 1995. *J. Mol. Biol.* 253, 530). This locking/freezing of the rotation/twist at one position by the attractant seems to inhibit the associated histidine kinase Che A, while the locking/freezing at another position by the repellent seems to activate the kinase activity (rotation/twist model).

We have also analyzed a molecular mechanism underlying the activation of the human epidermal growth factor receptor (EGFR) family of cell-surface receptor tyrosine kinases, also known as ErbB or HER. These EGF/ErbB receptors play a pivotal role in the development of organisms, and are frequently implicated in human cancers. Furthermore, these receptors also regulate neural activities, and mutations of these receptor genes are frequently associated with mental diseases. 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 long) 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 the preferred heterodimerization partner for other ErbB receptors. ErbB3, on the other hand, must associate with an ErbB family member that has an active tyrosine kinase in order to respond to its own ligand neuregulin (NRG).

Ligand-induced dimerization has widely been thought 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 this 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 exists as a preformed dimer on the cell surface. We also analyzed the receptor dimerization by inserting cysteine residues at strategic positions about the α-helix axis of the

extracellular juxtamembrane region. The mutant receptors spontaneously formed disulfide bridges and transformed NIH3T3 cells in the absence of ligand, depending upon the positions of the cysteine residues inserted. Kinetic analysis of the disulfide bonding indicates that ligand binding induces flexible rotation or twist of the juxtamembrane region of the receptor in the plane parallel with the lipid bilayer. The binding of an ATP competitor to the intracellular kinase domain also induced similar flexible rotation/twist of the juxtamembrane region. All the disulfide-bonded dimers had flexible ligand-binding domains with the same biphasic affinities for the ligand as the wild type. Based on these results, we have proposed an alternative 'rotation/twist' model for the molecular mechanism of the EGF receptor activation, in which ligand binding to the flexible extracellular domains of the receptor dimer induces rotation/twist of the juxtamembrane regions, hence the transmembrane domains, and rearrange the kinase domains for the receptor activation. Indeed, this rotation/twist model (Figure 1; Moriki *et al.,* 2001. *J. Mol. Biol.* 311, 1011) is consistent with the homodimeric structure of the receptor kinase, transmembrane and unactivated extracellular domains that have recently been determined by others.

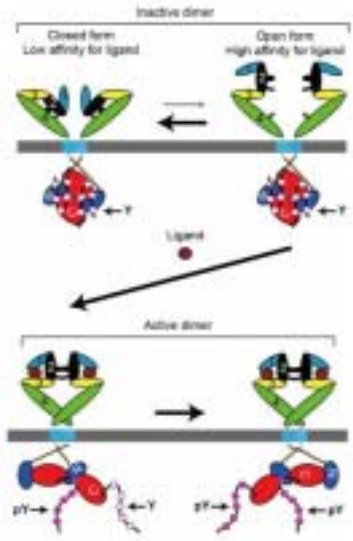


Figure 1: 'Rotation/twist' model for the mechanism of the ErbB receptor activation.

To support the 'rotation/twist' model, we have recently determined preformed homo- and heterodimeric structures of EGFR and ErbB2 at physiological expression levels (~10^4 molecules per cell), using fluorescence microscopy, fluorescence resonance energy transfer (FRET) and fluorescence cross-correlation spectroscopy (FCCS) (Figure 2; Liu *et al.,* 2007. *Biophys. J.* 93, 684). When EGFR and ErbB2 fused with a fluorescent protein (FP) were expressed on the cell surface of Chinese hamster ovary cells at physiological expression 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 ~5 x 10^6 molecules per cell.

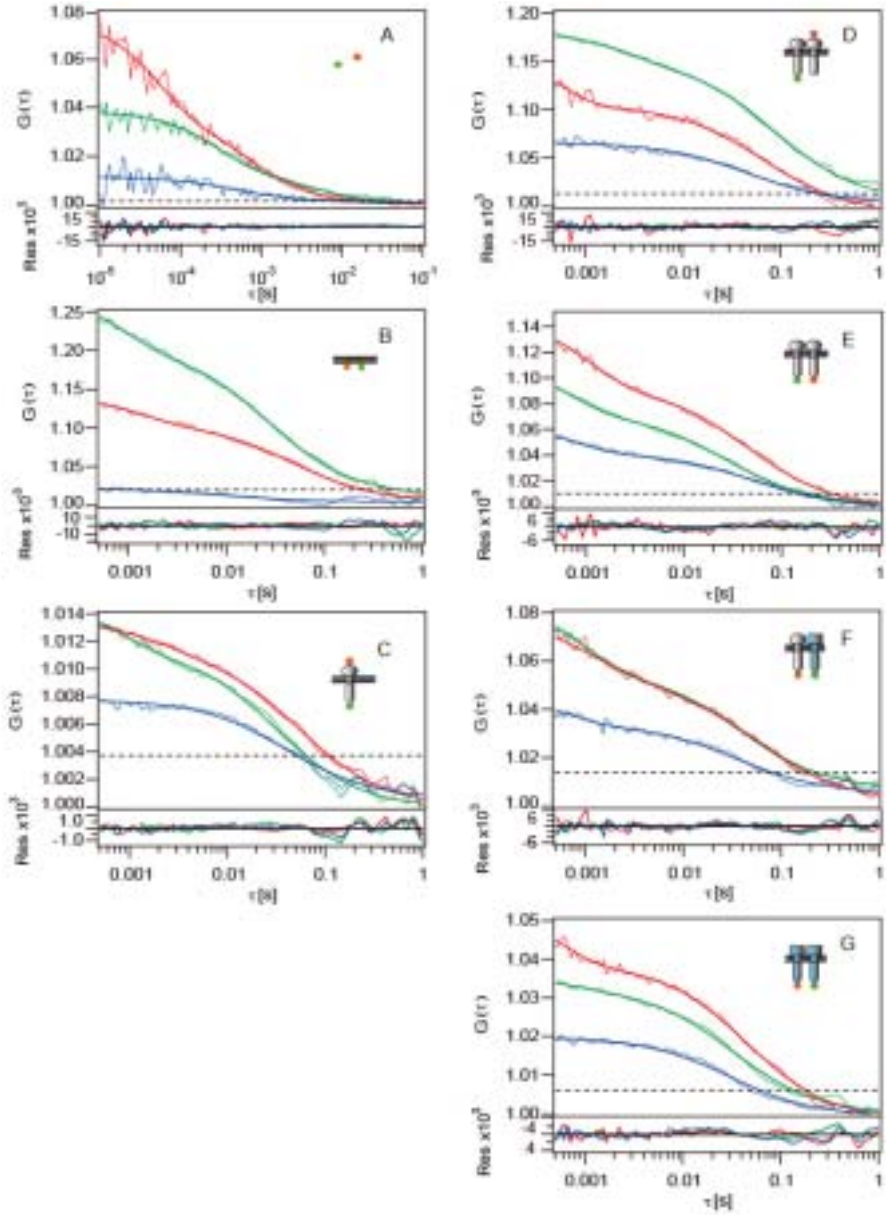


Figure 2: Auto- and cross-correlation curves measured from CHO cells expressing chimeric fluorescent proteins. Autocorrelation curves (dashed curves) of GFP (green) and mRFP (red), and cross-correlation curves (blue) between the two are shown with their fits (solid curves). (A) Cell coexpressing cytosolic free GFP and mRFP (negative control). (B) PMT-GFP/-mRFP (negative control). No cross-correlation, hence no interaction, between the two fluorescent proteins was observed. (C) Cell expressing mRFP-EGFR-GFP (positive control), in which mRFP and GFP were fused in tandem to N- and C-termini of EGFR, respectively. (D) Cell coexpressing EGFR-GFP /mRFP-EGFR. Similar auto-, and cross-correlation curves were also observed for cells co-expressing EGFR-GFP/-mRFP (E). (F) Cell co-expressing ErbB2-GFP/EGFR-mRFP. (G) Cell co-expressing ErbB2-GFP/-mRFP.

Furthermore, we have been analyzing 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, yet inactive, homo- and heterodimeric structures in the absence of bound ligand (Figure 3; Tao & Maruyama, 2008. *J. Cell Sci.* 121, 3207). The ligand-independent dimerization of the EGF/ErbB receptors occurs in the endoplasmic reticulum (ER) before newly synthesized receptor molecules reach the cell surface. 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. These results indicate that all the EGF/ErbB receptors have homo- and heterodimeric structures before ligand binding, and are consistent with the 'rotation/twist' model. The ErbB receptors exist as a dimer on the cell surface, mainly through interaction between the intracellular kinase domains and C-terminal tails. The receptor dimers have flexible extracellular domains, and presumably can take two major conformations with low and high affinities for ligand. Ligand binding to the high affinity receptor stabilizes the extracellular domains, induces approximately 140-degree rotation or twist of the transmembrane domains about its helix axis in parallel with the cell membrane, dissociate the symmetric back-to-back kinase domains, and then rearrange the kinase domains to take head-to-tail asymmetric configuration for the receptor activation (Figure 1).

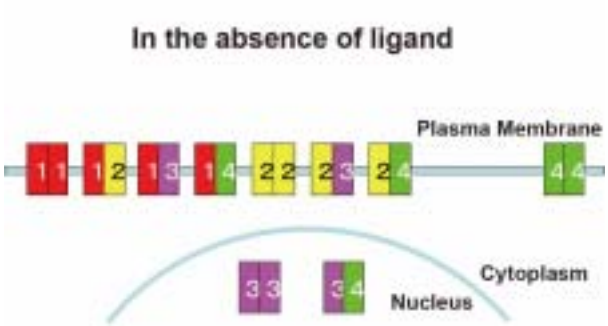


Figure 3. Preformed homo- and heterodimeric structures of the ErbB receptor family and their subcellular localization

Thus, different cell-surface receptors, bacterial Tar and human EGF/ErbB receptors, seem to be similarly regulated by their ligands in order to transmit the external information to the inside of the cell. Namely, their ligand binding regulates the rotation/twist of the receptor's transmembrane domain in parallel with the plane of the plasma membrane. Therefore, we have been continuing to test the "rotation/twist" model for the molecular mechanism of the activation of other cell-surface receptors including Tar, EGF/ErbB receptors and neurotrophin receptors as described below. Indeed, the neurotrophin receptor TrkA is present as a preformed, yet inactive, dimer in the living cells. We have also previously found that the intracellular domain of the EGF/ErbB receptors plays a crucial role for the spontaneous formation of the receptor dimers (Tao & Maruyama, 2008. *J. Cell Sci.* 121, 3207). This year, therefore, we have analyzed amino-acid residues in the intracellular domain, which are involved in the spontaneous formation of preformed dimers, and have found that negatively charged amino-acid residues of the receptor carboxyl termini stabilize the preformed receptor dimers through their electrostatic interaction with the back of the kinase domains.

3.2.1.2. Crystal structure of Tar with a repellent.

The three-dimensional structures of the extracellular ligand-binding domain of the bacterial

aspartate receptor Tar with or without bound ligands have been determined, and only a small (~2 Å) vertical shift of TM2 from each other has been detected by others. This indicates that the receptor structures with and without the ligand aspartate are the same each other, consistent with the 'rotation/twist' model for the mechanism of the Tar activity regulation by aspartate (Maruyama *et al.,* 1995. *J. Mol. Biol.* 253, 530). Furthermore, the 'rotation/twist' model predicts that another cofactor for Tar, nickel as a repellent in *E. coli* chemotaxis, stabilizes TM2 in a distinct rotational orientation against TM1. Namely, the repellent nickel could induce the rotation/twist of TM2 against TM1 in parallel with the plane of the cytoplasmic membrane. To test this hypothesis, we are currently trying to determine the three dimensional structure of the extracellular domain of Tar in the presence of bound nickel

3.2.1.3. EGFR domains required for its spontaneous dimerization.

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 are trying to identify domains and amino-acid residues required for the dimerization. When the amino acid residue Glu712 shown in Figure 4 was mutated to lysine, for instance, the kinase was fully activated at the similar level activated by EGF (Figure 5).

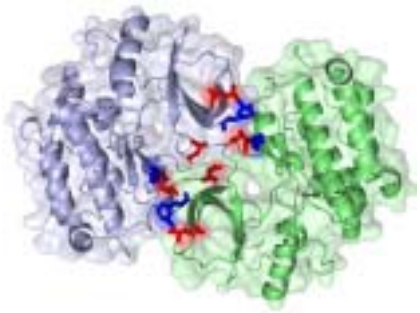


Figure 4. Symmetric structure of the EGFR kinase dimer. Amino acid residues positively and negatively charged, which reside in the symmetric dimer interface and were mutated in this study, are shown in blue and red, respectively. Two active sites of the kinase dimer are also shown in pink.

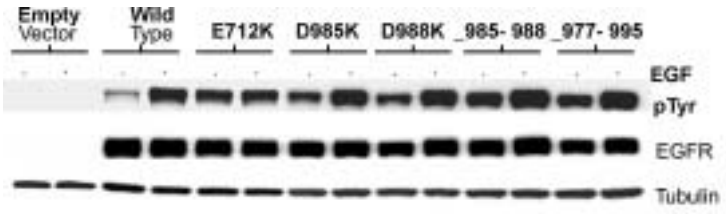


Figure 5. Ligand-independent activation of EGFR mutants with a point or deletion mutation. Amino acid residues residing in the interface of the symmetric dimeric structure were replaced or deleted as shown. Note that all the mutant receptors were activated prior to EGF binding.

3.2.1.4. Preformed, yet inactive, dimeric structures of receptors for neurotrophic factors.

The nerve growth factor (NGF) family of neurotrophins, which include NGF, brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and NT-4/5, plays a central role in the development, growth, and maintenance of the nervous system. These neurotrophins interact with two types of cell surface receptors, the tropomyosin-related kinase (Trk) family of tyrosine kinase receptors, TrkA, TrkB and TrkC, and the p75 neurotrophin receptor ($p75^{NTR}$). Trk receptor subtypes bind mature neurotrophins with different specificities: TrkA and TrkC preferentially bind NGF and NT-3, respectively, while TrkB mainly binds BDNF and NT-4. The $p75^{NTR}$ binds all mature neurotrophins with approximately equal low affinity and has been demonstrated to bind the proneurotrophins with high affinity. These receptors are present on the same cell, and modulate the responses of neurons to neurotrophins. Two classes of NGF-binding sites are present on the surface of respective neurons that are distinguished by greater than 100-fold differences in their equilibrium binding constants and have been termed the 'high affinity' (*Kd* ~10^{-11} M) and 'low affinity' (*Kd* ~10^{-9} M) receptor-binding sites. Cells expressing $p75^{NTR}$ by itself also exhibit only low affinity NGF binding (10^{-9} M) sites. When TrkA and $p75^{NTR}$ receptors are co-expressed, the association rate of NGF and TrkA is dramatically increased, creating a new site consistent with the NGF high affinity binding site (*Kd* ~10^{-11} M).

NGF-induced activation of TrkA is thought to be mediated by receptor dimerization. Cross-linking studies revealed the formation of TrkA homodimers upon NGF binding to PC12 cells and fibroblasts ectopically expressing TrkA receptors. Because NGF, as well as other neurotrophins, exist in solution as stable homodimers, it is thought that a single NGF dimer bridges two TrkA monomers. This model was supported by the symmetric crystal structure of the NGF complex with the extracellular IgG domain of TrkA. However, NGF mimetics and Fab fragments of antibody against TrkA, which are structurally incapable of dimerizing the receptor, could activate TrkA. Furthermore, an asymmetric chimeric NGF/Neurotrophin-4 heterodimer also was found to activate TrkA receptor. TrkA receptor molecules expressed in *Xenopus Laevis* oocytes were found to be present as oligomers in the plasma membrane in the absence of NGF. These previous results led us to examine whether neurotrophin receptors exist as monomers or preformed dimers on the cell surface prior to ligand binding.

In the present study, we have analyzed the structures of TrkA, TrkB and p75 receptors for NGF, BDNF and NT-4, and all neurotrophins, respectively, by bimolecular fluorescence complementation (BiFC) and luciferase fragment complementation assays. These analyses demonstrated that before ligand binding, the receptors exist as homo- and heterodimers in living cells (Figure 6). Furthermore, the intracellular domains of the receptors are necessary for interaction between two monomers. Using Brefeldin A, which disassembles the Golgi apparatus and blocks anterograde transport of the receptors from endoplasmic reticulum (ER) to Golgi, it was found the preformed dimers were formed in ER before reaching Golgi. This work provides new insights into understanding of transmembrane signaling by receptors for neurotrophic factors.

Figure 6: TrkA exists as a homodimer on the cell surface when analyzed by luciferase fragment complementation assay. TrkA-Nluc and TrkA-Cluc constructs were co-expressed in HEK 293 cells, and luciferase activity was assayed. Relative enzyme activity is shown on the vertical axis. As positive controls, luciferase activities of cells expressing the full-length luciferase (pGL3), and cells co-expressing EGFR-Nluc and EGFR-Cluc are also shown.

3.2.2. Information processing by the nervous system

3.2.2.1. Alkalinity sensation in *C. elegans.*

3.2.2.1.1. Background.

Like other animals, the nematode *Caenorhabditis elegans* detects various environmental cues such as tastes and odors mainly through its amphid sensilla. The amphids are the largest chemosensory organs, and each amphid includes 12 sensory neurons (ADF, ADL, AFD, ASE, ASG, ASH, ASI, ASJ, ASK, AWA, AWB, and AWC) with ciliated dendrites as well as one sheath and one socket cell. Apart from AFD, the sensory dendrites of 11 neurons penetrate the sheath-cell ending, and the cilia of eight of these neurons, except those of AWA, AWB, and AWC, extend into the doughnut-like pore created by the socket cell and are directly exposed to the external medium. These amphid neurons have roles in chemotaxis, thermotaxis, mechanosensation, osmotaxis, and dauer pheromone sensation.

Chemotaxis of *C. elegans* to cations, anions, cyclic nucleotides, and amino acids was first described, and this water-soluble attractant list has been extended further. The sensory neurons required for chemosensory responses have been identified by laser microsurgery of identified neurons. Killing one pair of the ASE sensory neurons resulted in reduced chemotaxis to water-soluble attractants including Na^+, Cl^-, cAMP, biotin, lysine, and serotonin. Amphid neurons belong to left-right pairs that are symmetrical at the structural level, but gene expression studies revealed asymmetry in the expression of transmembrane guanylyl cyclases in the left and right ASE neurons. For example, three transmembrane guanylyl cyclase genes, *gcy-5, gcy-6,* and *gcy-7*, are expressed only in ASE neurons, and each is expressed exclusively either in ASEL or ASER. A cyclic nucleotide-gated (CNG) cation channel encoded by the t*ax-4* and *tax-2* genes is essential for the function of many sensory neurons including ASE, and TAX-4 and TAX-2 are expressed in a subset of sensory neurons and localized to sensory cilia. The *tax-4* gene encodes a subunit that can form a channel on its own, while *tax-2* encodes a subunit that enhances TAX-4 activity. The *C. elegans* genome encodes 34 guanylyl cyclases that could produce cGMP to activate TAX-4/TAX-2 neurons.

Animals can survive in a narrow pH range, and continuously monitor the pH of environment and body fluids. However, little is known about how animals monitor alkaline pH in the environment, although channels that can sense intracellular alkaline pH have recently been found.

Environmental mild alkalinity is one of attractive cues for *C. elegans*. Between pH 2.8 to pH 10.4, *C. elegans* prefers higher alkaline pH ranges, while the animal avoids higher alkalinity than pH ~11.0 and acidic conditions lower than pH ~4.0. However, neurons and cell-surface molecules that sense the low and high alkaline pH ranges have not been elucidated in *C. elegans*. Neural networks responsible for worm's attraction to mild alkalinity and aversion from strong alkalinity can also be efficiently analyzed since the wiring diagrams of all neurons have been reconstructed from electron micrographs of serial thin sections of *C. elegans*.

3.2.2.1.2. Mild-alkalinity sensation in *C. elegans.*

To investigate cellular and molecular bases for *C. elegans* chemotaxis toward low-alkaline pH, we devised an agar plate assay with a linear pH gradient. Along the pH gradient from pH 6.8 to pH 8.5, wild-type worms were attracted to higher pH regions, whereas *che-1* mutants defective in chemosensory ASE neurons were not. To search for a molecular sensor for the low-alkaline pH, we have performed a series of RNAi knock down of genes encoding various channels, cell-surface receptors and guanylyl cyclases in conjunction with analysis of chemotaxis mutants previously known. Among the genes analyzed, *tax-2, tax-4* and *gcy-14* have been found to be involved in the *C. elegans* chemoattraction to the low-alkaline pH. By imaging of membrane voltage changes using Mermaid, furthermore, we found that a pair of ASE neurons sense alkaline pH up to pH 10.0. ASE-left (ASEL) is activated by pH up-shift (Figure 7A), and ASE-right (ASER) is activated and inactivated by pH down- and up-shift, respectively. GFP-tagged GCY-14 was localized to ASEL sensory cilia, and in the *gcy-14* mutant, ASEL did not respond to pH up-shift (Figure 7A). While ASI and ASK sensory neurons did not respond to pH up-shift, furthermore, ASI and ASK neurons ectopically expressing GCY-14 were activated by pH up-shift from pH 7.0 to pH10.0 (Figure 7B). This demonstrates that GCY-14 is sufficient for the alkaline pH sensation. Indeed, point mutations of conserved cysteines on the extracellular domain of GCY-14 could not rescue the chemotaxis defect of *gcy-14* mutations (Figures 7C&D). These results indicate that the transmembrane guanylyl cyclase GCY-14 acts as an alkaline pH sensor, and an increased concentration of cGMP opens the cGMP-gated cation channel TAX-2/TAX-4 for the activation of ASEL (Figure 7E).

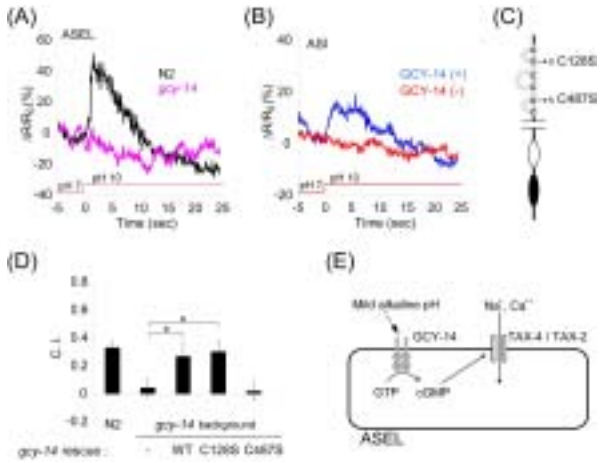


Figure 7. (A) Voltage transients in the ASEL neuron of wild-type and *gcy-14* mutants in response to pH up-shift from pH 7.0 to pH 10.0. **(B)** Voltage transients in ASI neurons of wild-type worms with or without GCY-14 ectopically expressed. **(C)** Diagram of GCY-14 domains. Three putative disulfide bonds in the extracellular domain are shown by 'C-C'. **(D)** Chemotaxis index along a linear alkaline pH gradient from pH 6.8 to pH 8.5. Chemotaxis defect of *gcy-14* was rescued by wild-type GCY-14 and C128S mutated GCY-14, but not by GCY-14 with C487S. **(E)** Model for the molecular mechanism of ASEL activation by environmental alkaline pH.

3.2.2.1.3. Strong-alkalinity sensation in *C. elegans.*

During the work on mild-alkalinity sensation described above, we have found that *C. elegans* avoids higher pH than ~11.0. To elucidate a molecular mechanism underlying this nociception in *C. elegans*, we designed the similar plate assay to that for the mild-alkaline chemotaxis, in which a pH gradient ranging from pH 7.0 to pH 11.5 was established by spreading 3 M KOH along a 1.5% agar plate as well as assay plates consisting of two quadrants each of 1.5% agar of pH 7.0 and pH 11.2. When analyzed by using these assay plates, we found that the following various chemotaxis mutants failed to avoid the high-alkaline pH range. (1) The *che-2* mutants defective in chemotaxis, dauer formation and longevity. The *che-2* gene encodes a novel protein, which contains G-protein beta-like WD-40 repeat, and is expressed in the ciliated sensory neurons and some head neurons. (2) The *osm-3* gene encodes a kinesin-2 family member, and is required for intraflagellar transport (IFT) for formation of the distal segment of amphid channel cilia. (3) The *dyf-3* gene encodes an ortholog of zebrafish cluap1 and human clusterin-associated protein 1 (CLUAP1), and is required for normal assembly of middle and distal ciliary segments, and is expressed in amphid and IL2 sensory neurons. The *dyf-3* mutants show truncated sensor cilia. (4) The *che-3* gene encodes a dynein heavy chain 1b isoform that affects the establishment and maintenance of the structural integrity of sensory cilia, and that also has a role in IFT, and is expressed in ciliated sensory neurons. (5) The *daf-3* gene encodes a co-SMAD protein, which functions as a transcriptional regulator, and is expressed in many tissues that undergo remodeling during dauer development, including the nervous system and gut. (6) The *che-11* gene encodes a novel protein, and is required for normal synthesis of sensory cilia (via IFT) in sensory neurons, osmotic avoidance and dauer formation. All of these genes except for *daf-3* are required for either IFT (*che-2, osm-3, che-3* and *che-11*) or for normal formation of sensory cilia (*dyf-3*). Furthermore, *daf-3* is a transcriptional regulator in the pathway mediated by TGFß receptor signaling in the

nervous system. All of these results indicate that the high-alkaline pH is sensed by ciliated sensory neuron(s) in the amphid.

Since *osm-9* mutants also failed to avoid the high-alkaline pH as shown in Figure 8, we tried to find sensory neurons that sense the high-alkaline pH by specifically expressing the *osm-9* gene in various sensory neurons including ASH, ADL and ASI. The *osm-9* gene encodes a V-type member of the transient receptor potential (TRP) cation channel. When *osm-9* was expressed in ASH by placing the gene under the promoter of *sra-6*, the strong-alkalinity sensation was almost completely recovered, indicating that the ASH sensory neurons are involved in the sensation. Indeed, the calcium concentration of the ASH sensory neuron was increased when stimulated by KOH solution (pH 11.0) as shown in Figure 9. Furthermore, this calcium concentration increase presumably through calcium influx was not observed in *osm-9* mutants. This is consistent with the *osm-9* mutants are insensitive to the high-alkaline pH (Figure 8). These results indicate that OSM-9 in the amphid sensory neuron ASH plays a vital role in the sensation of the strong-alkalinity in *C. elegans.*

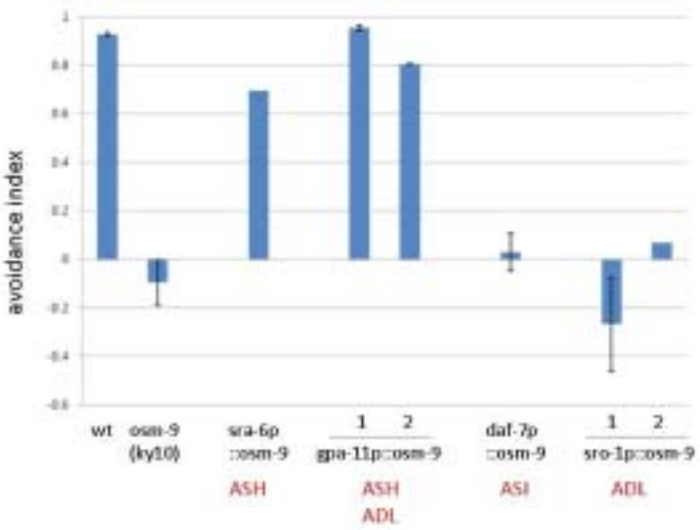


Figure 8: OSM-9 channels in ASH play a crucial role in strong-alkalinity sensation.
OSM-9 channel proteins were specifically expressed in three distinct sensory neurons, ASH, ADL and/or ASI by using the cell-specific promoter constructs. Note that the *osm-9* defect was almost completely rescued by expressing OSM-9 only in ASH.

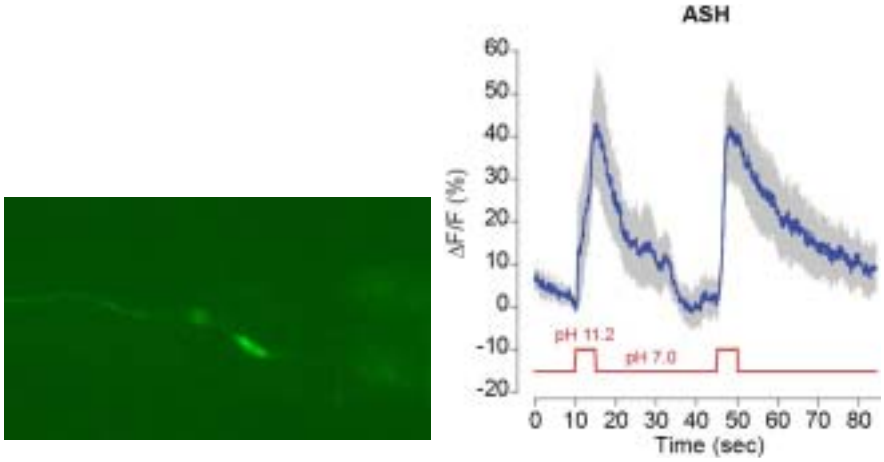


Figure 9. Calcium imaging of the ASH sensory neuron when stimulated by high-alkaline pH.

The imaging was carried out by expressing the calcium-sensitive fluorescent protein Chameleon in the wild-type N2 worms (Left). When the worms were stimulated by shifting pH from 7.5 to 11.0 as shown by red line, fluorescence intensity of the ASH sensory neuron was increased (Right).

3.2.2.2. Associative learning and memory in *C. elegans.*

3.2.2.2.1. Background.

Traditionally, learning has been divided into two categories: non-associative and associative. Non-associative learning occurs when an individual is exposed to a single type of stimulus and behavior is changed as a result of that exposure. Examples of non-associative learning include habituation and sensitization. Associative learning occurs when animals learn to link a stimulus or behavior with a second temporally associated stimulus. Whereas learning is a change in behavior as a result of experience, memory is the ability to store and recall those changes to behavior. Memory can last in various phases from as short as seconds as is found in short-term memory or as long as hours to a lifetime as is found in long-term memory. The cellular and molecular mechanisms behind these phases of memory seem to be distinct. For example, long-term but not short-term memory cannot be formed by treatment with inhibitors of transcription and translation.

C. elegans is an excellent model organism for the study of learning and memory for a number of reasons. For instance, the *C. elegans* hermaphrodite nervous system consists of only 302 neurons. The neural circuits of these invariant neurons have completely been reconstructed from serial thin sections using electron microscopy. The body is transparent throughout the life so that neural activities can be observed using Ca^{2+}- and voltage-sensitive fluorescent proteins in living animals.

Associative learning in *C. elegans* has first been suggested from the finding that worms return to their temperature of cultivation if they had food at that temperature. Most of learning paradigms in *C. elegans* are based on pairing chemical cues or cultivation temperature with food or starvation. Conditioning worms with NaCl in the absence of food leads to a significant reduction in chemotaxis, compared to conditioning in the presence of food. Similar observations have been made in olfactory paradigms. *C. elegans* can also learn to avoid odors associated with infection by

pathogenic bacteria, a behavior analogous to mammalian conditioned taste aversion. Serotonin production by ADF sensory neurons is required for this form of learning; pathogenic infection leads to upregulation of serotonin in ADF. Mutant screens for animals defective in learning have resulted in the identification of *lrn-1* and *lrn-2*, which affect both taste learning and olfactory learning. Screens based on these complex behaviors should be useful in the identification of many new genes. In some cases, the *C. elegans* learning paradigms meet strict criteria for associative learning set forth in the psychology literature. More often, however, *C. elegans* learning paradigms have a mixed character in which the distinction between associative learning and non-associative sensitization, habituation, and adaptation can be blurry, particularly when pairing chemical cues or cultivation temperature with food or starvation. Rather than pairing chemical cues with food or starvation, therefore, it would be preferable for subsequent analysis of neuronal circuits responsible for associative learning and memory that two defined chemical cues are used for conditioning of worms in associative learning paradigms.

3.2.2.2.2. Reward learning and long-term associative memory.

In this section, we studied learning and memory in *C. elegans* by classical conditioning of worms with nonanol, as a conditioned stimulus (CS), and potassium chloride (KCl) as an unconditioned stimulus (US). Before the conditioning, worms avoided nonanol, an aversive olfactory stimulus, and were attracted by KCl, an appetitive gustatory stimulus, in chemotaxis assay. After eight-cycle massed (without intertrial intervals, ITI) or spaced (with 10-min ITI) training, in contrast, worms were attracted to nonanol. Memory induced by the massed training was extinguished within an hour, while the spaced training induced the memory which was retained for more than 12 hours (Figure 10A). Worms treated with cycloheximide or actinomycin D failed to form the long-lasting memory by the spaced training, whereas the memory induced by the massed training was not significantly affected (Figure 10B). These results indicated that the memory formation by the spaced training, but not by the massed training, required protein synthesis and mRNA transcription. Therefore, the memories induced by the massed and spaced training are classified as short-term and long-term memories, respectively. In support of this, *C. elegans* mutants defective in *nmr-1* encoding an NMDA receptor subunit failed to form both of the short-term and long-term memory, while mutations in *crh-1* encoding CREB, a transcription factor, affected only the formation of the long-term memory (Figure 10C).

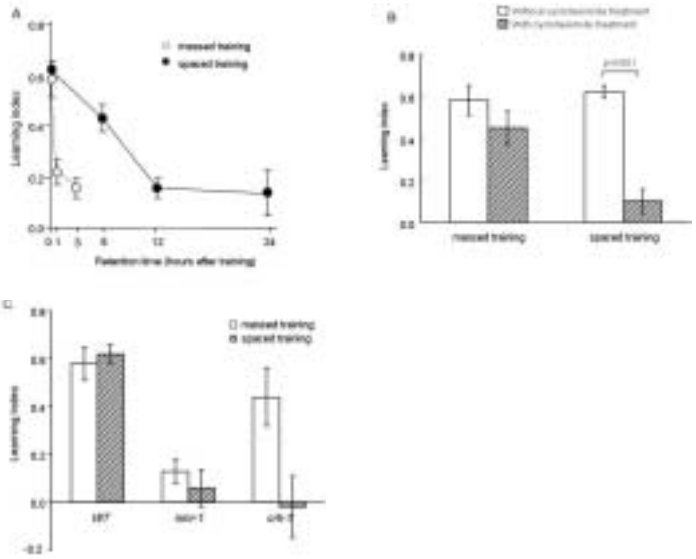


Figure 10. (A) Memory retention after massed or spaced training. **(B)** Learning index of worms treated with or without cycloheximide. Note that the cycloheximide treatment significantly affects learning after spaced training. **(C)** Mutants defect in learning. Note that *nmr-1* mutants showed defects both in learning after massed and spaced training, while *crh-1* showed a defect only in learning after spaced training.

3.2.2.2.3. Aversive olfactory learning and long-term associative memory.

To understand cellular and molecular bases for learning and memory in *C. elegans*, we also studied classical conditioning of worms with propanol, as a conditioned stimulus, and hydrochloride (HCl), as an unconditioned stimulus. Before the conditioning, worms were attracted to propanol, an appetitive olfactory stimulus, and avoided HCl, an aversive gustatory stimulus, in chemotaxis assay. After ten-cycle massed (without ITI) or spaced (with 10-min ITI) training, in contrast, worms avoided propanol on the assay plate. Furthermore, interstimulus interval (ISI) between the two stimuli were crucial for the conditioning. The memory after the massed training was extinguished within a few hours, while the memory after the spaced training was retained for 24 hours. Worms treated with cycloheximide or anisomycin failed to form the memory by the spaced training, whereas the memory after the massed training was not significantly affected. Furthermore, the memory after the spaced training was resistant to cold shock, while the memory after the massed training was disrupted by cold shock. Since the memory after the spaced training was consolidated by protein synthesis and was resistant to cold shock, it is classified as long-term memory. In contrast, the memory after the massed training is classified as short-term memory since it did not require protein synthesis and was sensitive to cold shock. Moreover, *C. elegans* mutants defective in *nmr-1* encoding an NMDA receptor subunit failed to form both of the short-term and long-term memory, while mutations in *crh-1* encoding the CREB transcription factor affected only on the formation of the long-term memory. These results are consistent with results previously observed in other model organisms such as *Aplysia, Drosophila*, and mice.

Thus, in the olfactory and gustatory associative learning paradigm in this study, we have successfully shown that worms can learn to be attracted to a previously aversive nonanol after it has been paired with an attractive KCl solution (positive associative learning). This associative

memory lasts for at least 12 hours. Furthermore, we have also shown that worms can learn to avoid a previously attractive propanol after it has been paired with acid as an aversive stimulus (negative associative learning). This associative memory also lasts for 24 hours as a long-term memory.

4. Publications

4.1 Journals

Adenan, A., Miyagi, H. & Maruyama, I. Unraveling the role of preformed epidermal growth factor receptor dimers by site-directed mutatgenesis. *Neuroscience Research* 65, S69-S69, doi:DOI 10.1016/j.neures.2009.09.228 (2009).

Maruyama, I., N. `Rotation/twist` model for the EGF/ErbB receptor family activation. *The FASEB Journal* 23, 844.2 (2009).

Murayama, T., Fujiwara, M. & Maruyama, I. Cellular and molecular mechanism underlying *C. elegans* chemotaxis toward mild alkaline pH. *Neuroscience Research* 65, S174-S174, doi:DOI 10.1016/j.neures.2009.09.920 (2009).

Sanehisa, S., Fujiwara, M., Murayama, T. & Maruyama, I. *C. elegans* mutants defective in high-alkaline pH avoidance. *Neuroscience Research* 65, S174-S174, doi:DOI 10.1016/j.neures.2009.09.919 (2009).

Shen, J. Y. & Maruyama, I. Preformed, yet inactive, dimeric structures of receptors for neurotrophic factors. *Neuroscience Research* 65, S47-S47, doi:DOI 10.1016/j.neures.2009.09.082 (2009).

Moriki, T., Maruyama, I. N., Igari, A., Ikeda, Y. & Murata, M. Identification of ADAMTS13 peptide sequences binding to von Willebrand factor. *Biochemical and Biophysical Research Communications* 391, 783-788, doi:DOI 10.1016/j.bbrc.2009.11.138 (2010).

Pan, Y. F., Viklund, I. M., Tsai, H. H., Pettersson, S. & Maruyama, I. N. The ulcerative colitis marker protein WAFL interacts with accessory proteins in endocytosis. *International Journal of Biological Sciences* 6, 163-171 (2010).

Gallegos, G., Nakata, K., Yan, D., Maruyama, I., Jin, Y. Ubiquitin E2 Variant Protein Acts in Axon Termination and Synaptogenesis in *C. elegans. Genetics* (in press).

Miyagi, H., Maruyama, I. N. Analysis of ligand-receptor interaction on the surface of living cells by fluorescence correlation spectroscopy. *Open Spectroscopy Journal* 4. 28-31(2010).

4.2 Books and other one-time publications

None.

4.3 Oral presentations

Maruyama, I. *Rotation/twist model for the EGF/ErbB receptor family activation*, Experimental Biology 2009, Ernest N. Morial Convention Center, New Orleans, Louisiana, USA, April 18-22, 2009

Maruyama, I. *Life sciences and model organisms*, Asian Youth Exchange Program 2009, Jichi Kaikan, Naha, Okinawa, Japan, August 23, 2009

Adenan, A. S., Miyagi, H., Maruyama, I. *Unraveling the role of preformed epidermal growth factor receptor dimers by site-directed mutagenesis*, 32nd Annual Meeting of the Japan Neuroscience Society, Nagoya International Convention Center, Aichi, Japan, September 16-18, 2009

Shen, J., Maruyama, I. *Preformed, yet inactive, dimeric structures of receptors for neurotrophic factors*, 32nd Annual Meeting of the Japan Neuroscience Society, Nagoya International Convention Center, Aichi, Japan, September 16-18, 2009

Maruyama, I. *Transmembrane signaling by cell-surface receptors,* Undergraduate Program, Ryukyu University School of Medicine, Nishihara, Okinawa, Japan, December 17, 2009

Maruyama, I. *'Rotation/twist' model for the molecular mechanism underlying the activation of the EGF/ErbB receptor family,* 3rd Annual World Congress of Pepcon (Protein & Peptide Conference) 2010, International Convention Center, Beijing, China, March 21-23, 2010

4.4 Posters

Maruyama, I. *Rotation/twist model for the EGF/ErbB receptor family activation*, Experimental Biology 2009, Ernest N. Morial Convention Center, New Orleans, Louisiana, USA, April 18-22, 2009

Murayama, T., Fujiwara, M., Maruyama, I. *Cellular and molecular mechanism underlying C. elegans chemotaxis toward mild alkaline pH*, 17th International C. elegans Meeting, University of California at Los Angeles, California, USA, June 24-28, 2009

Sanehisa, S., Fujiwara, M., Murayama, T., Maruyama, I. *C. elegans mutants defective in high-alkaline pH avoidance*, 17th International C. elegans Meeting, University of California at Los Angeles, California, USA, June 24-28, 2009

Adenan, A. S., Maruyama, I. *'Rotation/twist' model for the mechanism of the EGF/ErbB receptor activation*, FASEB summer Research Conference, Protein Kinases & Protein Phosphorylation, Snowmass, Colorado, USA, July 26-31, 2009

Shen, J., Maruyama, I. *Preformed, yet inactive, dimeric structure of receptors for neurotrophic factors*, 21st IUBMB and 12th FAOBMB International Congress of Biochemistry and Molecular Biology; Biomolecules for Quality of Life; Shanghai International Conventional Center, Shanghai, China, August 2-7, 2009

Adenan, A. S., Miyagi, H., Maruyama, I. *Unraveling the role of preformed epidermal growth factor receptor dimers by site-directed mutagenesis*, 21st IUBMB and 12th FAOBMB International Congress of Biochemistry and Molecular Biology; Biomolecules for Quality of Life; Shanghai International Conventional Center, Shanghai, China, August 2-7, 2009

Murayama, T., Fujiwara, M., Maruyama, I. *Cellular and molecular mechanisms underlying C. elegans chemotaxis toward mild alkaline pH*, 32nd Annual Meeting of the Japan Neuroscience Society, Nagoya International Convention Center, Aichi, Japan, September 16-18, 2009

Sanehisa, S., Fujiwara, M., Murayama, T., Maruyama, I. *C. elegans mutants defective in high-alkaline pH avoidance*, 32nd Annual Meeting of the Japan Neuroscience Society, Nagoya International Convention Center, Aichi, Japan, September 16-18, 2009

Maruyama, I., Adenan, A. S., Miyagi, H. *'Rotation/twist' model for the mechanism of the EGF/ErbB receptor activation*, 49th Annual Meeting of the American Society for Cell Biology, San Diego Convention Center, San Diego, California, USA, December 5-9, 2009

5. Intellectual Property Rights and Other Specific Achievements

Nothing to report

6. Meetings and Events

6.1 Seminar entitled "Migration of epithelia: The mechanobiology of cells and tissues"

Date: April 7, 2009
Venue: OIST IRP Conference Room
Speakers: Paul Matsudaira, Department of Biological Sciences,
National University of Singapore, Singapore

6.2 Seminar entitled "Hox gene clusters in a basal jawed vertebrate and the evolution of Hox gene clusters in vertebrates"

Date: October 26, 2009
Venue: Biocenter 1F, Meeting Room
Speakers: Byrappa Venkatesh, Institute of Molecular and Cell Biology, Singapore

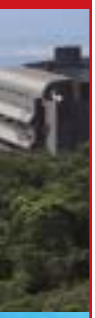
Developmental Neurobiology Unit

Principal Investigator:
Ichiro Masai

Research Theme:
Mechanisms underlying neuronal differentiation in the zebrafish retina

Abstract

The vertebrate retina consists of six major classes of neurons and forms neural circuits that mediate phototransduction. Because of such simple neural circuits, the retina provides a good model for studying neuronal differentiation in the nervous system. In the developing retina, multipotent progenitor cells generate all retinal cell types. However, the mechanism underlying the generation of diverse retinal cell types remains unclarified. To elucidate this mechanism, we study retinal development using zebrafish (*Danio rerio*) as an animal model. We have focused on the initial step of neuronal differentiation, that is, neurogenesis. Previous studies including ours revealed that at least five signaling molecules, namely Hedgehog (Hh), Fibroblast growth factor, Wnt, Notch and Histone deacetylase 1 (HDAC1), regulate the spatial and temporal pattern of neurogenesis in the zebrafish retina. One of aims of our research unit is to elucidate the molecular mechanism that regulates neurogenesis. First, we examine factors that modulate the HDAC1-dependent regulation of retinal neurogenesis in zebrafish. Recently, we identified a new zebrafish mutation, *oki150*, which enhances neurogenic defects in the zebrafish *hdac1* mutant retina. Second, we examine the role of cell polarity in retinal neurogenesis in zebrafish. We found that zebrafish cell-polarity-defective mutations modulate retinal neurogenesis through the Notch and Hh signaling pathway. Third, we examine the surveillance mechanism that monitors abnormalities of retinal neurogensis in zebrafish. Recently, we found that the DNA damage checkpoint and its downstream factor p53 determine whether retinal cells continue to differentiate or undergo apoptosis in the zebrafish retina. Through these projects, we will elucidate molecular networks that regulate neurogenesis in the zebrafish retina. In addition, we are currently initiating a project on zebrafish lens development, because proliferation of retinal stem cells is correlated with the growth of the lens.



1. Staff

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Technical Staff: Dr. Masaya Morita, Ms. Asuka Yoshizawa, Dr. Shohei Suzuki, Ms. Eri Oguri (temporal worker)

Research Assistant / Graduate Student: Mr. Yutaka Kojima, Ms. Yayoi Tomoyose, Ms. Yuki Tamae

Research Administrator / Secretary: Ms. Ayako Gima

2. Partner Organizations

None.

3. Activities and Findings

3.1 Mechanism regulting 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 regulating 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 (Fig. 1). Fibroblast growth factors (Fgfs) are expressed in the optic stalk and required for the initial induction of retinal neurogenesis in zebrafish. The Hedgehog (Hh) 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 *histone deacetylase 1 (hadc1)* mutant, in which retinal progenitor cells fail to exit from the cell cycle and continue to proliferate. HDAC1 promotes retinal neurogenesis by suppressing both Wnt and Notch signaling pathways. HDAC1 is recruited to several transcription repressor and corepressor complexes other than the Wnt and Notch pathways. To elucidate the HDAC1-dependent regulation of retinal neurogenesis, we have identified a new mutation, namely *oki150*, that modify the *hdac1* mutant-mediated neurogenic defects in heterozygous. We are currently cloning the *oki150* mutant gene.

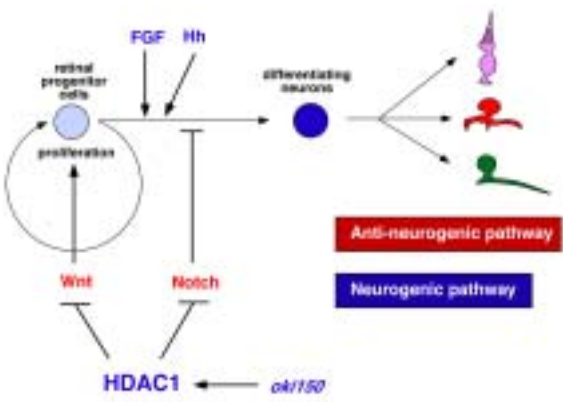
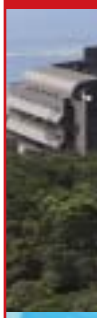


Figure 1: Molecular network regulating retinal neuroegnesis in zebrafish.

FGF and Hh regulates the induction and progression of retinal neurogenesis in zebrafish. Wnt and Notch signaling pathways promote cell proliferation and neurogenic inhibition, respectively. HDAC1 promotes retinal neurogenesis by suppressing both Wnt and Notch signaling pathways. A novel mutation namely *oki150* may interact with HDAC1 to promote neurogenesis.





3.2 Roles of apicobasal cell polarity in retinal neurogenesis

Cell and epithelial polarity is compromised in various human cancers. The loss of apicobasal cell polarity and the disruption of adherens junctions induce hyperplasia in the mouse developing brain and *Drosophila* imaginal discs. However, the relationship among the loss of cell polarity, proliferation, and differentiation has been discussed for a long time. Three groups of proteins play a central role in the establishment and maintenance of apicobasal cell polarity. The Crumbs-Stardust and Par3-Par6-aPKC protein complex promotes apical-membrane domain identity, whereas Lethal giant larvae-Scribble-Discs large protein complex, which promotes basolateral membrane identity. The apical junctional complex separates the apical and basolateral membrane domains and links between internal cell polarity and three-dimensional tissue organization. We examined the role of cell polarity and epithelial integrity in retinal neurogenesis. We focused on two zebrafish mutants, *n-cadherin (ncad)* and *nagie oko (nok)*, in both of which the apicobasal cell polarity and adherens junctions are disrupted in the retinal epithelium. We found that the fraction of proliferating cells increased in these mutant retinas during neurogenic stages (Fig. 2A and 2B). We examined the mode of cell division during neurogenesis, and found that the switching from proliferative cell division to neurogenic cell division is compromised in these mutant retinas (Fig. 2C-2F), resulting in the reduction of retinal neurogenesis. Furthermore, Notch and Hh-PKA signaling pathways are involved in the neurogenic defects in these mutant retinas. These findings suggest that the loss of apicobasal cell polarity affect the mode of cell division during retinal neurogenesis in the zebrafish through the Notch and Hh-PKA signaling pathways. Cell polarity regulators function as neurogenic promoting factors in the zebrafish retina.

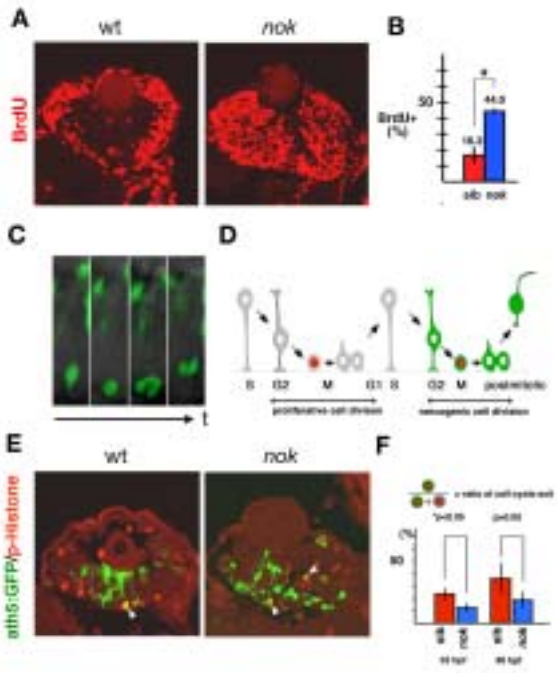
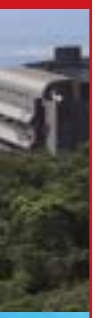


Figure 2: Retinal neurogenesis is affected in the *nok* mutant.
(A) BrdU incorporation in 58 hpf wild-type and the *nok* mutant retina. (B) The percentage of BrdU incorporation is higher in the *nok* mutant retina than in wild-type retina. (C) Time-lapse imaging of GFP expression under the control of ath5 enhancer (ath5:GFP). GFP expression is initiated at the G2 phase and inherited by two postmitotic daughter cells. (D) Schematic drawing of proliferative cell division and neurogenic cell division in the zebrafish retina. The double labeling with ath5:GFP and anti-phosphorylated histone H3 (pH3) antibody enables us to distinct proliferative cell division from neurogenic cell division. (E) The double labeling of wild-type and the *nok* mutant retinas with ath5:GFP and anti-pH3 antibody. Arrowheads indicate ath5:GFP and pH3-double positive cells.



(F) The percentage of ath5:GFP and pH3-double positive cell to total pH3-positive cells is lower in the *nok* mutant than in wild-type, suggesting that the switching from proliferative cell division to neurogenic cell division is affected in the *nok* mutant retina.

3.3 In vivo imaging of cell-cycle progression and exit in the zebrafish retina

The cell cycle consists of the G1, S, G2 and M phases. After the cell division, one or two daughter cells exit from the cell-cycle and start the differentiation of retinal neurons. However, the mechanisms underlying the switch from proliferative cell division to neurogenic cell division remains to be elucidated. We previously identified a zebrafish gene encoding a basic helix-loop-helix transcription factor, *ath5*, and established a zebrafish transgenic line carrying GFP under the control *ath5* enhancer. In the ath5:GFP transgenic embryos, GFP is initiated in the G2 phase just before the final cell division and inherited by two generated postmitotic daughter cells (Fig. 2C and 2D), suggesting that the decision between cell-cycle re-entry or exit is regulated at the G2 phase in the zebrafish retina. Recently, it was reported that a zebrafish Cecyil transgenic line visualizes the cell-cycle progression in vivo (Sugiyama et al., 2009 PNAS 106, 20812-20817.). Cell-cycle regulators, Cdt1 and Geminin, are expressed in the G1 and the S-G2-M phase, respectively. In the Cecyil transgenic line, the cell-cycle dependent Cdt1 and Geminin expressions are illuminated with fluorescent proteins. We are currently establishing the zebrafish ath5:GFP transgenic line combined with the Cecyil system, which will enable us to trace the cell-cycle progression and subsequent neurogenesis in the zebrafish retina in vivo.

3.4 Mechanism regulating apoptosis during retinal development in zebrafis

Apoptosis is observed in a developing retina, and is believed to remove abnormal differentiated cells. However, it is unknown how the apoptotic pathway is regulated during neurogenesis. Previously, we identified four zebrafish mutants, which show severe apoptosis of differentiating retinal neurons. Previously, we characterized one of these apoptotic mutants, *pinball eye (piy)*. The *piy* mutant gene encodes the small subunit of DNA primase (Prim1). Prim1 is essential for DNA replication and also required for the activation of the DNA damage response. We found that apoptosis in the *piy* mutant depends on the DNA damage response pathway, which activate a series of signaling molecules, Ataxia telangiectacia mutated (ATM), Checkpoint kinase 2 (Chk2), and the tumor suppressor p53. These findings suggest that the DNA damage response and its downstream target p53 determine whether retinal cells will differentiate normally or undergo apoptosis during retinal neurogenesis (Fig. 3). In addition to the *piy* mutant, retinal apoptosis of three other apoptotic mutants, namely, *rw329*, *rw337*, and *rw564*, depends on p53. To elucidate the p53-dependent molecular

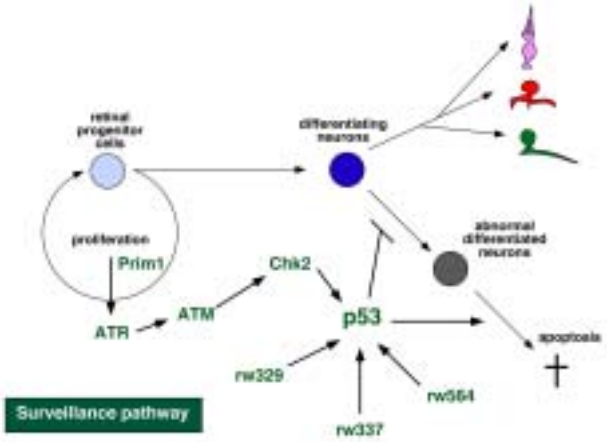


Figure 3: Surveillance pathway in the developing zebrafish retina.
Prim1 activates DNA replication checkpoint ATR. ATR activates the DNA damage response, ATM and Chk2. Chk2 activate p53, which repair DNA damages or induce apoptosis to eliminate abnormal differentiated cells.

mechanism that determines whether retinal cells are eliminated by apoptosis, we are cloning these mutant genes. Recently, we identified *rw564* gene, which encodes a novel protein containing WD40 repeats and are charactering its function.

3.5 Mechanism regulating the differentiation of lens fibers

Lens is an intraocular tissue that supports the vision. During development, lens delaminates from epidermal ectoderm and forms the lens vesicle. In the lens vesicle, the posterior epithelial cells differentiate into primary lens fiber cells, whereas, the anterior epithelial cells are maintained as proliferating lens stem cells. At the boundary between the anterior and posterior epithelium, called the equator, epithelial cells start to differentiate into secondary lens fiber cells, which elongate and cover the old lens fiber core like an onion. It is expected that the growth of the lens correlates with that of the retina for focusing visual images on the photoreceptor layer through lens-mediated refraction. However, the mechanisms that coordinate the cell-cycle progression and exit of retinal and lens stem cells are largely unknown.

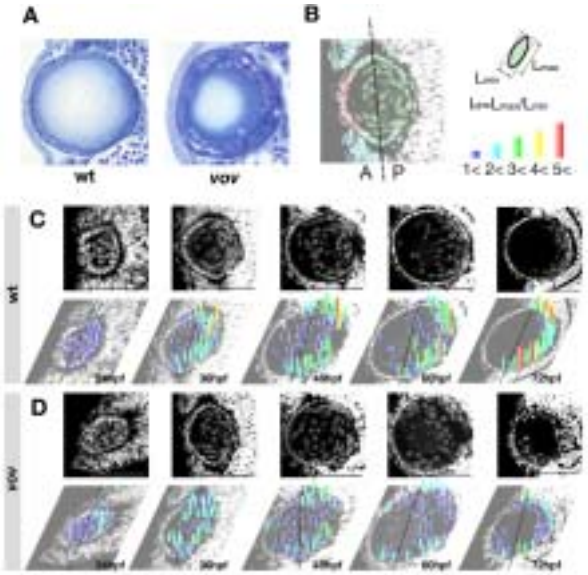
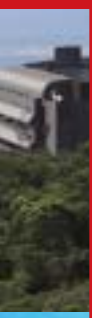


Figure 4: Wild-type and *vov* mutant lenses.
(A) Plastic section of 72 hpf wild-type and *vov* mutant lens. Round-shaped nuclei surround a small lens fiver core in the *vov* mutant. (B) Red and green colors indicate lens epithelial cells and lens fiber cells, respectively. Light blue indicates the retinal stem cell region, which associates with the lens equator, where lens cell differentiation starts. The flatness of lens fiber cells are defined as the ratio of the maximum thickness to the minimum thickness of lens fiber cells (Nuclear flatness index, Inf). (C, D) Nucleic labeling of wild-type (C) and the *vov* mutant (D) lens with DAPI (upper panels) at 24, 36, 48, 60 and 72 hpf. Lower panels indicate the Inf of individual lens fiber cells by color indication (red, the highest; green, intermediate; blue, the lowest). The nucleus positioned in the posterior lens become flat according as developmental stages proceed in wild-type, but not in the *vov* mutant.

Furthermore, the lens fiber cells lose organelles including the nucleus during their maturation. However, the mechanism underlying organelle elimination is unknown.
To elucidate the issues above, we examined zebrafish lens cell differentiation with histological methods. First, we examined the number, position and shape of lens fiber cell nuclei (Fig. 4). The number of lens fiber cell nuclei increases by 48 hpf, maintains a plateau within 48-60 hpf and



decreases at 72 hpf, suggesting that the number of lens fiber cell nuclei is dynamically regulated by the balance between generation from lens epithelium and degradation by denucleation (nuclear degradation). During lens fiber cell differentiation, lens fiber nuclei become more flat and positioned more posteriorly in the lens sphere (Fig. 4B and 4C), suggesting lens nuclei initially positioned in the anterior lens region are eliminated probably by denucleation. Recently, we identified a zebrafish lens-defective mutant, *volvox (vov)* (Fig. 4A). In the vov mutant, nuclear shape does not become flat and a significant number of lens fiber cell nuclei are retained in the anterior half of the lens sphere (Fig, 4D). Furthermore, the lens fiber cells do not fully elongate in the *vov* mutant. These findings suggest that morphogenesis and denucleation of lens fiber cells are affected in the *vov* mutant. The *vov* mutant gene encodes a regulatory subunit of the proteasome, Psmd6, and the proteasome activity is reduced in the *vov* mutant. These data suggest that the ubiquitin-proteasome system is required for morphogenesis and denucleation of lens fiber cells in zebrafish. Previous studies done by other groups have revealed that DNA degradation of lens fiber cells is regulated by DNase II-like acid DNase (DLAD), suggesting that DNA degradation occurs in an acidic organelle such as the lysosome. This raised the possibility that cellular organelles are degraded by autophagy in the cells' own lysosomes during lens fiber differentiation. However, organelle degradation normally occurs in the lens of autophagy-deficient Atg5 knockout mouse. It will be interesting to address how the ubiquitin proteasome system regulates lens denucleation and whether autophagy is involved in the organelle degradation of lens fiber cells in zebrafish.

4. Publications

4.1 Journals

Yamaguchi, M., Imai, F., Tonou-Fujimori, N. and Masai, I. Mutations in N-cadherin and a Stardust Homolog, Nagie oko, Affect Cell-cycle Exit in Zebrafish Retina. *Mechanisms of Development* 127, 247-264 (2010).

4.2 Book(s) and other one-time publications

Masai, I. ゼブラフィッシュ網膜における神経細胞分化の誘導および補償機構 (特集 細胞周期研究の新たなステージ) Mechanism that induces and ensures retinal neurogenesis in zebrafish[In Japanese]. *Cell Technology* 28, 52-58 (2009).

4.3 Oral presentations

Imai, F., Yoshizawa, A., Masai, I. *Ubiquitin proteasome system is essential for lens fiber differentiation*, 42nd Annual meeting for the Japanese society of developmental biologists, Niigata, Japan, May 28-31, 2009

Imai, F., Yoshizawa, A., Masai, I. *Ubiquitin proteasome system is essential for lens fiber differentiation*, 15th Annual meeting of zebarfish and medaka researchers in Japan. Nagoya, Japan, September 12-13, 2009

Masai, I. *Molecular mechanism underlying the induction and surveillance of retinal neurogenesis in zebrafish*. The MEXT "Cell proliferation control" International Symposium, Kyoto, Japan, March 15-17, 2010

Masai, I. *Mechanism underlying neural development in the zebrafish retina*, 5th “Sensory organ” symposium of the National Institute of Sensory Organ, National Tokyo Medical Center, Tokyo, Japan, March 11, 2010

Masai, I. *Surveillance mechanisms of neurogenesis in the zebarfish retina*, 4th Asia-Oceania zebrafish meeting, Jeju, Korea, August 31 - September 2, 2009

Ohata, S., Aoki, R., Kinoshita, S., Tsuruoka-Kinoshita, S., Yamaguchi, M., Tanaka, H., Wada, H., Masai, I., Okamoto, H. *Crumbs complex coordinately regulates neurogenesis and neuroepithelial polarity through canonica; and non-canoical Notch pathway*, 6th European zebrafish genetic and development meeting, Rome, Italy, July 15-19, 2009

Ohata, S., Aoki, R., Kinoshita, S., Tsuruoka-Kinoshita, S., Yamaguchi, M., Tanaka, H., Wada, H., Masai, I., Okamoto, H. *Crumbs complex coordinately regulates neurogenesis and neuroepithelial polarity through canonica; and non-canoical Notch pathway*, 42nd Annual meeting for the Japanese society of developmental biologists, Niigata, Japan, May 28-31, 2009

Yoshimura, Y., Hanahara, N., Yamaguchi, M., Fujimori, N., Masai, I. *Mechanisms regulating neuronal apoptosis in the developing zebrafish retina*, 42nd Annual meeting for the Japanese society of developmental biologists, Niigata, Japan, May 28-31, 2009

4.4 Posters

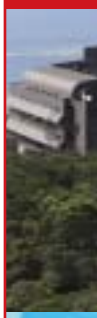
Imai, F., Yoshizawa, A., Masai, I. *Ubiquitin proteasome system is essential for lens fiber differentiation,* 6th European zebrafish genetic and development meeting, Rome, Italy, July 15-19, 2009

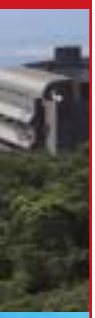
Imai, F., Yoshizawa, A., Masai, I. *Ubiquitin proteasome system is essential for lens fiber differentiation*, 32nd Annual meeting for the molecular biology society of Japan, Yokohama, Japan, December 1-4, 2009, (in Japanese)

Mochizuki, T., Yamaguchi, M., Masai, I. *Zebrafish mutant showing defects in lens epithelial integrity and fiber differentiation*, 6th European zebrafish genetic and development meeting, Rome, Italy, July 15-19, 2009

Ohata, S., Aoki, R., Kinoshita, S., Tsuruoka-Kinoshita, S., Yamaguchi, M., Tanaka, H., Wada, H., Masai, I., Okamoto, H. *Roles of Mosaic eyes in coordination of proliferation with the apicobasal polarity of neuroepithelial cells*, 32nd Annual meeting for the molecular biology society of Japan, Yokohama, Japan, December 1-4, 2009 (in Japanese)

Shiraki, T., Kojima, D., Wada, Y., Kawamura, S. Vogalis, F., Lamb, T., Nishiwaki, Y., Masai, I., Fukada, Y. *Role of GRK1 and GRK7 in the regulation of photoresponse properties of rods and cones in zebrafish*, 32nd Annual meeting for the molecular biology society of Japan, Yokohama, Japan, December 1-4, 2009 (in Japanese)





Yoshimura, Y., Hanahara, N., Yamaguchi, M., Fujimori, N., Masai, I. *Mechanisms regulating neuronal apoptosis in the developing zebrafish retina*, 6th European zebrafish genetic and development meeting, Rome, Italy, July 15-19, 2009

5. Intellectual Property Rights and Other Specific Achievements

None to report.

6. Meetings and Events

6.1 Workshop: The retina – neural stem cells and photoreceptor degeneration

Date: 9-13 Nov 2009

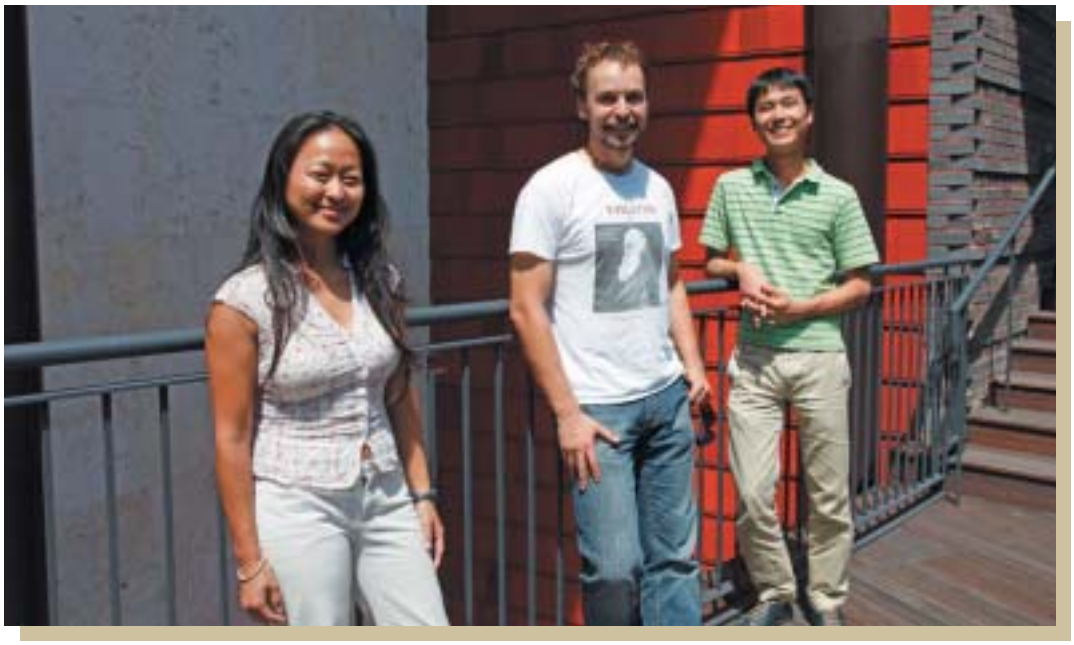
Venue: OIST Seaside House

Co-organizer: Sumiko Watanabe (University of Tokyo)

Speakers: Robin R Ali (University College London, UK)
Seth Blackshaw (Johns Hopkins Univ., USA)
Kwang-Wook Choi (KAIST, Korea)
Takahisa Furukawa (Osaka Bioscience Institute, Japan)
Florian Gekeler (Univ. Eye Hospital Centre for Opthal., Germany)
Brian Link (Medical College Wisconsin, USA)
Jarema Malicki (Tufts University, USA)
Kunimasa Ohta (Kumamoto Univ., Japan)
Thomas A. Reh (Washington Univ., USA)
Ching-Hwa Sung (Cornell Univ., USA)
Anand Swaroop (National Eye Institute, NIH, USA)
Masayo Takahashi (RIKEN CDB, Japan)
Valerie Wallace (Ottawa Health Res. Institute, Canada)
Sumiko Watanabe (Univ. Tokyo, Japan)

Ecology and Evolution Unit

Independent New Investigator:
Alexander Mikheyev
Research Theme:
Evolution of horizonally acquired genes in bdelloid rotifers



Abstract

Bdelloid rotifers are unique among multicellular animals in that they acquire large numbers of genes from other organisms, such as plants, bacteria and fungi. We are investigating the fate of these genes, in order to determine whether they have evolutionary consequences. In parallel, we are also investigating aspects of basic rotifer biology, such as population genetics and the presence of sexual reproduction. This research project is still only beginning, and more findings are expected in 2010.

1. Staff

Researcher: Yutaka Watanabe
Technical Staff: Tanya Vo

2. Partner Organizations

Nothing to report.

3. Activities and Findings

3.1 We are screening nearly 100 rotifer isolates form in the genus *Adenita* for the presence of 30 horizontally acquired genes.

4. Publications

4.1 Journals

None yet.

4.2 Books and other one-time publications

None yet.

4.3 Oral presentations

Mikheyev, A. S. *Rare sexual reproduction by a facultatively asexual ant facilitates invasion of novel habitats*, The OIST International Workshop, Okinawa, Japan, January 15, 2009

4.4 Posters

Mikheyev, A. S. *Horizontal gene transfer in bdelloid rotifers*, The OIST Opening Ceremony, Okinawa, Japan, March 28, 2010

5. Intellectual Property Rights and Other Specific Achievements

None yet.

6. Meetings and Events

None yet.

Physics and Biology Unit

Principal Investigator:

Dr. Jonathan Miller

Research Theme:

Quantitative Comparative, Evolutionary and Biomedical Genomics.

Abstract

Comparative sequence analysis - the inference of the action of selection by comparing sequences from diverse species – has long been one of the most effective tools of modern biology. Today, its practice is based primarily on local models of uncorrelated, single-nucleotide substitution; however, our work has demonstrated that such models fail the simplest qualitative tests of consistency with whole-genome sequence data. The sequence data – in particular, “ultraconservation,” a phenomenon first described by Brenner and colleagues when comparing fragments of the human, mouse, and pufferfish genomes in the 1990s - can only be accounted for by linkage. This inference is drawn from the ubiquitous algebraic tail of the conserved sequence length distribution. Linkage, which can be thought of as the association of alleles within a population or of sequences among diverse genomes is, within the context of a single chromosome, a signature of recombination. Over the last year, we have found that an algebraic tail in the length distribution of duplicated sequence within a genome is also ubiquitous, yielding strong support for the inference that the algebraic tail is in itself a a signature of recombination.

1. Staff

Researchers: Kun Gao, PhD (from 2008/12).
Sathish Venkatesan, PhD (from 2009/3).
Maxim Koroteev, PhD (from 2009/7).
Eddy Taillefer, PhD (from 2009/9).

Research Administrator / Secretary: Midori Tanahara

2. Partner Organizations

None.

3. Activities and Findings

3.1 Introduction.

Ultraconserved elements were first characterized as anomalously long sequences of DNA common to multiple divergent genomes [1,2]. As we first reported in 2005 [3], the sequence lengths are anomalous in a distinctive way: they exhibit a 'heavy tail' that is inconsistent with an evolutionary dynamics dominated by point mutations or short indels. To a physical scientist, such a heavy tail represents the hallmark of strong non-local spatial correlation of conserved bases - in biologists' jargon, "linkage." Although we at first believed the linkage reflected functional interaction [4,5,6], our more recent report that heavily repetitive sequence shares this feature [7] suggests that it derives from a neutral mechanism. The neutral mechanism associated with linkage is recombination, whose role in sequence evolution and comparative genomics has largely been treated as intractable or of only marginal relevance.

Although we reported observation of the distinctive heavy tail of ultraconservation in mammals, plants and bacteria [4] and more generally in vertebrates and yeast [5], we had no independent evidence that genomic recombination and repair processes could possibly yield such distributions. Here, we report on the first exhaustive studies of the length distributions of exact and nearly exact duplicate sequences within a single genome (E. Taillefer and J. Miller, 2010; to be submitted; K. Gao and J. Miller, 2010; to be submitted). We identify an analogous heavy tail in this distribution - shared among metazoans, plants, and bacteria - a phenomenon we call "ultraduplication." The elements comprising this tail include, for example, homeobox sequences. We show that the form of this tail is remarkably constrained, and we exhibit a minimal model that reproduces the observed constraint (M. Koroteev and J. Miller, 2010; to be submitted). The essential property of the model is the occurrence of long duplication events on time scales comparable to those of point mutation.

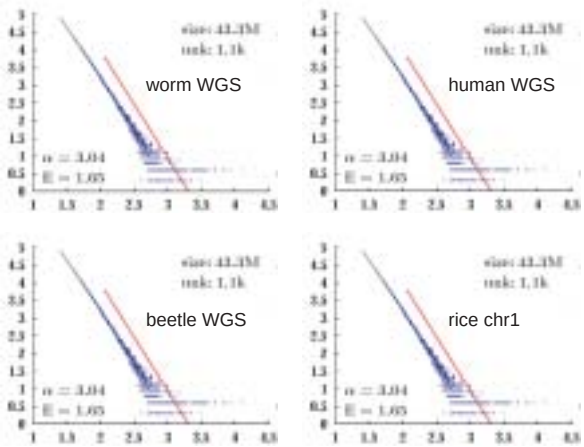


Figure 1: Log/log (base 10) plots of the number of duplicated sequences (y-axis) as a function of length (x-axis). Clockwise from upper left: worm (C. elegans) whole genome; human (H. sapiens) whole genome; rice (O. sativa japonicus) chromosome 1; beetle (T. castaneum) whole genome. The red lines are maximum likelihood linear fits with extent *E* and slope -α. Duplication counts were obtained using the software tool *SEQANALYSIS* (E.Taillefer and J. Miller, 2010; manuscript in preparation).

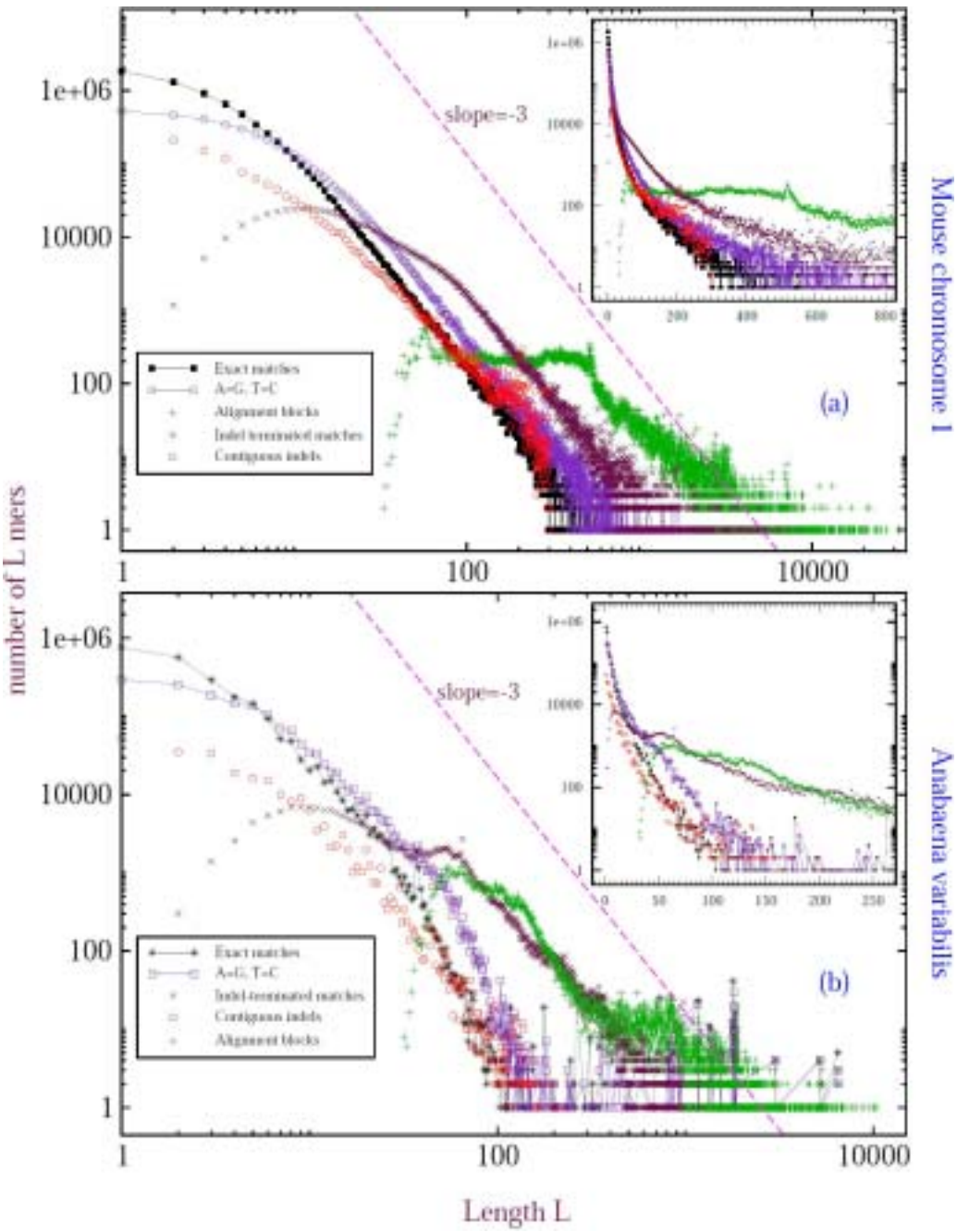


Figure 2: Length distribution of exact and approximately duplicated sequence from (a) chromosome 1 of mouse; and (b) whole genome of the cyanobacterium, Anabaena variabilis. They are log/log (base 10) plots, except for the insets, which are log/linear. From K. Gao and J. Miller, 2010; to be submitted.

3.2 Ultraduplication.

Ultraconserved sequences are sequences having at least a single copy in each of two or more genomes; their length distribution for widely separated species exhibits a characteristic algebraic tail that we first described in 2005. The inference, that this length distribution reflects the lengths of contiguous sequences that have migrated as integral units over the course of evolution, is indirect. Our discovery this year (K. Gao and J. Miller, 2010; to be submitted; E. Taillefer and J. Miller, 2010; to be submitted) that algebraic tails are ubiquitous in the length distributions of sequences having at least two copies within a *single* genome supports this inference, because it is the conventional wisdom that duplication within genomes involves transfer of contiguous sequence as an integral unit. We call the sequences comprising this algebraic tail, *ultraduplicated* sequences. As illustrated in figure 1, for a diverse set of organisms, the algebraic tail shows an exponent (slope $-\alpha$ in figure 1) very close to -3. In rice, chromosome 1 only is displayed, but the plots obtained from the other chromosomes by themselves, or from whole genome, are nearly indistinguishable in shape from chromosome 1.

3.3 Approximate match lengths.

[Please see figure 2 on preceding page.]

The phenomenological similarities of ultraconserved and ultraduplicated sequences extend beyond the algebraic tail observed in the lengths of exact matches. In figure 2, we plot the distributions of lengths of approximate matches for ultraduplicated sequence, to be compared to the distributions of lengths of approximate matches for ultraconserved sequence that we described in our 2008 OIST annual report and in [7].

3.4 A simple model.

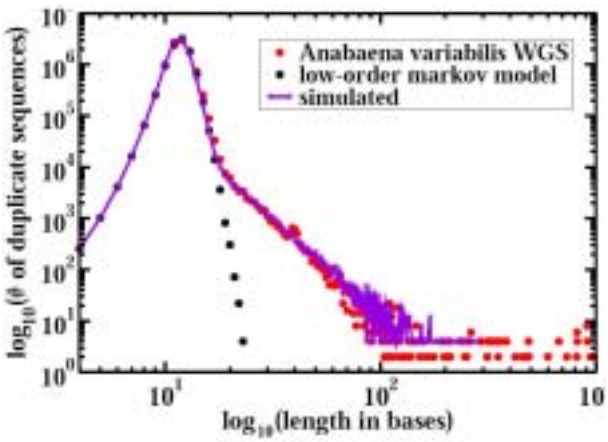


Figure 3: Lengths of ultraduplicated sequence from (red) *A. variabilis* whole-genome; (black) a sequence of the same total length as the *A. variabilis* genome, but randomly-generated by a low-order Markov model; (purple) the steady-state of a dynamical model for genome sequence evolution proposed by M. Koroteev and J. Miller, 2010; to be submitted. Observe the close correspondence among all three curves for short lengths, and between real genome and simulated genome in the tail region from around 20 bases to 200 bases.

3.5 Ultraduplication and Ultraconservation.

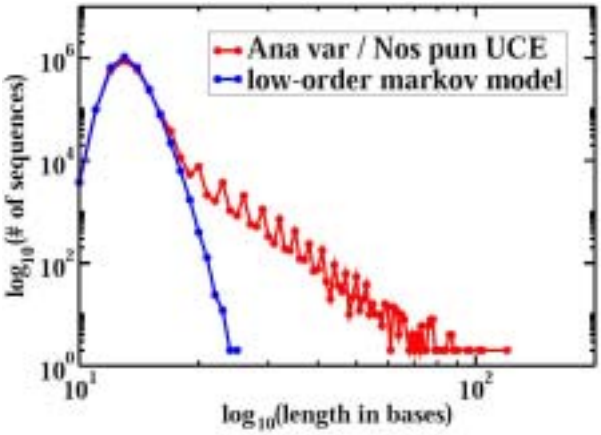


Figure 4: Lengths of ultraconserved sequence shared between (red) the cyanobacteria *Anabaena variabilis* and *Nostoc punctiforme*; (blue) a low-order Markov model for the *A. variablis* genome and a low-order Markov model for the *N. punctiforme* genome. Observe the close correspondence between the ultraconserved sequence for the real genomes and the low-order markov models at short length, and the qualitatitive similarity of the shape of the tail for ultraconservation in this figure to the shape of the tail for ultraduplication in figure 3.

References:

1. B. Kammandel et al. (1999). Developmental Biology 205, 79–97.
2. G. Bejerano et al. (2004). Science 304(5675), 1321-5.
3. J. Miller and P. Havlak (2005). CSHL Genome Informatics Meeting.
4. W. Salerno et al. (2006). Proc Natl Acad Sci USA, 103(35), 13121-5.
5. J. Miller (2007). http://www.icsb-2007.org/proceedings/abstracts/H56.pdf
6. J. Miller (2007). First annual q-bio conference on cellular information processing Santa Fe, NM (2007). http://cnls.lanl.gov/q-bio/wiki/images/e/e2/Poster-miller.pdf
7. J. Miller (2009). IEICE technical report, Neurocomputing, 109(53) (20090518).

4. Publications

4.1 Journals

None.

4.2 Book(s) and other one-time publications

None.

4.3 Oral presentations

Miller, J. *Colossal ultraconservation and super-colossal ultraconservation*, Technical Committee on Neurocomputing, OIST Seaside House, Okinawa, May 25, 2009

4.4 Posters

Gao, K., Miller, J. *Are Hox genes representative of the genome-wide distribution of duplication lengths?*, OIST Opening Ceremony, Okinawa, Japan, March 28, 2010

5. Intellectual Property Rights and Other Specific Achievements

Nothing to be reported.

6. Meetings and Events

6.1 Seminar: "The physics of whole genome sequences and its implications for genome growth and evolution"

Date: May 11, 2009
Venue: IRP Conference Room, OIST
Speaker: H.C. Paul Lee (National Central University)

6.2 Seminar: "Coupled Exclusion Processes for the Modeling of Intracellular Particle Transport"

Date: May 28, 2009
Venue: IRP Conference Room, OIST
Speaker: Konstantinos Tsekouras (Rice University)

6.3 Seminar: "Full Azimuth Direction-of-Arrival Estimation with Successive-Selection Technique"

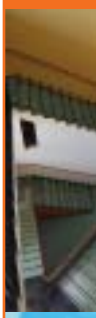
Date: June 10, 2009
Venue: IRP Conference Room, OIST
Speaker: Eddy Taillefer (Fujitsu)

6.4 Seminar: "Identification and characterization of evolutionarily conserved cis-regulatory elements in the human genome"

Date: Jan 21, 2010
Venue: Bio Center, OIST
Speaker: Byrappa Venkatesh (Institute Molecular Cell Biology, Singapore)

Marine Biophysics Unit

Independent New Investigator:
Satoshi Mitarai
Research Theme:
Marine Ecology



Abstract

We propose to develop real-time forecasting system for the coastal ocean circulation processes around Okinawa and implement a validated connectivity assessment tool, coupling available modeling and observation techniques. This modeling tool will …

1. Help marine biologists and geneticists estimate rates of gene flow among coral reefs,
2. Enable public agencies to build and evaluate marine protected areas based on population connectivity, and
3. Provide marine ecologists with a modeling system for predicting the global and long-term dynamics of the coral reef ecosystem.

1. Staff

Researcher: Masako Nakamura
Technical Staff: Taichiro Sakagami
Research Administrator / Secretary: Shizuka Kuda

2. Partner Organizations

University of California, Santa Barbara
Type of partnership: Collaboration
Name of researchers: Dave Siegel, Robert Warner, Carter Ohlmann
Research theme: Marine Ecology

University of Ryukyus
Type of partnership: Collaboration
Name of researchers: Profs. Tsuchiya, Suda, Kurihara, Sakai
Research theme: Marine Ecology

3. Activities and Findings

3.1 Marine Biophysics Unit was established September 1, 2009 with my arrival in Okinawa. Taichiro Sakagami joined the unit as of February 1, 2010. Masako Nakamura joined us on March 24, 2010. Shizuka Kuda has been serving as a secretary for Marine Biophysics Unit since February 2010.

4. Publications

4.1 Journals

Mitarai, S., Siegel, D. A., Watson, J. R., Dong, C. & McWilliams, J. C. Quantifying connectivity in the coastal ocean with application to the Southern California Bight. *J Geophys Res-Oceans* 114, -, doi:Artn C10026 Doi 10.1029/2008jc005166 (2009).

Berkley, H. A., Kendall, B. E., Mitarai, S. & Siegel, D. A. Turbulent dispersal promotes species coexistence. *Ecol Lett* 13, 360-371, doi:DOI 10.1111/j.1461-0248.2009.01427.x (2010).

Watson, J. R., Mitarai, S., Siegel, D. A., Caselle, J. E., Dong, C. & McWilliams, J. C. Realized and potential larval connectivity in the Southern California Bight. *Mar Ecol-Prog Ser* 401, 31-48, doi:Doi 10.3354/Meps08376 (2010).

Selkoe, K., Watson, J., White, C., Ben-Horin, T., Iacchei, M., Mitarai, S., Siegel, D., Gaines, S., Toonen, R. Taking the chaos out of genetic patchiness: revealing ecological and oceanographic drivers of seascape genetics in Southern California kelp forests. *Molecular Ecology* (in press).

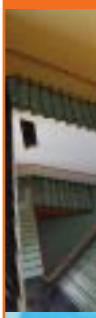
Ohlmann, C., Mitarai, S. An emiprical examination of simulated dispersal patterns, with application to Southern California Bight. *Geophysical Review Letters* (in review).

4.2 Books and other one-time publications

Yamazaki, H., and Mitarai, S. *The carrying capacity of a biological species in an environment and sustainable fishers (In Japanese)*. (Kodansha, in press).

4.3 Oral presentations

Mitarai, S., Ohlmann, J.C., Dong, C., Siegel, D.A., and McWilliams, J.C. *An Empirical Examination of Simulated Connectivity, with Application to the Southern California Bight*,

2010 Ocean Sciences Meeting, Portland, Oregon, USA, Feb 22-26, 2010

Watson, J. R., Siegel, D.A., Raimondi, P., Hays, C., Mitarai, S., Dong, C., McWillimas, J.C. *Communities connected by currents: a study of nearshore marine species in the Southern California Bight*, 2010 Ocean Sciences Meeting, Portland, Oregon, USA, Feb 22-26, 2010

Mitarai, S. *Larval Dispersal in the Turbulent Coastal Ocean: Dynamics and Impacts*, Life Science Special Seminar, Departments of Ecology and Evolutionary Biology and Atmospheric & Oceanic Sciences, University of California, Los Angeles, USA, Dec 7, 2009

4.4 Posters

None.

5. Intellectual Property Rights and Other Specific Achievements

None.

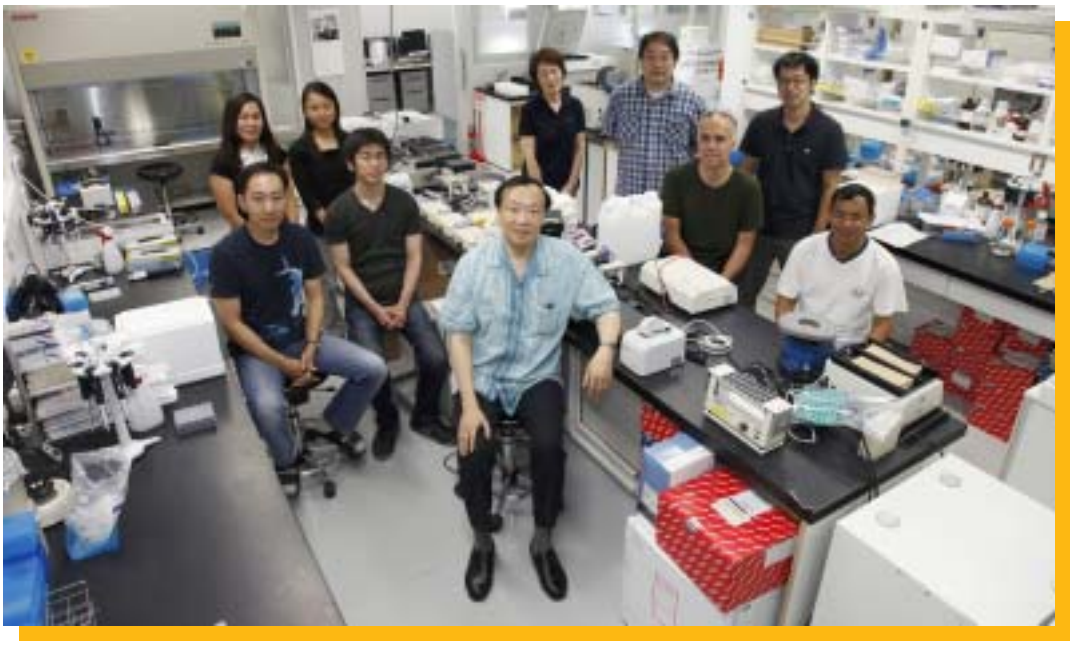
6. Meetings and Events

None.

Molecular Neurobiology Unit

Principal Investigator:
Takayuki Naito

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



Abstract

"Single-cell biochemistry and molecular analysis of brain functions" is the title of our research. Our research aims to provide link between genetic and molecular events occurring in cells and brain functions.

In FY2009, we carried out mainly the following research to obtain the basic information for neuroscience, (1) gene expression study, (2) mass spectrometry (MS) related study, and (3) the salamander project. (1) We demonstrated a gene expression profile of cerebellar Purkinje cells using a combination of DNA microarray and real-time qPCR. We studied the effect of cholecystokinin (the most abundant neuropeptide in hippocampus) on the gene expression in hippocampal networks. (2) We examined imaging-MS of phospholipids using salamander retina and showed a possibility of imaging-MS at a single-cell level. We developed a newly microsampling device based on a microdialysis probe for peptide sampling to elucidate dynamics of peptides in living animals. We studied the character of the probe. (3) We are establishing molecular neuroscience of salamander. Because salamander has large cells, it is thought to be a good material for imaging studies, electrophysiology, and single-cell analysis of the nervous system. In this year, we carried out a preliminary trial of gene expression analysis at a single-cell level using the salamander *(Ambystoma mexicanum*) cells. And, we constructed a 3D brain atlas of the salamander. These results are described in the following sections.

1. Staff

Researchers: Kiyotaka Akiyama, Nozomu Nakamura, Michael Chandro Roy,
Makoto Araki, (Setsuko Nakanishi)
Technical Staff: Seiko Kuraba, Saori Ishida, David Alan Richter, Shotaro Kasai,
Research Administrator / Secretary: Kaori Yamashiro



2. Partner Organizations

Okinawa Institute of Science and Technology, PC
Type of partnership: Joint research
Name of 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 researcher: Dr. Ryo Taguchi
Research theme: Lipids in the nervous system

Hamamatsu University School of Medicine
Type of partnership: Joint research
Name of researcher: Dr. Mitsutoshi Setou
Research theme: Mass Imaging

University of Oulu, Finland
Type of partnership: Joint research
Name of researcher: Dr. Kazuhiko Tatemoto, Dr. Karl-Heinz Herzig
Research theme: Peptidome in the brain

Keio University, Department of Biosciences and Informatics
Type of partnership: Joint research
Name of researcher: Dr. Daisuke Uemura
Research theme: Marine microorganism project

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

The University of Tokyo, Department of Psychology
Type of partnership: Joint research
Name of researcher: Dr. Masao Tachibana
Research theme: Study of retina

3. Activities and Findings

3.1 Gene expression analysis

3.1.1 Gene expression profiling of Purkinje cells

Purkinje cell in the cerebellum is one of the well-studied neuronal types anatomically and physiologically. It is one of the largest cells in the mammalian CNS. We have demonstrated a “single-type cell” gene expression profile of mouse Purkinje cells using a combination of DNA microarray and real-time qPCR.

According to DNA microarray analysis, we found that at least 7,055 genes (corresponded to 9,487 probe sets tagged with P call in all four samples) were expressed in Purkinje cells, which covered 41.0% of total genes on the microarray (17,197 genes; 26,351 probe sets). More than 80% of all identified genes was categorized using PANTHER Molecular Function database.

To characterize Purkinje cells, we compared gene expression profile of Purkinje cells with the cerebellar granule cell layer. The correlation analysis between Purkinje cells and the granule cell layer showed that 5 categories had significantly lower distribution of correlation coefficients than the other 24 categories of PANTHER Molecular Function database (Fig.1A, B): (1) Signaling molecule (644 genes), (2) Cell adhesion molecule (282 genes), (3) Ion channels (276 genes), (4) Select calcium binding protein (219 genes), and (5) Receptor (1059 genes). These categories mainly represent the “neural communication system”, such as neuropeptides, voltage-gated ion channels, ligand-gated ion channels, G protein-coupled receptors (GPCRs), and neurotransmitter synthases.

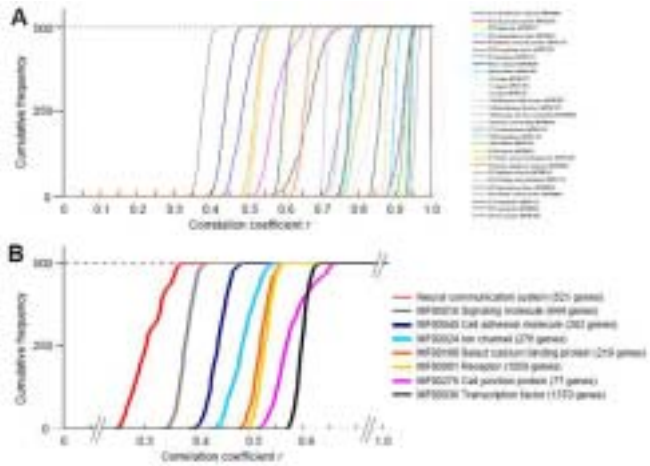


Figure 1 [A] Genes expressed in Purkinje cells are classified into 29 categories of PANTHER Molecular Function database. Each cumulative curve shows the distribution of correlation coefficients in each category between Purkinje cells and cerebellar granule cell layer. [B] The cumulative curves are selected from lower distributions of the correlation coefficients in A, and the distribution of the neural communication system is added.

The “ligand-receptor system” or “neural communication system” is considered to cause functional differences of signal transmission between neurons or neuronal types. It is important to elucidate precise expression of these genes in each neuron and neuronal type to characterize them. We carried out real-time qPCR analysis for 125 genes of neural communication system. The following genes expressed over 100 copies per 1 ng of total RNA: (i) 10 voltage-gated ion channels (Clcn3, Clcn4-2, Kcnk1, Kcnab1, Kcnab2, Kcnb1, Kcnc3, Kcna1, Kcnd3, and Scn8a), (ii) 6 ligand-gated ion channels (Gabra1, Gabrg1, Gabrb2, Gabrg2, Gria3, Grik1, and Glrb), (iii) 9 GPCRs (Gabbr1, Gabbr2, Grm1, Grm7, Gpr37l1, Gprc5b, Gpr176, Bai3, and Opn3), (iv) 6 neurotransmitter synthases (Gad1, Gad2, Abat, Got1, Glul and Glud1), and (v) 1 neuropeptide (Cck). We think these genes can characterize the function of Purkinje cells.

3.1.2 Suppression of cAMP-dependent gene expression in the hippocampus by cholecystokinin and extracellular regulatory machinery

Our gene expression analysis suggests that the “neuropeptidergic system” might promote a diversity of regulations of signal transmission in hippocampal networks. Cholecystokinin (CCK) is

the mostly expressed neuropeptide and represents 66% of all neuropeptides expressed in the CA1 and CA3 regions. It is known that extraneous CCK octapeptide sulfated, the active form of CCK, regulates electrophysiological action potentials in the hippocampus. However, despite the fact that CCKBRs induce Gq protein signaling, a role of CCK-CCKBR signaling for the gene regulation in hippocampal networks remains unknown. Inducible genes called immediate-early genes (IEGs; e.g. *Fos, Egr1/zif268,* and *Arc*) are identified as critical indicators of neuronal activity. To understand "transcription level" neuronal activity in hippocampal networks, we have investigated the IEG regulation by CCK in hippocampal slices.

We identified the amount of CCK octapeptide sulfated in the hippocampus of the intact adult mouse (0.58 ± 0.21 pmol/mg tissue), using LC-mass spectrometry. CCK increased luciferase activity dose-dependently in PC12 cells transfected with CCKBR and Egr1 promoter-luciferase. Although we confirmed that CCK increased IEG expression in cells having CCKBR, the IEG regulation by CCK in neural networks remains unclear.

We examined CCK regulatory effects on IEG expression in organotypic hippocampal slices. CCK suppressed forskolin-induced expression levels of *Fos* (Fig. 2C) and *Egr1/zif268* (Fig. 2D), but it did not suppress DMSO-treated and PMA-induced mRNA expression levels of *Fos* and *Egr1/zif268* (Fig. 2A, B). In addition, Western blotting showed that forskolin-induced Fos protein expression level was decreased by CCK (Fig. 2E, F). In acute hippocampal slices, CCK suppressed forskolin-induced IEG expression levels. We observed that CCK significantly suppressed cAMP-induced IEG expression in living hippocampal slices.

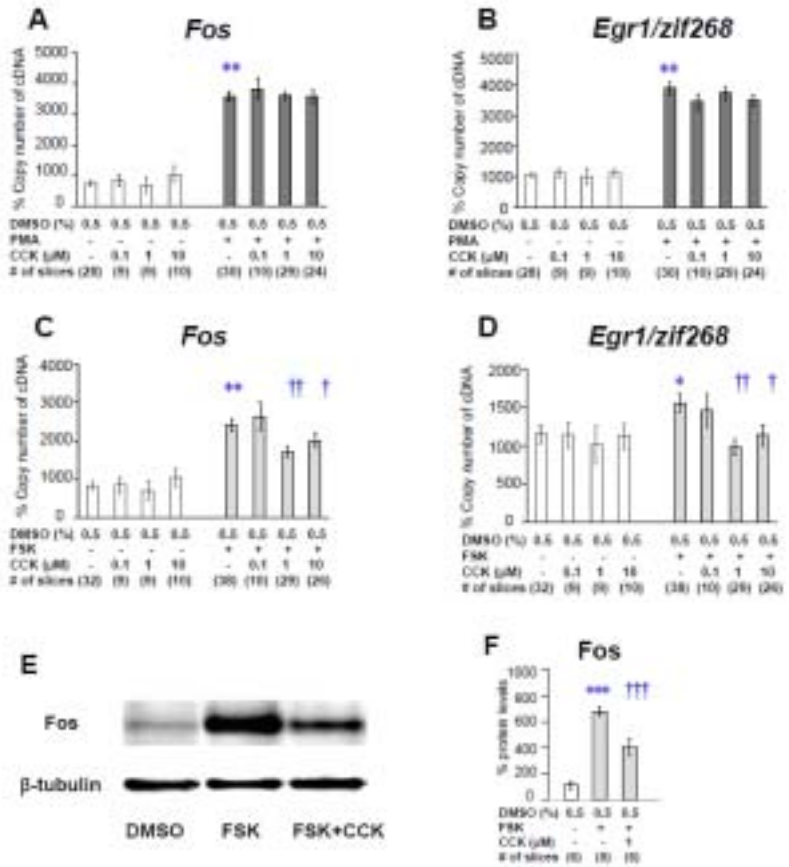


Figure 2: [A, B] Bar-plots show PMA (1 µM)-induced expression levels of *Fos* (A) and *Egr1/zif268* (B) with different doses of CCK application. [C, D] Bar-plots show forskolin (FSK, 10 µM)-induced expression levels of *Fos* (C) and *Egr1/zif268* (D) with different doses of CCK application. [E]

Western blotting shows the intensity of bands of Fos and ß-tubulin proteins. [F] Bar-plots show Fos protein expression levels with a combination application of forskolin and CCK.

We also showed that CCK induces CCKB receptor activation and indirectly suppresses cAMP-induced IEG expression via activation of GABAB and cannabinoid CB1 receptors in living hippocampal slices. Therefore, we suggest that cAMP-induced IEG expression may be suppressed by a complex mechanism of hippocampal networks via CCK-CCKBR signaling. Further work is necessary to investigate the detailed mechanism of CCK suppression of cAMP-dependent gene expression in hippocampal networks.

3.2 Mass spectrometric analysis of biomolecules in the nervous system

3.2.1 Imaging mass spectrometry to visualize phospholipids in the salamander retina section

Imaging mass spectrometry (IMS) is one of good technologies for studying distribution of biomolecules (lipids, proteins and peptides) in tissue to understand their functions more precisely. Lipidomics is gaining much attention in recent days due to crucial roles of lipids in the living systems. Lipids serve variety of cellular functions and are principal components of cell membranes and control biomembrane physiology. The central nervous system is composed of diverse lipids and defective lipid metabolisms are related to various diseases of the brain and peripheral nervous systems.

To observe the phospholipids distribution by imaging mass spectrometry, we selected salamander retina because salamander has large cells compared to other vertebrates. We visualized and identified distinct layers of phospholipids distribution in the salamander retina section (5 µm). In the spectrum obtained from a raster scan, six signals were selected by principal components analysis (PCA) and characterized them as phosphatidylcholines (PC) by MS/MS. They were identified as PC (32:0), PC (32:1), PC (34:1), PC (36:4), PC (38:6), and PC (40:6). A reconstructed ion images of the salamander retina showed that PC (32:0), PC (32:1), and PC (34:1) distributed in the outer and inner plexiform layers. Whereas, PC (36:4) distributed in the outer segment and retina pigment epithelium (RPE) layers, and PC (38:6) and PC (40:6) distributed in the outer and inner segments (OS & IS). These PC molecules clearly form distinct layers in the retina. The different distribution patterns of PCs in the retina suggest that they might have specific function. However, the full function of fatty acids in the retina is unclear. We speculate that PC (38:6) and (40:6) with a polyunsaturated fatty acid DHA (22:6) might play a key role in phototransduction mechanisms in the visual cells by increasing membrane fluidity and enhancing conformational change of rhodopsin to metarhodopsin.

Due to the large cells (average diameter 14.5 µm) of salamander retina, it is possible to visualize and identify lipids in a single cell level. By reducing MALDI MS laser size (<10 µm), we have detected scattered distribution of phospholipids in the OS, IS, and RPE layers. The three major phospholipids containing unsaturated fatty acyl groups at *m/z* 820.5 (PC 36:4), 844.5 (PC 38:6), and 872.5 (PC 40:6) are distributed in the OS and IS regions, as shown in the Fig. 3. This mosaic distribution of lipids in these three layers could be best explained by the layers intercrossing. The mosaic distribution of phospholipids [PC (36:4), PC (38:6), and PC (40:6)] also implies their cell and cell parts specific distribution.

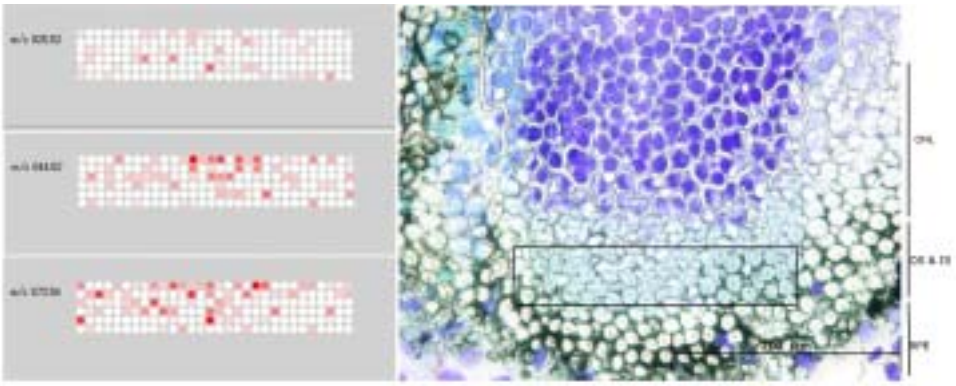


Figure 3. Distribution pattern of phospholipids in the outer segment, inner segment, and retinal pigment epithelium layers. Mosaic distribution of phospholipids PC (36:4), PC (38:6), and PC (40:6) implies their cell and cell parts specific distribution.

3.2.2 Method development for the collection of endogenous peptide from living tissues

Neuropeptides are important signal molecules for intercellular communication and play significant roles in animal behavior and physiology. A small amount of neuropeptides is produced by distinct neurons and released into the extracellular space, and they reach their target receptors by diffusion. Due to their high affinity, a very small amount of neuropeptides is enough to exert their action in the brain. To study their dynamics in living brain, it is necessary to deploy a sensitive tool for their sampling, detection, and identification in the brain extracellular fluid. Microdialysis is a widely used research tool for sampling molecules of interest (such as neurotransmitters, peptides, proteins, drugs and its metabolites) from the extracellular space in vivo and it has broad applications from basic research to clinical usage. However, a high recovery of peptides by microdialysis has not been achieved yet. To overcome recovery problem we devised a high recovery microsampling probe.

The high recovery microfiltration probe based on microdialysis was devised (Fig. 4). The new probe showed a high recovery (100%, Fig. 5) of peptides in vitro at different perfusion flow rates (0.1–1.0 µl/min). At a high flow rate 1.0 µl/min, a 10-fold increased in recovery of peptides compared to the conventional microdialysis probe was achieved. The microsampling probe can be a useful tool for high recovery of peptides from living tissues.

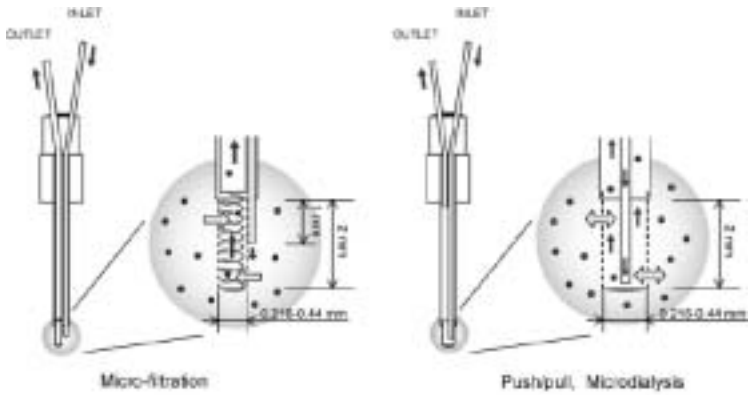


Figure 4. A schematic diagram of the new and conventional probes (membrane active length 2 mm, width 0.21–0.44 mm).

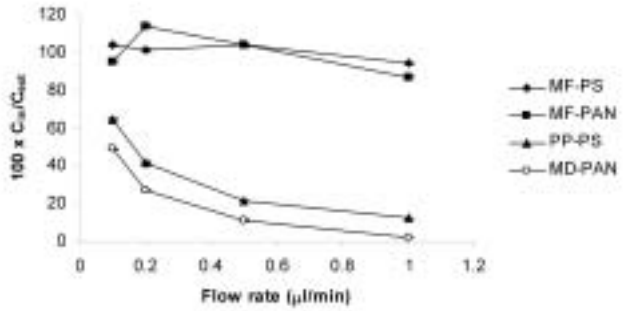


Figure 5. Comparison of relative recoveries (%) of peptides (Leu-enkephalin, 10 μM) by different probes (MF-PS, microfiltration probe made of PS membrane; MF-PAN, microfiltration probe made of PAN membrane; PP-PS, push-pull probe made of PS membrane; MD-PAN, microdialysis probe made of PAN membrane).

3.3 Salamander project

We are establishing molecular neuroscience of salamander. The salamander *(Ambystoma mexicanum)* was selected as a model organism for the study of neuroscience. The salamander's cell size, genome size, and mRNA content per cell are about 10-fold higher than those of the mouse. The number of cells composing the brain and retina of the salamander is fewer than the mouse, suggesting a simple neuronal network of the salamander.

3.3.1 3D brain map

A brain atlas is necessary for neuronal research. We have constructed a three-dimensional brain atlas of *Ambystoma mexicanum* using AZIVO software (ver. 5.1.0, Mercury Computer Systems, Berlin, Germany) from 277 serial sections (Fig. 6).

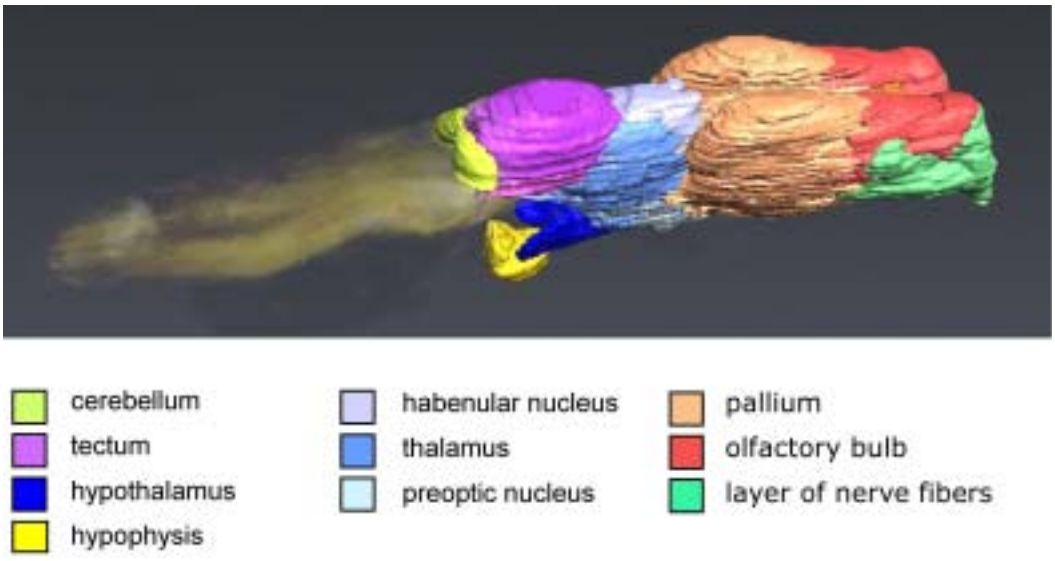


Figure 6. 3D Brain Map of salamander. A three dimensional representation was done from 277 serial sections with AZIVO software (ver. 5.1.0, Mercury Computer Systems, Berlin, Germany). The following regions were presented in different colors: layer of olfactory nerve fibers (green); olfactory bulb (orange); pallium (light orange); habenular nucleus (mauve); thalamus (blue); preoptic nucleus (light blue); hypothalamus (deep blue); tectum (purple); cerebellum (light green); hypophysis (yellow).

3.3.2 Single-cell analysis

To understand neural mechanisms underlying brain functions, precise classification of cell types composing brain system is indispensible. Information about gene expression in single neurons enables cell classification depending on their gene expression patterns. Salamander has simple nervous system and large mRNA content per cell; therefore, it is thought to be an ideal animal for the single cell gene expression analysis. Retina processes complex optic information with many different cell types, and is an ideal subject for the analysis.

We have tried to classify salamander's retinal ganglion cells (neurons sending messages to brain through optic nerve), depending on their gene expression pattern. Ganglion cells were stained by backfilling fluorescent dye from optic nerve cut end (Fig. 7A). Single retinal ganglion cells were collected under fluorescent binoculars from dissociated retinal cell-suspension (Fig. 7B). Total RNA was extracted from the collected single-ganglion cell and cDNA was synthesized with reverse transcriptase. After cDNA amplification, copy numbers of the cDNAs were analyzed using qPCR method.

Figure 7. Staining and collection of retinal ganglion cells. [A] Fluorescent labeled retinal ganglion cells after backfill staining. [B] Dissociated retinal cell-suspension was mixed with low-melting point agarose (B_1) and fluorescent labeled ganglion cells were collected under fluorescent binoculars (B_2).

In a preliminary small scale experiment, we used 33 cells and 35 genes as markers. Individual ganglion cells were classified depending on their gene expression pattern by using software for clustering (Gene Spring GX11) (Fig. 8). These results showed feasibility of cell classification by gene expression pattern at a single-cell level. We are now performing large scale experiment.

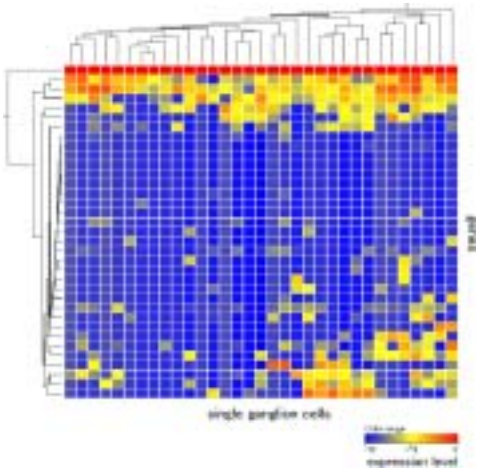


Figure 8. Clustering analysis of gene expression patterns of single-ganglion cells. After normalizing the gene expression levels to rRNA copy number, gene expression patterns of single-ganglion cells were clustered using clustering software. Cells were classified into some groups depending on their gene expression patterns.

4. Publications

4.1 Journals

Roy, M. C., Ikimura, K., Nishino, H. & Naito, T. A high recovery microsampling device based on a microdialysis probe for peptide sampling. *Anal Biochem* 399, 305-307, doi:Doi 10.1016/J.Ab.2009.12.036 (2010).

Nakamura NH., Akiyama, K., Naito T. Quantitative gene expression analysis of the ligand-receptor system for classical neurotransmitters and neuropeptides in hippocampal CA1, CA3 and dentate gyrus. *Hippocampus* (2010, in press).

4.2 Books and other one-time publications

None.

4.3 Oral presentations

None.

4.4 Posters

Naito, T., Akiyama, K., Nakanishi, S., Araki, M., Brenner, S. *Molecular neuroscience of salamander*, The 36th International congress of Physiological Sciences, Kyoto, Japan, July 27 - August 1, 2009

Nakamura NH., Akiyama, K., Naito T. *Cholecystokinin suppresses cAMP-dependent immediate-early gene expression in the hippocampus*, The 32nd Annual Meeting of the Japan Neuroscience Society, Nagoya Congress Center, Nagoya, September 16-18, 2009

Roy, M. C., Ikimura, K., Nishino, H., Naito, T. *A New Type of Micro-Sampling Probe for Quantitative Sampling of Brain Peptides in vivo and its Application for Peptide Profiling of a Rat Brain by NanoLCMS*, The 32nd Annual Meeting of the Japan Neuroscience Society, Nagoya Congress Center, Nagoya, September 16-18, 2009

Akiyama K., Nakanishi, S., Naito T. *Gene expression profiling of mouse cerebellar Purkinje cells*, The 32nd Annual Meeting of the Japan Neuroscience Society, Nagoya Congress Center, Nagoya, September 16-18, 2009

Akiyama K., Nakanishi, S., Nakamura NH., Naito T. *Gene expression profiling of mouse retina*, The 32nd Annual Meeting of the Molecular Biology Society of Japan, Pacifico Yokohama, Yokohama, December 9-12, 2009

Araki, M., Akiyama, K., Naito T. *Classification of neuron types by gene expression at single cell level,* The 32nd Annual Meeting of the Molecular Biology Society of Japan, Yokohama, Japan, December 9-12, 2009

Nakamura NH., Akiyama, K., Naito T. *Cholecystokinin suppresses cAMP-dependent immediate-early gene expression in the hippocampus*, The 32nd Annual Meeting of the Molecular Biology Society of Japan, Pacifico Yokohama, Yokohama, December 9-12, 2009

5. Intellectual Property Rights and Other Specific Achievements

Nothing to report

6. Meetings and Events

6.1 Special lecture at the PREST meeting in Okinawa Title; Molecular Neuroscience of Salamander

Date: September 23-25, 2009

Venue: Zanpa Misaki Royal Hotel

Speaker: Takayuki Naito

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),

•understanding the subcellular localization of Hh signalling components, and how this is regulated by Hh signalling

•elucidating the mechanism of partial degradation of Ci by the proteasome, including an exploration of the evolutionary conservation of Ci processing in primitive metazoans

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

•determining whether Hh plays a role in T-cell proliferation and/or differentiation in adult humans as it does in the early development of T-cells in mice.

1. Staff

Researchers: Dr. Ingo Lehman, Dr. Yawer Hussein, Dr. Spencer Spratt,
Dr. Olga Elisseeva, Dr. Chiemi Spratt
Technical Staff: Dr. Satoshi Hasegawa, Ms. Michiko Arai
Research Administrator /Secretary: Ms. Chika Azama



2. Partner Organizations

None.

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 cells. In vertebrates, the Hh family members pattern tissues such as the limb and neural tube and are involved in the development of many organs. More recently, Hh signalling has been postulated to be involved in the organization of the body plan in basal metazoa such as sea anemone, suggesting that the pathway has ancient evolutionary roots in the animal kingdom. 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. In vertebrates, the role of Ci is played by the three Gli proteins: Gli1, Gli2, and Gli3. Gli1 acts solely as a transcriptional activator and is not processed to a repressor form, while both Gli2 and Gli3 can be processed to repressors. My lab has been focused on the regulation of Ci/Gli, primarily their processing to the repressor form.

During the FY2009, one new post-doc joined the lab, Dr. Olga Elisseeva, and two post-docs left, Drs. Yawer Hussein and Chiemi Spratt (the latter only worked in the lab for one month in FY2009). Other than Dr. Elisseeva's project, our aims remain largely the same as reported in the FY2007 and FY2008 annual reports. To avoid repetition, I have only briefly introduced the recurring topics here; please see the FY2007 report for further information.

Project 1: Identification and characterization of Ci binding partners and post-translational modifications of Ci. Previously, Dr. Alexander Soloviev used tandem affinity purification (TAP) followed by mass spectrometry (MS) to identify novel binding partners for Ci. Dr. Lehmann has continued to develop the TAP-MS approach that Dr. Soloviev initiated in the lab. As Dr. Soloviev previously saw, Dr. Lehmann detects known Ci binding partners (Cos2, fused, Su(fu)) in TAP-Ci complexes, but otherwise there is little overlap between their "hit" lists. Dr. Lehmann is attempting to use a new TAP tag (GS-TAP), which has been shown to improve yield and purity. In order to semi-quantitatively compare Ci binding partners under different conditions (such as the presence and absence of Hh), he will use the ITRAQ method. In this technique the lysines of proteins or peptides from two different samples are labeled with tags having slightly different molecular

weight. The samples are then mixed and analyzed in a single LC/MS/MS run. The fragmentation of the attached tag generates a low molecular mass reporter ion that can be used to relatively quantify the peptides and the proteins from which they originated. To optimize the method, he first tried labeling a mixture of BSA and hemoglobin. The shift in the major band in Figure 1 illustrates that he has successfully labeled the proteins.

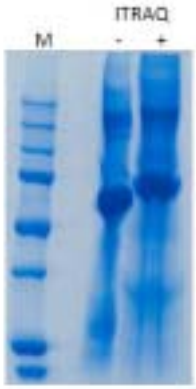


Figure 1: ITRAQ labeling of intact BSA and hemoglobin.

Dr. Lehmann is also using MS approaches to identify post-translational modifications of Ci. In particular, we are interested in determining whether a particular lysine residue (K750) is ubiquitinated. K750 is part of the protection signal that prevents complete degradation of Ci-75 by the proteasome. In order to determine whether this residue is ubiquitinated, Dr. Lehmann will look for a di-glycine signature left by ubiquitin on the peptide after trypsin cleavage. He has not yet been able to detect this signature in Ci peptides, but can see it in the peptide arising from K48 in ubiquitin (Figure 2), a site of polyubiquitin chain formation. This is a good first step toward detecting ubiquitination of Ci peptides.

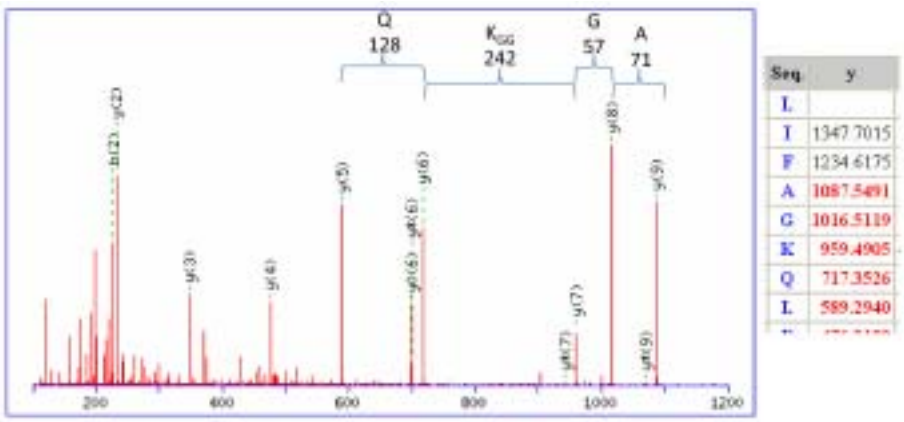


Figure 2: MS/MS spectrum of the LIFAK(GG)QLEDGR peptide of ubiquitin, showing the linkage at K48.

Project 2: Subcellular localization of Hh signalling components. Full-length Ci, like its binding partner Costal2 (a microtubule motor protein), has been reported to partially localize to intracellular vesicles in cultured *Drosophila* cells. In order to determine whether Ci localizes to vesicles in the wing imaginal disc, and if so, to identify these vesicles, Dr. Yawer Hussein examined the localization of full-length Ci (using the 2A1 monoclonal antibody that recognizes only full-length Ci) in wing discs using high-resolution confocal microscopy. He found that some Ci does localize to puncta, which may represent intracellular vesicles. In order to identify the vesicles, and to determine whether Ci puncta are regulated by Hh, he did double-staining with 2A1 and antibodies to vesicle compartment markers such as rab5 (early endosome), rab11 (recycling endosome), and rab7 (multi-vesicular body/lysozome) and examined colocalization both at the far anterior of the disc where there is no Hh signal and at the A/P border where Hh signals. Of the

markers examined so far, Ci appears to partially co-localize with rab5 and this colocalization is regulated by Hh signalling. In the future, we hope to determine whether other Hh signalling components localize in Ci puncta, and whether Ci localization to punta is dependent on other Hh components, such as Costal2. Finally, to determine whether Ci localization to puncta is important for its regulation, we will disrupt Ci localization here and determine whether it affects Ci processing or transcription.

Project 3: Mechanism of Ci partial proteolysis by the proteasome and its evolutionary conservation. Previously, we determined the regions of Ci required for Ci-75 formation, either for initiation of proteasomal degradation or for prevention of the complete degradation of Ci once the proteasome begins degrading the Ci C-terminus. The Ci zinc finger region and a particular lysine residue (K750) together form a protection signal that prevents the complete degradation of Ci by the proteasome. Furthermore, the zinc fingers, the C-terminal domain, and a PKA-, GSK3-, and CK1-phosphorylated region are each required for initiation of proteasomal degradation. Furthermore, we find that most of these regions (the zinc fingers, the phosphorylated region, and the C-terminus) are conserved in Ci from limpet (Lottia gigantea), Ci from a cnidarian (Nematostella vectensis) (Figure 3), and Gli3 from vertebrates (not shown).

Figure 3: Aligment of the C-terminus of Ci from Drosophila melanogaster (Dme), Lottia gigantea (Lgi), and Nematostella vectensis (Nve).

In order to test whether Ci from a basal metazoan is processed, we expressed N-terminally HA tagged coral Ci (another cnidarian) in *Drosophila* Kc cells and determined whether a Ci-75-like product is formed. While we consistently see a product slightly larger than Ci-75, its production is not Hh sensitive (Figure 4). We thus tentatively conclude that coral Ci is not processed in *Drosophila* cells. In this case a negative result is not very informative, since the *Drosophila* version of proteins required for processing may not be compatible with coral Ci (though vertebrate Gli2 and Gli3 are processed in *Drosophila* cells). Thus in the future we would like to study cnidarian Ci processing in cnidarian embryos (most likely Nematostella).

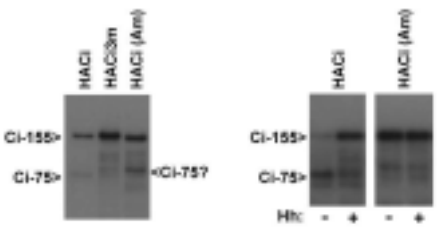


Figure 4: Western blots (with anti-HA antibody) of immunoprecipitates from cells expressing *Drosophila* HACi, HACi3m (lacking 3 PKA sites required for processing), or coral HACi. In the figure on the right, cells have also been transfected with either empty vector or Actin-Hh as indicated.

Project 4: Genome wide RNAi screen to identify novel regulators of Ci proteolysis.
Drs. Hasegawa and Spratt (with the help of Ms. Arai) are continuing with the validation and secondary screens of the ~240 hits Dr. Yifei Wang obtained in his genome-wide RNAi screen for genes involved in Ci processing. Briefly, the assay used in the screen involves monitoring the

Renilla/firefly luciferase ratio of double-stranded RNA (dsRNA) treated cells expressing our Firefly-Ci-Renilla reporter. DsRNAs corresponding to genes required for processing increase the ratio (blue and purple bars, Fig. 5), and dsRNAs corresponding to genes whose absence enhances processing decrease the ratio (red bar, Fig. 5).

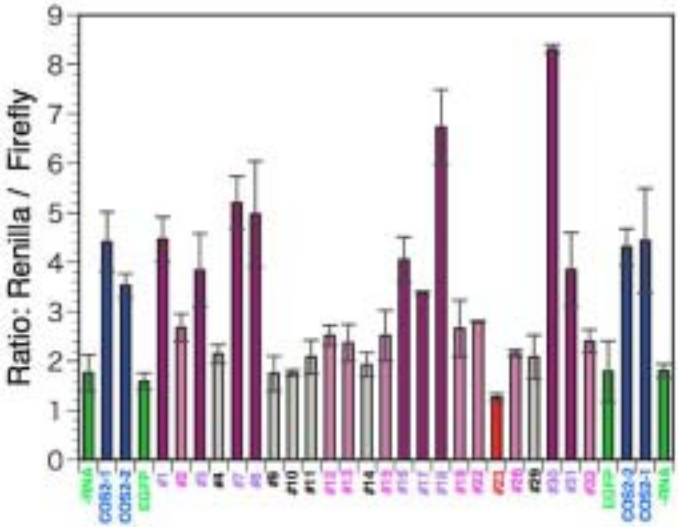


Figure 5: A bar chart showing the Renilla/ Firefly luciferase ratio for negative controls (green), cos2, a positive control (blue), strong hits which block Ci processing (dark purple), weaker hits which block Ci processing (light purple), hits which enhance Ci processing (red), and hits having no effect (gray).

Retesting ~140 dsRNAs used in the initial screen, Dr. Hasegawa finds that 55 block processing (38%) and 31 enhance processing (22%). Dr. Hasegawa is also performing IP-WB assays to test directly for Ci-75 formation. These assays yield examples of genes whose absence blocks processing (Figure 6, #124 and #195), but also genes whose absence stabilizes Ci-155 without affecting Ci-75 (Figure 6, #151, #152, #154, and #157).

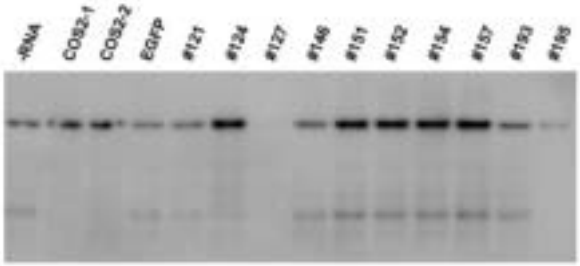


Figure 6: IP-WB of Kc cells expressing HA-Ci treated with no RNA or various dsRNAs, as indicated. The upper band is Ci-155, and the lower is Ci-75. Lanes marked “-RNA” and “EGFP” show the normal extent of Ci processing in these cells.

In the wing imaginal disc, Ci is expressed in the anterior (A) compartment, while Hh is expressed in the posterior (P) compartment. Hh diffuses to cells in the A compartment, near the A/P border, blocking Ci processing and leading to higher levels of full-length Ci in a stripe along the A/P border (Figure 7A). Cells lacking components required for Ci processing located in the A compartment even at a distance from the A/P border will accumulate high levels of full-length Ci. In order to validate our "hits" in vivo, Dr. Spratt is currently using the MS1096 GAL4 line to drive expression in the wing disc of hairpin RNAs corresponding to our “hits”. MS1096 GAL4 drives expression in the wing pouch region of the disc (blue dotted line oval, Figure 7A), with some dorsal bias (the top half of the oval). We have found “hits” whose removal leads to a block of Ci processing (Fig. 7, CK1α control (B), RNAi 1 (D) and RNAi 2 (E)) and “hits” whose knock-down leads to a prevention of the up-regulation of Ci levels at the A/P border (Fig. 7, smo control (C) and RNAi 3 (F)).

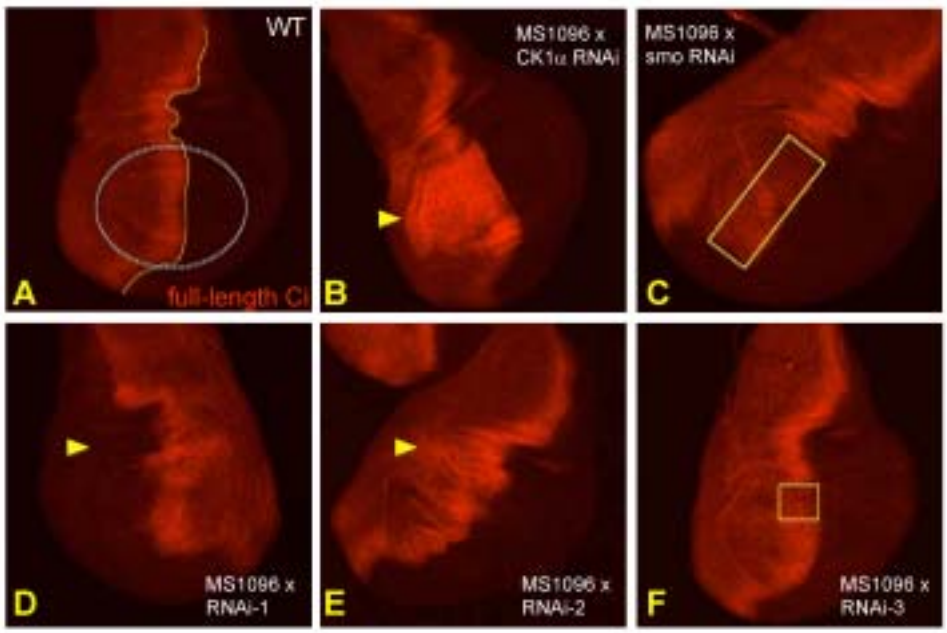


Figure 7: 2A1 (full-length Ci) staining of wing discs expressing hairpin RNAs driven by MS1096 GAL4 in the wing pouch. Anterior is to the left and dorsal is to the top. The yellow dotted line in A marks the A/P boundary and the blue oval marks the wing pouch; the yellow arrowheads in B, D, and E mark ectopic up-regulation of full-length Ci levels; the yellow dotted line boxes in C and F mark areas where full-length Ci levels would normally be up-regulated.

We will continue testing "hits" from our screen (particularly those validated by Dr. Hasegawa's secondary screens) in vivo. Genes that show activity in our assay will be further characterized by epistasis with known pathway genes. For example, RNAi-3 will be combined with CK1α RNAi to determine whether the increase in Ci levels when CK1α is knocked-down depends on the presence of "hit" 3.

Project 5: The role of Hh signalling in T cell differentiation and proliferation. Hh signalling has been shown to be involved in the maturation of T-cells in the thymus in mouse. In order to test whether Hh plays a role in the further differentiation and proliferation of circulating T-cells, Dr. Elisseeva will study T-cells purified from peripheral human blood or umbilical cord blood. She will test whether the expression of a variety of differentiation markers changes when Hh signalling is activated or blocked either on its own or with co-activation of the T-cell receptor signalling pathway (the primary regulator of T-cells). So far, Dr. Elisseeva, who joined the lab in December, has obtained approval for our project from the OIST human subjects ethical committee, and is optimizing conditions for detecting Hh pathway components, Hh pathway targets, and T-cell differentiation markers.

4. Publications

4.1 Journals

None.

4.2 Book(s) and other one-time publications

None.

4.3 Oral presentations

Price, M. A., Wang, Y. *Mechanism of the partial proteolysis of Cubitus interruptus and its evolutionary conservation*, The 9th Japanese Drosophila Research Conference (JDRC9), Tsumagoi, Japan, July 6-8, 2009

Price, M. A., Wang, Y., Arai, M., Hasegawa, S., Spratt, S. J. *The mechanism of Ci repressor formation*, Hedgehog Signaling: From developmental biology to anti-cancer drugs, St. Jean, France, March 27-31, 2010

Price, M. A., Arai, M., Hasegawa, S., Lehmann, I. T., Azama, C. *DNA: the Blueprint of Life*, OIST Open House, OITC, Okinawa Japan, Nov. 15, 2009

Price, M. A., Hussein, Y. S. *Green Fluorescent Protein*, OIST Open House, OITC, Okinawa Japan, Nov. 15, 2009

Price, M. A., Hasegawa, S., Lehmann, I. T., Watanabe, Y., Kasai, S. *DNA: the Blueprint of Life*, Kumoji Community Center, Naha, Okinawa, Japan, February 28, 2010

4.4 Posters

Arai, M., Hasegawa, S., Spratt, S. J., Wang, Y., Price, M. A. *A genome-wide search for novel Hedgehog signaling regulators involved in Ci processing*, The 9th Japanese Drosophila Research Conference (JDRC9), Tsumagoi, Japan, July 6-8, 2009

Price, M. A., Wang, Y. *Mechanism of the partial proteolysis of Cubitus interruptus and its evolutionary conservation*, The 9th Japanese Drosophila Research Conference (JDRC9), Tsumagoi, Japan, July 6-8, 2009

5. Intellectual Property Rights and Other Specific Achievements

Nothing to be reported

6. Meetings and Events

6.1 Seminar

Date: July 29, 2009
Venue: IRP Conference Room
Speaker: Dr. Nick Baker, Albert Einstein College of Medicine
Other remarks: Title: Cell engulfment and cell competition in Drosophila growth, homeostasis, and tumor suppression

6.2 Seminar

Date: April 23, 2009
Venue: IRP Conference Room
Speaker: Dr. Ayako Yamaguchi, Boston University
Other remarks: Title: Sexually distinct vocal pattern generation in African clawed frog

6.3 Seminar

Date: April 24, 2009
Venue: IRP Conference Room
Speaker: Dr. Matthew Wachowiak, Boston University
Other remarks: Title: Seeing what the nose tells the brain:
Active sensing and odor coding imaged in the awake rat and mouse

6.4 Seminar/ mini-symposium

Date: August 5, 2009
Venue: 2F Salon, OITC
Co-organizer: Dr. Ichiro Masai
Speaker: Dr. Akinao Nose
Other remarks: Title: Development and plasticity of motor circuits in Drosophila:
from synapses to function

6.5 Seminar/ mini-symposium

Date: August 5, 2009
Venue: 2F Salon, OITC
Co-organizer: Dr. Ichiro Masai
Speaker: Dr. David Van Vactor, Harvard Medical School
Other remarks: Title: The Receptor Protein Tyrosine Phosphatase LAR regulates Myosin II
function to Influence Axon Guidance

Trans-membrane Trafficking Unit

Principal Investigator:
Fadel A. Samatey
Research Theme:
Functional study of bacterial export apparatus

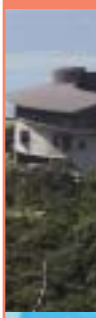
Abstract

Bacteria move in their living environment by the mean of an organelle called the flagellum. The flagellum is widely spread throughout the bacterial world. Flagella come in different sizes; their length and diameter vary among species. They can be polar, peritrichous, and, in some species, sheathed. The first observation of a flagellum obtained by electron microscopy in the early 1940's brought lots of excitement and questions on its structure and from where on the cell it originated. Many of the questions from that time have been answered. The flagellum is a complex structure made by the association of many different proteins. It can be divided into three parts: 1) the filament: a long tubular structure that works as a helical propeller, 2) the hook: a short, highly flexible tubular segment that works as a universal joint, 3) the basal body: a rotary motor embedded in the cell membrane. During the construction of the flagellum, all the flagellar axial proteins are exported from the cytoplasm to the flagellum distal end through a 2 -3 nm channel located at its centre. This export mechanism is regulated by a specialized protein export system located on the cytoplasmic side of the basal body: the type III secretion system (T3SS). In the case of *Salmonella*, this export aparatus is made by six membrane proteins: FlhA, FlhB, FliO, FliP, FliQ, FliR, and three cytoplasmic proteins: FliI, FliH and FliJ. Among these six membrane proteins, FliO is the only one not found in all the different bacteria T3SS, fuelling specifications on whether or not it is essential for substrates export. We, hereafter, present part of our functional study on FliO and preliminary structural study on FlhB.

1. Staff

Researchers: Clive S. Barker, Hideyuki Matsunami, Vladimir Meshcheryakov, Young-Ho Yoon
Technical Staff: Yasuji Kido, Irina Meshcheriakova
Research Administrator / Secretary: Saeko Hedo





2. Partner Organizations

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

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
Name of researchers: Jean-Romé Voulhoux, Gérard Michel
Research theme: Structural Study of the bacterial Type II secretion system

Robert Wood Johnson Medical School, New Jersey, USA
Type of partnership: Collaboration
Name of principal researcher: Dr. Alla Kostyukova
Name of researcher: Alla Kostyukova
Research theme: Structural investigation of disordered proteins

Institut de Biologie Physico-Chimique, CNRS, Paris, France
Type of partnership: Collaboration
Name of principal researcher: Dr. Bruno Miroux
Name of researchers: Bruno Miroux, Jean-Luc Popot, Francesca Zito
Research theme: *In vitro, in vivo* high-yield production of membrane proteins

3. Activities and Findings

3.1 Topology and Functional Characteriization of FliO

The flagellum is the dominant organelle of motility in the Bacteria domain. Export of the components of the flagellum across the cytoplasmic membrane requires a specialized secretion apparatus, which shares homology to the type III secretion apparatus of the bacterial needle used by some Gram-negative bacteria in pathogenesis (Macnab, 2004). The type III secretion system of the *Salmonella* flagellum consists of 6 integral membrane proteins: FlhA, FlhB, FliO, FliP, FliQ, and FliR. FlhA, FlhB, FliP, and FliR have been shown by biochemical or genetic techniques to be present in the flagellar basal body (Fan et al., 1997; Kihara et al., 2001; Van Arnam et al., 2004). FlhA and FlhB are large proteins, 692-amino acid residues, and 383-amino acid residues, respectively, and both have large C-terminal cytoplasmic domains. The atomic structures of the cytoplasmic domains of FlhA, and close virulence type III secretion system homologs of FlhB, have recently been published (Deane et al., 2008; Lountos et al., 2009; Saijo-Hamano et al., 2010;

Zarivach et al., 2008). However, it is not known how they work. Even less is understood about FliOPQR. FliP, FliQ, and FliR are predicted to be predominately embedded in the cytoplasmic membrane. We predicted the membrane topology of the 125-amino acid residue FliO protein using various topology prediction programs, and it is expected to have a bitopic membrane topology with an approximately 80 residue C-terminal cytoplasmic domain. Since, it was predicted to have a large cytoplasmic domain like FlhA and FlhB, it suggests that this protein has an important role in transport so it seemed important to investigate the function of this protein. Furthermore, in some other type III secretion systems a homologue of FliO is apparently absent, suggesting it might be an accessory protein with a specialized role in Salmonella flagellum assembly.

Firstly, we experimentally confirmed the predicted membrane topology of FliO using alkaline phosphatase reporter gene fusions at permissive sites into the FliO protein (Figure 1). Alkaline phosphatase is known to be active when found in the periplasm, and fusions to membrane proteins can be used to determine topology since a blue pigmentation is produced when alkaline phosphatase is found in the periplasm for bacteria inoculated onto L-agar plates containing 5-Bromo-4-chloro-3-indolyl phosphate (X-Pho) (Manoil and Beckwith, 1986).

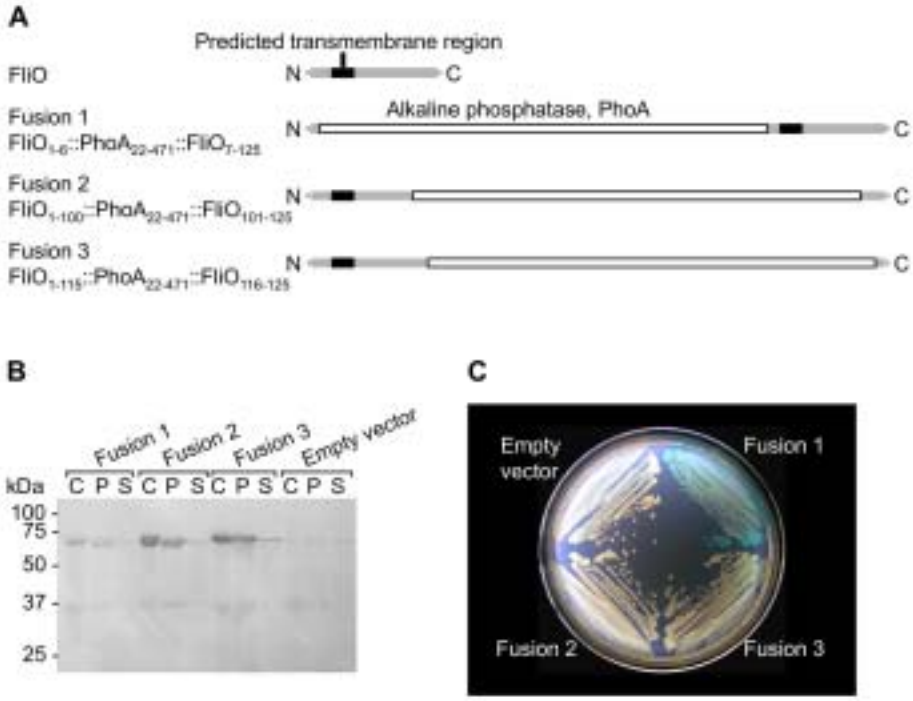


Figure 1. Determination of the membrane topology of FliO. (A) A schematic overview of different FliO/alkaline phosphatase chimeric fusions is shown. (B) Immunoblotting using anti-alkaline phosphatase antibody of strains expressing the FliO/alkaline phosphatase chimeras (60.3 kDa): C, whole cells; P, insoluble membrane pellet fraction; S, soluble supernatant fraction. (C) The chimeric fusions were expressed in cells from plasmid pTrc99A-FF4 on L-agar containing 40 μg ml^{-1} 5-Bromo-4-chloro-3-indolyl phosphate to detect alkaline phosphatase activity after overnight incubation at 37ºC.

We then constructed a Δ*fliO* mutant as a background for complementation analysis to understand the important regions of the protein. It revealed that deletion of the *fliO* gene from the chromosome of a motile strain of *Salmonella* resulted in a drastic decrease of motility, but the cells

were not completely non-motile. Furthermore, incubation of the Δ*fliO* mutant strain in motility agar, gave rise to pseudorevertants containing extragenic bypass mutations that help to restore motility. One group of bypass mutations was located in the *fliP* gene; either FliP(R143H) or FliP(F190L) mutations were encoded (Figure 2). The FliP(R143H) mutation was not responsible for the improved motility by itself and another secondary mutation must be encoded since an engineered ΔfliO fliP(R143H) mutant was not as motile as the isolated pseudorevertant, but forms pseudorevertants after a much earlier time. However, an engineered ΔfliO fliP(F190L) mutant was as motile as the isolated pseudorevertant, so the FliP(F190L) mutation was responsible for the improved motility. This result suggested that FliO and FliP naturally interact with each other, either directly, or indirectly through another protein. Since, FliP has been shown to be present in the final secretion apparatus it indicates FliO is at least involved in secretion apparatus assembly.

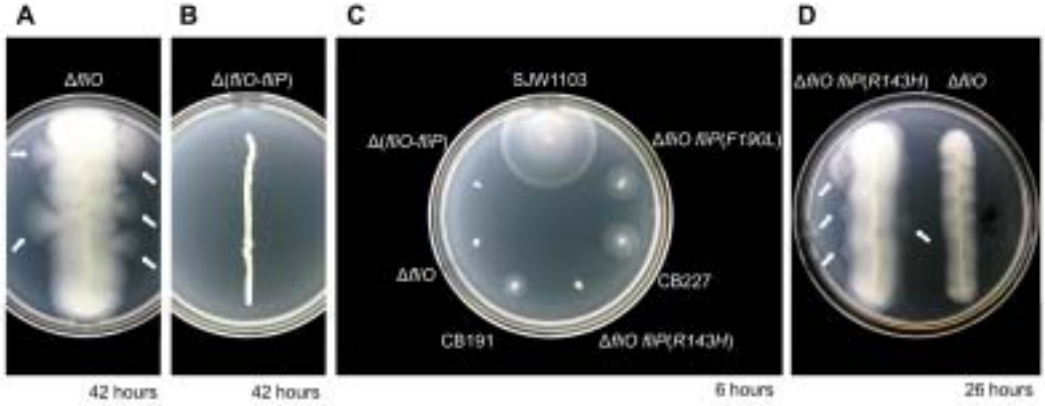


Figure 2. Isolation of motile pseudorevertant strains from a poorly motile ΔfliO strain. (A) Incubation of strain CB186 (ΔfliO22252) in soft-tryptone motility agar after 42 hours. The strain is extremely poorly motile, but outgrowths of pseudorevertants containing suppressor mutations can be observed as motility halos (indicated by white arrows). (B) Incubation of a non-motile strain, CB184 (Δ[fliO-fliP]22251), in motility agar after 42 hours. (C) Comparison of motility of pseudorevertant strains after 6 hours in motility agar: SJW1103 is wild-type for motility, CB191 is a pseudorevertant strain and encodes ΔfliO fliP(R143H), CB227 is a pseudorevertant strain and encodes ΔfliO fliP(F190L). Other strains were engineered mutants constructed from SJW1103 by λ-Red genetic engineering (D) Formation of pseudorevertants (indicated by white arrows) by the ΔfliO fliP(R143H) engineered mutant strain after 26 hours. All plates were incubated at 30°C.

To determine which regions of FliO are important for function we performed N- and C-terminal truncation analysis of FliO, and examined the ability of the proteins to complement the ΔfliO mutant for motility in soft-tryptone agar. We showed that $FliO_{22\text{-}125}$, or $FliO_{1\text{-}95}$ were able to rescue motility when expressed from a plasmid vector similar to full-length protein (Figure 3). Over-expression of the cytoplasmic domain only of FliO, $FliO_{43\text{-}125}$, also was able to partially restore motility. Based on secondary structure prediction the cytoplasmic domain, $FliO_{43\text{-}125}$, should contain beta-structure and alpha-helices. $FliO_{43\text{-}125}$-Ala was purified and studied using circular dichroism spectroscopy, however, we showed that this domain is disordered and its structure is a mixture of beta-sheet and random coil (Figure 4). Presumably, it becomes structured while interacting with its binding partners. When $FliO_{43\text{-}125}$ was overexpressed in the ΔfliO fliP(R143H) or ΔfliO fliP(F190L) engineered mutants an additive effect was observed, and near wild-type levels of motility were regained. Therefore, the FliP(R143H) and FliP(F190L) bypass mutations have not fully replaced the function of the cytoplasmic domain of FliO. We suggest that the transmembrane region of FliO, is responsible for the interaction with FliP, and also the transmembrane region of

FliO is important for localizing the cytoplasmic domain to the cytoplasmic membrane. A summary of our findings is presented (Figure 5).

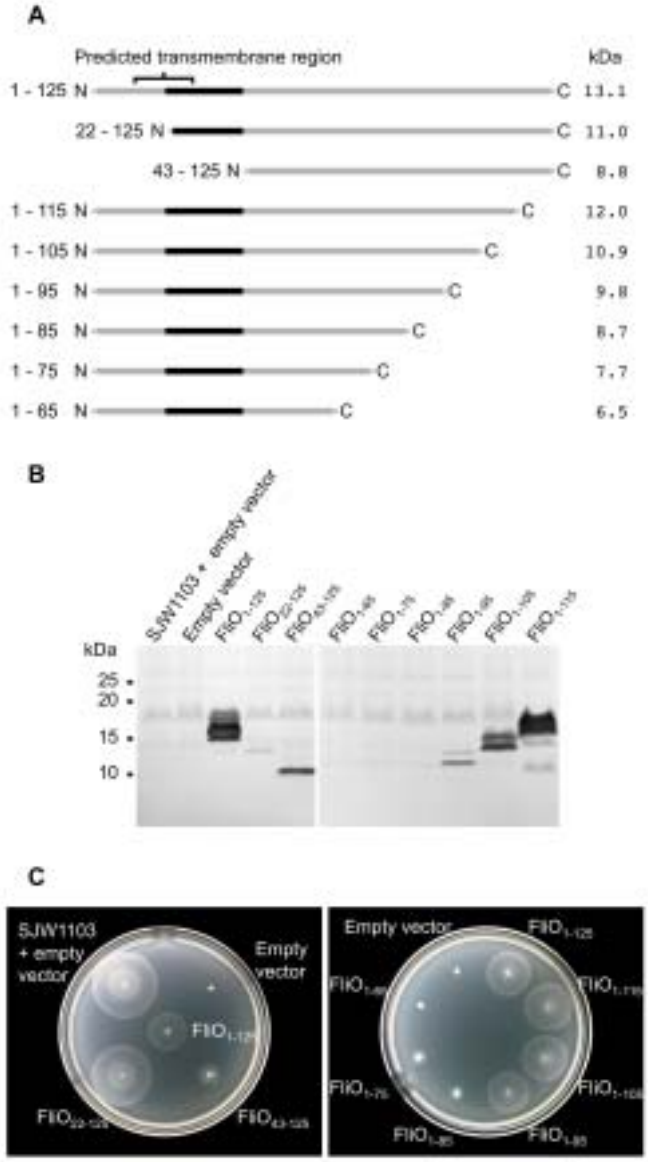


Figure 3. Complementation of a ΔfliO strain by FliO truncated at the N-terminus or C-terminus. (A) A schematic overview of the different FliO deletion mutants is shown with the corresponding molecular weight of the protein. (B) Immunoblotting of 10-20% gradient SDS-PAGE separated whole cell lysates of the wild-type strain SJW1103, containing empty pTrc99A-FF4 vector, or strain CB186 (DfliO22252) containing empty vector, or expressing the FliO mutants from plasmid pTrc99A-FF4 (without IPTG induction) using polyclonal anti-$FliO_{43-125}$-6xHis antibody. (C) Complementation of strain CB186 by the FliO deletion mutants expressed from plasmid pTrc99A-FF4 (without IPTG induction) in soft-tryptone motility agar plates incubated for 6 hours at 30 °C after inoculation with bacteria.

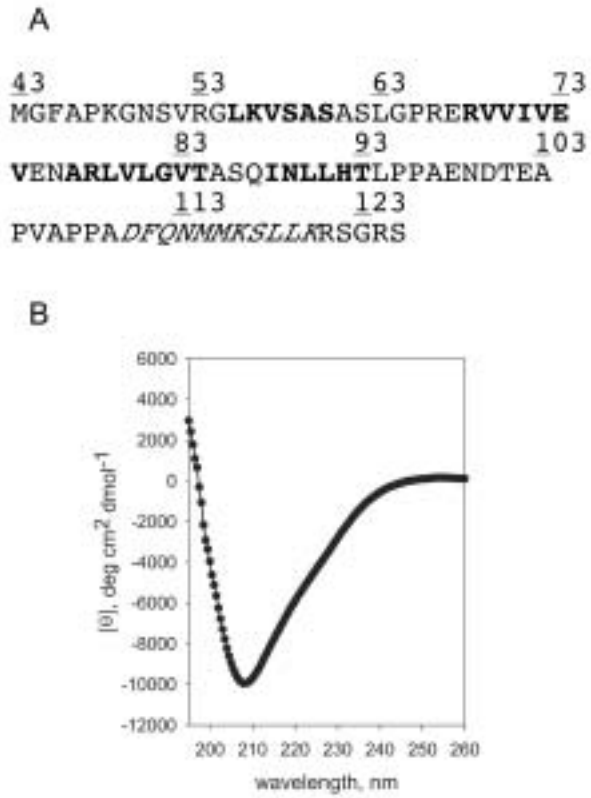


Figure 4. Circular Dichroism (CD) Spectroscopy analysis of the cytoplasmic domain of FliO. (A) Predicted regions of secondary structure of $FliO_{43-125}$: Bold, regions with predicted β-structure; Italic, regions with predicted α-helix. (B) CD spectrum (mean residue ellipticity vs wavelength) of $FliO_{43-125}$-Ala in 20 mM Na/K phosphate, pH 6.2; 100 mM NaCl.

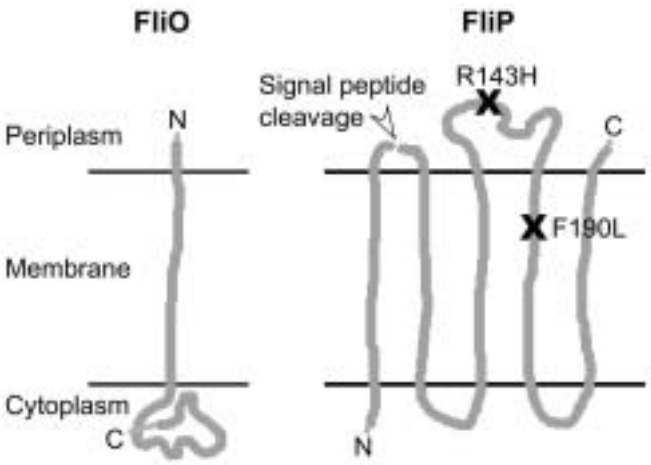


Figure 5. Membrane topology of FliO, and location of bypass mutations in FliP that partially restore motility to a Salmonella serovar Typhimurium ΔfliO mutant.

3.2 Structural study of the cytoplasmic domain of FlhB

The flagellar export apparatus consists of six membrane proteins, FlhA, FlhB, FliO, FliP, FliQ, and FliR, and three cytoplasmic proteins, FliH, FliI, and FliJ. The export apparatus is responsible not

only for translocation of flagellar proteins across cell membrane but it also regulates the order in which the proteins are exported. The membrane protein FlhB has been found to play a key role in the regulation process.
This protein is an about 40 kDa and consists of two domains: the hydrophobic N-terminal part that is predicted to have four transmembrane helices and the C-terminal cytoplasmic domain. FlhB homologues were found in all type III secretion systems.

Recently, several different structures of the cytoplasmic domain of FlhB paralogues from bacterial needles have been published. Sequence homology (about 20% of identity and 35% of similarity) between flagellar FlhB and its needle paralogue assumes that general spatial organization may be the same for the two types of the protein. Nevertheless there are also differences. Thus it was shown in our unit that SsaU and SpaS proteins (FlhB paralogues from *Salmonella typhimurium* needles) couldn't substitute *Salmonella* flagellar FlhB (C. Barker *et al.,* unpublished observations). To reveal the difference between flagellar FlhB and its needle paralogue we attempted to get crystal structure of cytoplasmic domain of *Salmonella* FlhB.
Cytoplasmic fragment of FlhB from *S. typhimurium* has been purified and successfully crystallized. The crystals obtained diffract beyond 2.8Å. Structural determination is in progress.

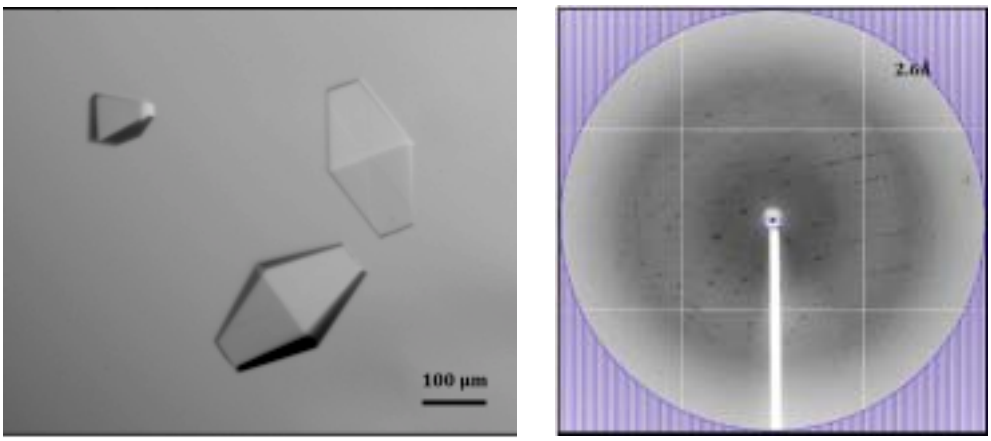


Figure 6. Crystals and diffraction pattern of FlhB cytoplasmic fragment from S. *typhimurium*.

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4. Publications

4.1 Journals

None.

4.2 Book(s) and other one-time publications

Samatey, F. A. in *PILI and Flagella: Current Research and Future Trends* (ed K. Jarrell) Ch. 9, 137-153 (Caister Academic Press, 2009).

4.3 Oral presentations

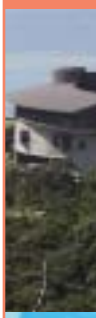
Matsunami, H. *X-ray crystal structure reveals the self-assembly mechanism of the bacterial hook capping protein*, 16th Japanese Flagellar Meeting, Nagoya, Japan, March 14-16, 2010

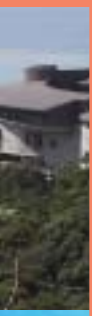
Barker, C. S. *Coexpression of the cytoplasmic domain of FliO, and suppressors in FliP can restore motility to a Salmonella enterica fliO mutant*, 16th Japanese Flagellar Meeting, Nagoya, Japan, March 14-16, 2010

4.4 Posters

Matsunami, H. *Crystal Structure and Mutational Analysis of the Periplasmic Flagellar Protein Flga*, Biophysical Society, San Francisco, U.S.A., February 20-24, 2010

Matsunami, H. *X-ray Crystal Structure reveals the self-assembly mechanism of the Bacterial Hook Capping Protein (in Japanese)*, Flagella Meeting, Nagoya, Japan, March 14-16, 2010





Barker, C. S. *Flio is not required for Motility in Salmonella if its cytoplasmic Domain and FliP Mutant Suppressores are Expressed*, Biophysical Society, San Francisco, U.S.A., February 20-24, 2010

Barker, C. S. *Coexpression of the cytoplasmic domain of Flio, and suppressors in FliP can restore motility to a Salumonella enterica fliO mutant*, Flagella Meeting, Nagoya, Japan, March 14-16, 2010

5. Intellectual Property Rights and Other Specific Achievements

Nothing to be reported

6. Meetings and Events

6.1 Seminar

Date: April 21, 2009
Venue: IRP Conference Room
Speaker: Dr. Young-Ho Yoon (Yokohama City University)
Other remarks: Title of the seminar : "Expression and structure analysis of malaria circumsporozoite protein using E.coli for drug and vaccine development"

6.2 Seminar

Date: December 14, 2009
Venue: IRP Conference Room
Speaker: Prof. Shin-Ichi Aizawa (Prefectural University of Hiroshima)
Other remarks: Title of the seminar : "Unusual flagella in the beautiful flagellar world"

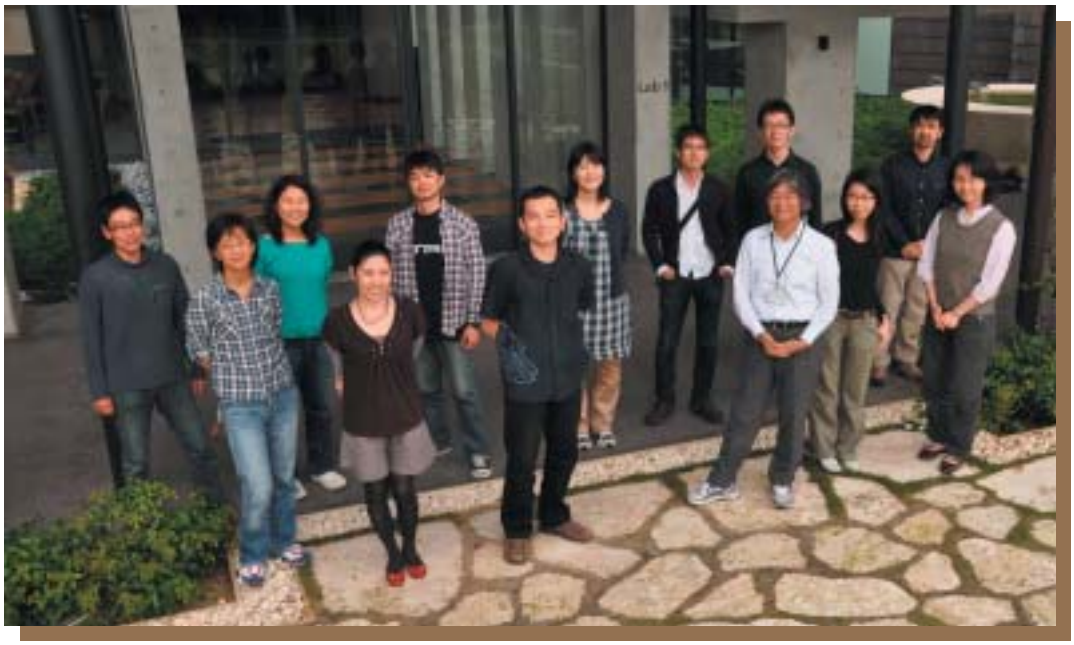
6.3 Seminar

Date: December 17, 2009
Venue: IRP Conference Room
Speaker: Dr. Alla Kostyukova (Robert Wood Johnson Medical school)
Other remarks: Title of the seminar : "Isoform Dependent Tropomodulin/Tropomyosin Binding as a Regulatory Mechanism"

Marine Genomics Unit

Principal Investigator:
Noriyuki Satoh

Research Theme:
Evolutionary, Developmental, and Environmental Genomics of Marine Invertebrates



Abstract

The genome contains all the genetic information of a given organism. Decoding the genome therefore provides the molecular basis for understanding every biological phenomenon. Since the turn of the 21st century, genomes of various metazoans have been sequenced, and consequently studies progressed efficiently in the fields of evolutionary biology, developmental biology, and environmental biology. The objective of the Marine Genomic Unit is to decode the genomes of target marine invertebrates so as to comprehensively elucidate the molecular mechanisms underlying the evolution, development, and environmental responses of marine animals. The major achievements of the Unit in fiscal year 2009 were as follows.

(a) Evolutionary genomics: We are interested in the origin and evolution of chordates. The phylum Chordata consists of three subphyla: urochordates (ascidians), cephalochordates (amphioxus), and vertebrates. The evolution of chordates has been debated for more than 150 years, since Charles Darwin proposed his idea of animal evolution by natural selection. To investigate this enigmatic question, we decoded the genome of the urochordate *Ciona intestinalis* in 2002 and that of the cephalochordate *Branchiostoma floridae* in 2008. Comparison of the genomes with those of echinoderms and vertebrates revealed that cephalochordates are basal among chordates, suggesting that chordates share a free-living ancestor. This year, we were able to show that shuffling of protein domains has played a significant role in the evolution of vertebrates from a common ancestor of chordates.

(b) Developmental genomics: The notochord is the most prominent feature of chordates, and these organisms were named after this organ. Previously we revealed that a T-box transcription factor, Brachyury (Bra), plays a pivotal role in the formation of the notochord in *Ciona* embryos and that Ci-Bra regulates more than 400 downstream genes that are involved in the formation of the notochord. This year we focused on the function of one of these downstream genes, which is to encode a fibrinogen-like protein (Ci-fibrn). We showed that Ci-fibrn interacts with CNS-derived

Notch and this interaction is essential for the localization of neuronal cells in the dorsal region of the larval head.

(c) Environmental genomics: The coral reefs of the Okinawa islands are amongst the most biologically diverse ecosystems in the world. The key organisms in their establishment, the scleractinian corals, increasingly face a range of human-caused challenges including ocean acidification and seawater temperature rises. In 2009, most of my Unit members have contributed to decoding the genome of the coral *Acropora digitifera*, an organism that suffered one of the highest mortality rates following the anomalous seawater warming that occurred in the summer of 1998. With the aid of next-generation sequencing technologies, we have almost decoded the coral's entire 420-Mbp-long genome.

1. Staff

Researchers: Eiichi Shoguchi, Takeshi Kawashima, Mayuko Hamada, Chuya Shinzato, Atsuko Sato
Technical Staff: Ken Maeda, Kanako Hisata, Makiko Tanaka, Mayuki Fujiwara
Research Assistant / Graduate Student: Shigehiro Yamada, Naoki Shimozono
Research Administrator / Secretary: Shoko Yamakawa

2. Partner Organizations

University of California at Berkeley, USA
Type of partnership: Collaboration
Name of researcher: Daniel S. Rokhsar
Research theme: Decoding of the hemichordate genome

CNRS & UPMC, Roscoff Biological Station
Type of partnership: Collaboration
Name of esearcher: Francois Lallier
Research theme: Analyses of genomes and transcriptomes of deep-sea mussel

National Institute for Basic Biology, Japan
Type of partnership: Collaboration
Name of researcher: Hiroki Takahashi
Research theme: Analysis of *Ci-Bra* target genes

Shimoda Marine Research Center, University of Tsukuba, Japan
Type of partnership: Collaboration
Name of researcher: Yasunori Sasakura
Research theme: Analysis of *Ciona* genes with transgenic lines

Graduate School of Life and Environmental Scineces, University of Tsukuba, Japan
Type of partnership: Collaboration
Name of principal researcher: Hiroshi Wada
Research theme: Analysis of the evolution of chordate genomes

3. Activities and Findings

3.1 Evolutionary Genomics:

Vertebrate evolution has included a number of important events: the development of cartilage, the immune system, and complicated craniofacial structures. This past year, we examined domain shuffling as one of the mechanisms that contributed the novel genetic material required for vertebrate evolution. We mapped domain-shuffling events during the evolution of deuterostomes, with a focus on how domain shuffling contributed to the evolution of vertebrate- and chordate-specific characteristics. We identified approximately1000 new domain pairs in the vertebrate lineage, including approximately 100 that were shared by all seven of the vertebrate species examined (Figure 1). Some of these pairs occur in the protein components of vertebrate-specific structures, such as cartilage and the inner ear. This suggests that domain shuffling contributed significantly to the evolution of vertebrate-specific characteristics.

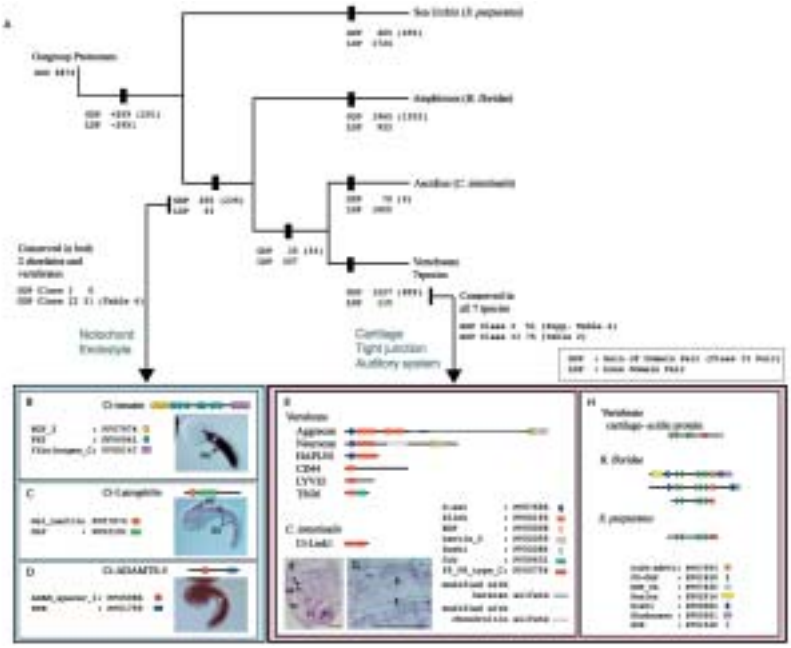


Figure 1: Domain shuffling during the evolution of deuterostomes. (A) Gain and loss of the domain pairs were mapped on the deuterostome phylogenetic tree constructed using molecular phylogenetic analyses of multiple gene sequences (Putnam et al. 2008). Numbers on each node present gains and loss of domain pairs after screening CI < 0.5. Gains of Class II domain pairs are shown in brackets. (Arrows) Domain pairs acquired in the ancestral chordates, and those in ancestral vertebrates, that were examined in detail. Among 1227 pairs that were acquired in the ancestral vertebrates, 51 class I domain pairs and 75 class II domain pairs were conserved in seven vertebrate species examined. Some of these were involved in vertebrate-specific characters such as cartilage (E–H), tight junctions, and auditory systems. Among 256 domain pairs unique to the chordates, 31 pairs were conserved in ascidian, amphioxus, and more than one species of vertebrates. Some of them were involved in chordate-specific characters such as the notochord (B–D) and endostyle. (B–D) Tenascin, latrophilin, and ADAMTS-5, which were created by domain shuffling in ancestral chordates, are expressed in the notochords of ascidian embryos. Symbols are indicated with the domain ID and Pfam accession numbers (Finn et al.

2006). (B) Ci-tenascin is expressed in the ascidian notochord (N) and muscle cells (Mu). (C) Ci-latrophilin expression was detected in the notochord, neural tube (NT), and endodermal strand (ES) of ascidian larvae. (D) Expression of Ci-ADAMTS-5 was restricted to the ascidian notochord. (E) Domain structures of the proteins that include an Xlink domain. (F, G) Expression of Ci-link1 was observed in some of the blood cells from an ascidian juvenile. (Arrowheads) Some of the Ci-link1-positive cells. Scale bars, 50 mm. (En) Endostyle, (GS) gill slits, (St) stomach, (It) intestine. (H) Domain structures of cartilage acidic protein and proteins containing an ASPIC-and-UnbV domain encoded by amphioxus gene models. (From Kawashima *et al.,* Genome Res. 19: 1393-1403, 2009)

The evolutionary history of domain pairs is traceable. For example, the Xlink domain of aggrecan, one of the major components of cartilage, was originally a functional domain of a blood cell surface molecule in protochordate ancestors, and was recruited by a cartilage matrix component protein in the vertebrate ancestor. We also identified genes that were created as a result of domain shuffling in ancestral chordates. Some of these are involved in the functions of chordate structures, such as the endostyle, Reissner's fiber of the neural tube, and the notochord. Therefore, this analysis shed new light on the role of domain shuffling, especially in the evolution of vertebrates and chordates.

3.2 Developmental Genomics:

We have been carrying out developmental genomics of the ascidian *Ciona intestinalis.* After decoding the genome in 2002, we showed that the *Ciona* genome contains approximately 700 genes for transcription factors. Of them, a T-box transcription factor, Brachyury (Bra), plays a pivotal role in the formation of the notochord in *Ciona* embryos, and Ci-Bra regulates more than 400 downstream genes that are involved in the formation of the notochord. This year we focused on the function of one of the downstream genes, which encodes a fibrinogen-like protein (Ci-fibrn). The notochord and its overlying dorsal neural tube are the most prominent features of the chordate body plan. Although the molecular mechanisms involved in the formation of the central nervous system (CNS) have been studied extensively in vertebrate embryos, none of the genes that are expressed exclusively in notochord cells has been shown to play a role in this process. The examination of the expression and function of Ci-fibrn resulted in an interesting suggestion regarding notochord-CNS interaction (Figure 2).

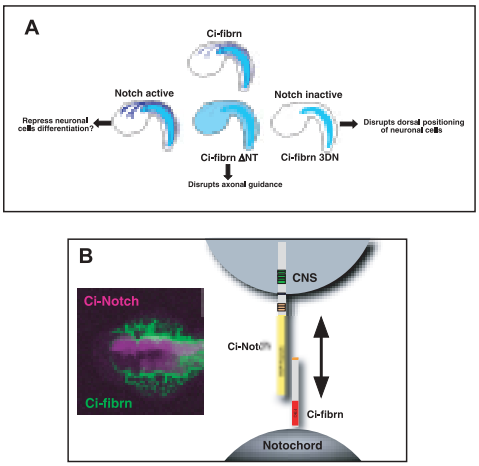


Figure 2: Model of proposed Ci-fibrn and Ci-Notch interactions. (A) Diagram illustrating results obtained in the study of Yamada *et al.* (Develop. Biol., 328:1-12, 2009). (B) Diagram illustrating the role of Notch signaling in the function of Ci-fibrn.

Ci-fibrn is expressed exclusively in *Ciona* embryo notochord cells but its protein product is not confined to these cells, but rather is distributed beneath the CNS as fibril-like protrusions. In *Drosophila* embryos it has been shown that a fibrinogen-like protein interacts with Notch and this interaction plays a role in the formation of compound eyes. We also demonstrated that Ci-fibrn interacts physically and functionally with the Ci-Notch expressed in the CNS. With the aid of various Ci-fibrn deletion constructs and chemical reagents that affect the Ci-fibrn function, we were able to show that correct distribution of the Ci-fibrn protein is dependent on Notch signaling. Disturbance of the Ci-fibrn distribution caused an abnormal positioning of neuronal cells and abnormal axon extension tracking. Therefore, it is highly likely that the interaction between the notochord-based fibrinogen-like protein and neural tube-based Notch signaling plays an essential role in the proper patterning of the CNS. This is the first demonstration in chordates of a significant role of interaction between a notochord-specific molecule and a CNS-derived molecule.

3.3 Environmental Genomics:

Coral reefs are amongst the most biologically diverse ecosystems on the planet, estimated to harbour around one-third of all described marine species, and their productivity supports around one-quarter of marine fisheries. Reef-building corals (Scleractinia) are anthozoan cnidarians, and have been the major architects of coral reefs since the mid-Triassic. Their spectacular ecological success is attributed to their forming obligate endosymbioses with dinoflagellates of the genus *Symbiodinium*. Much of the detail of the interaction between coral host and dinoflagellate symbiont is unclear, but this association enables the massive rates of calcification that distinguish reef-building corals from other anthozoans such as sea anemones and zoanthids. Photosynthesis by the symbionts enables the coral holobiont to become essentially phototrophic, but restricts the association to the warm shallow waters of the tropics. Moreover, corals operate near their upper thermal tolerance, and the symbiosis collapses under elevated seawater temperatures, leading to mass coral bleaching (expulsion of symbionts) and mortality. Such bleaching events have become increasingly common and will continue to do so. Furthermore, they are exacerbated by high levels of solar irradiation. Declines in coral abundance lead to wholesale loss of reef habitats and this is one of the most pressing environmental problems of our time. Our ability to address coral decline is limited by a lack of basic molecular data on corals and their symbionts, and our poor

understanding of many aspects of the interactions between the two partners. Therefore, this year, my Unit has spent most of its research time decoding the coral genome. We selected *Acropora digitifera* as a target for genome sequencing because this is a dominant species in Okinawan reefs (Figure 3). *A. digitifera* suffered amongst the highest mortality rates as a result of the exceptional seawater warming that occurred in the summer of 1998.

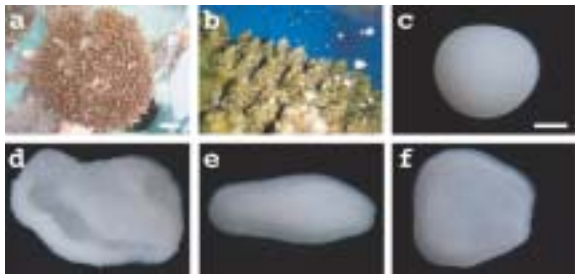


Figure 3: The coral *Acropora digitifera*. a, The colony for which the genome was decoded in the present study. This colony is maintained in aquarium culture at the Sesoko Station, University of the Ryukyus, and is thus available for further investigation of the genome. Scale bar, 5 cm. b, Natural spawning of the coral. c-f, Eggs, embryos, larva and primary polyp of *A. digitifera*, from which mRNA was extracted for transcriptome analyses. Scale bar, 200 mm. c, Fertilized egg; d, blastula at the prawn chip stage; e, planula larva; and f, primary polyp.

OIST has next-generation sequencing machines, namely the Roche 454 GS-FLX and Illumina Genome Analyzer IIx. With the aid of next-generation sequencing technologies, we decoded the approximately 420-Mbp genome of *Acropora digitifera*. We obtained ~12-fold coverage of the *A. digitifera* genome sequences. After assembling the sequences, we generated a set of gene models by employing gene prediction programs. Our results showed that the genome contains approximately 21,000 protein-coding genes. We are currently attempting to identify the coral genes involved in the establishment of symbiosis, those associated with response to environmental changes, and those responsible for mineralization.

4. Publications

4.1 Journals

Chiba, S., Jiang, D., Satoh, N. & Smith, W. C. *brachyury* null mutant-induced defects in juvenile ascidian endodermal organs. *Development* 136, 35-39, doi:Doi 10.1242/Dev.030981 (2009).

Ikuta, T., Miyamoto, N., Saito, Y., Wada, H., Satoh, N. & Saiga, H. Ambulacrarian prototypical *Hox* and *ParaHox* gene complements of the indirect-developing hemichordate *Balanoglossus simodensis. Dev Genes Evol* 219, 383-389, doi:DOI 10.1007/s00427-009-0298-4 (2009).

Kawashima, T., Kawashima, S., Tanaka, C., Murai, M., Yoneda, M., Putnam, N. H., Rokhsar, D. S., Kanehisa, M., Satoh, N. & Wada, H. Domain shuffling and the evolution of vertebrates. *Genome Res* 19, 1393-1403, doi:DOI 10.1101/gr.087072.108 (2009).

Keduka, E., Kaiho, A., Hamada, M., Watanabe-Takano, H., Takano, K., Ogasawara, M., Satou, Y., Satoh, N. & Endo, T. M-Ras evolved independently of R-Ras and its neural function is conserved between mammalian and ascidian, which lacks classical Ras. *Gene* 429, 49-58, doi:DOI 10.1016/j.gene.2008.10.001 (2009).

Nakayama-Ishimura, A., Chambon, J. P., Hofie, T., Satoh, N. & Sasakura, Y. Delineating metamorphic pathways in the ascidian *Ciona intestinalis. Developmental Biology* 326, 357-367, doi:DOI 10.1016/j.ydbio.2008.11.026 (2009).

Noda, T., Hamada, M., Hamaguchi, M., Fujie, M. & Satoh, N. Early zygotic expression of transcription factors and signal molecules in fully dissociated embryonic cells of *Ciona intestinalis*: A microarray analysis. *Dev Growth Differ* 51, 639-655, doi:DOI 10.1111/j.1440-169X.2009.01124.x (2009).

Paix, A., Yamada, L., Dru, P., Lecordier, H., Pruliere, G., Chenevert, J., Satoh, N. & Sardet, C. Cortical anchorages and cell type segregations of maternal postplasmic/PEM RNAs in ascidians. *Developmental Biology* 336, 96-111, doi:DOI 10.1016/j.ydbio.2009.09.001 (2009).

Sasakura, Y., Inaba, K., Satoh, N., Kondo, M. & Akasaka, K. *Ciona intestinalis* and *Oxycomanthus japonicus,* Representatives of Marine Invertebrates. *Experimental Animals* 58, 459-469 (2009).

Satoh, N. An Advanced Filter-Feeder Hypothesis for Urochordate Evolution. *Zoological Science* 26, 97-111, doi:Doi 10.2108/Zsj.26.97 (2009).

Satou, Y., Satoh, N. & Imai, K. S. Gene regulatory networks in the early ascidian embryo. *Biochimica Et Biophysica Acta-Gene Regulatory Mechanisms* 1789, 268-273, doi:DOI 10.1016/j.bbagrm.2008.03.005 (2009).

Yamada, S., Hotta, K., Yamamoto, T. S., Ueno, N., Satoh, N. & Takahashi, H. Interaction of notochord-derived fibrinogen-like protein with Notch regulates the patterning of the central nervous system of *Ciona intestinalis* embryos. *Developmental Biology* 328, 1-12, doi:10.1016/j.ydbio.2008.12.037 (2009).

4.2 Books and other one-time publications

Satoh, N. *Analysis of the Amphioxus Genome in Encyclopedia of Life Sciences* (John Wiley & Sons, Ltd, 2009).

4.3 Oral presentations

Kawashima, T. *Evolution of Metazoan Development revealed by Comparative Genomics*, The Medical Research Institute of Tokyo Medical and Dental University, Tokyo, Japan, April 28, 2009

Satoh, N. *An advanced filter-feeder hypothesis for urochordate evolution*, 5th International Tunicate Meeting. Okinawa Industry Support Center, Okinawa, Japan, June 21-25, 2009

Satoh, N. "今ここにあるということ", 第48回九州高等学校理科教育研究会沖縄大会). Pacific Hotel Okinawa, Okinawa, Japan, July 30, 2009

Shinzato, C., Satoh, N. *The coral Acropora digitifera genome project*, The International Workshop on"The Evolution of Multicellularity."Tutzing, Germany, September 14-17, 2009

Satoh, N. *Gene network underlying the formation of notochord and the origin of chordates*, International Titisee Conferences, Genome evolution and the origin of novel gene functions. Schwarzwald hotel Titisee, Germany, October 14-18, 2009

Shoguchi, E. *Chromosomal map of organ-enriched genes and regulatory genes reveals a dispersed pattern of small gene-clusters in the ascidian Ciona intestinalis*, International Symposium on Marine Genomics 2009. Hotel Southern Plaza Kaiho, Okinawa, December 15-18, 2009

Satoh, N. *Genetic cascade underlying the formation of notochord in urochordate embryos*, International Symposium on Marine Genomics 2009. Hotel Southern Plaza Kaiho, Okinawa, December 15-18, 2009

Shinzato, C. *The coral Acropora digitifera genome project*., International Symposium on Marine Genomics 2009. Hotel Southern Plaza Kaiho, Okinawa, December 15-18, 2009

4.4 Posters

Shoguchi, E., Fujie, M., Satoh, N. *Chromosomal distribution of regulatory genes and organ-expressed genes in the ascidian Ciona intestinalis*, 5th International Tunicate Meeting. Okinawa Industry Support Center, Okinawa, Japan, June 21–25, 2009

Hamada, M., Shimozono, N., Ohta, N., Horie, T., Sasakura, Y., Fujie, M., Satoh, N. *Transcriptome analysis of brain-specific genes in the Ciona intestinalis larva*, 5th International Tunicate Meeting. Okinawa Industry Support Center, Okinawa, Japan, June 21–25, 2009

Tagawa-Sasaki, A., Humphreys, T., Kawashima, T., Tagawa, K. *Analysis of genes highly expressed in the cDNA library of regenerating bud i nthe hemichordates Ptychodera flava*, The 80th Annual Meeting of the Zoological Society of Japan. Granship, Shizuoka, Japan, September 17-20, 2009

Tagawa, K., Kawashima, T., Kawashima, T., Kubota, H., Tagawa-Sasaki, A., Humphreys, T., Satoh, N. *What is the stomochord? The answer from gene expression analysis*, The 80th Annual Meeting of the Zoological Society of Japan. Granship, Shizuoka, Japan, September 17-20, 2009

Yamada, S., Ueno, N., Satoh, N. *Ciona intestinalis Noto4 contains a phosphotyrosine interaction domain and is involved in the midline intercalation of notochord cells*, The 80th Annual Meeting of the Zoological Society of Japan. Granship, Shizuoka, Japan, September 17-20, 2009

Shoguchi, E., Itoh, N., Tagawa, K., Satoh, N. *The analysis of chromosomes of the hemichordate Ptychodera flava*, The 80th Annual Meeting of the Zoological Society of Japan. Granship, Shizuoka, Japan, September 17-20, 2009

Shinzato, C., Satoh, N., Takahashi, H. T*he coral Acropora digitifera genome project*, International Symposium on Marine Genomics 2009. Hotel Southern Plaza Kaiho, Okinawa, December 15-18, 2009

Yamada, S., Ueno, N., Satoh, N., Takahashi, H. *Ciona intestinalis Noto4 contains a phosphotyrosine interaction domain and is involved in the midline intercalation of notochord cells*, International Symposium on Marine Genomics 2009. Hotel Southern Plaza Kaiho, Okinawa, December 15-18, 2009

Hamada, M., Shimozono, N., Ohta, N., Fujie, M., Satou, Y., Horie, T., Kawada, T., Satake, H., Sasakura, Y., Satoh, N. *Transcriptome Analysis of Ciona intertinalis to Idntify Genes Preferentially Expressed in the Larval Brain*, International Symposium on Marine Genomics 2009. Hotel Southern Plaza Kaiho, Okinawa, December 15-18, 2009

Kawashima, T., Tagawa, K., Satoh, N. *Whole Genome Shotgun Assembly of Acornworm, Ptychodera flava*, International Symposium on Marine Genomics 2009. Hotel Southern Plaza Kaiho, Okinawa, December 15-18, 2009

5. Intellectual Property Rights and Other Specific Achievements

Nothing to report

6. Meetings and Events

6.1 Okinawa Genomics Workshop, Public Symposium

Date: April 25, 2009
Venue: Pacific Hotel Okinawa, Okinawa, Japan
Co-organizer: Nori Satoh (OIST, Japan)
Co-sponsors: OIST, University of the Ryukyus
Speakesr: Toru Shimada (The University of Tokyo,Japan)
Satoshi Tabata (Kazusa DNA Research Institute, Japan)
Asao Fujiyama (National Institute of Informatics, Japan)

6.2 The 5th International Tunicate Meeting

Date: June 21-25, 2009
Venue: Okinawa Industry Support Center, Okinawa, Japan
Co-organizers: Nori Satoh (OIST, Japan)
Hiroki Nishida (Osaka University, Japan)

Euichi Hirose (University of the Ryukyus, Japan)
Plenary Speakers: William R. Jeffery (University of Mayland, USA)
Hiroki Nishida (Osaka University, Japan)
Other remarks: Oral presentations: 61 other speakers.
Poster presentations: 72 participants.

6.3 OIST Seminar

Date: June 26, 2009
Venue: IRP Conference Room, OIST, Okinawa, Japan
Speaker: François H. Lallier (UPMC Paris, France)

6.4 OIST Internal Seminar

Date: October 16, 2009
Venue: IRP Lounge, OIST, Okinawa, Japan
Speaker: Chuya Shinzato (OIST, Japan)

6.5 OIST Winter Course "Evolution of Complex Systems" 2009

Date: December 7-12, 2009
Venue: Seaside House, OIST, Okinawa, Japan
Co-organizers: Sydney Brenner (OIST, Japan)
Nori Satoh (OIST, Japan)
Michael Levine (UC Berkeley, USA)
Lecturers: Nori Satoh (OIST, Japan)
Nipam H. Patel (UC Berkeley, USA)
Thomas C.G. Bosch (Christian-Albrechts-University Kiel, Germany)
Michael Levine (UC Berkeley, USA)
Robb Krumlauf (SIMR, USA)
Shigeru Kuratani (RIKEN, Japan)
Hiroshi Wada (University of Tsukuba, Japan)
Jr-Kai Sky Yu (Academia Sinicam Taiwan)
Other remarks: Workshop: 19 speakers.
Participants: 26 graduate students and postgraduate researchers.

6.6 International Symposium Marine Genomics

Date: December 15-18, 2009
Venue: Hotel Southern Plaza Kaiho, Okinawa, Japan
Co-organizers: NIG/ROIS, Japan
OIST, Japan
Fisheries Research Agency, Japan
Okinawa Prefecture, Japan
University of the Ryukyus, Japan
International Union of Biological Science, France
Invited Speakers : Charles N. David (Ludweg-Maximilians University, Germany)
Daniel Rokhsar (UC Berkeley, USA)
Giorgio Bernardi (Stazione Zoologica "Anton Dohrn", Italy)
Hiroshi Wada (Tsukuba University, Japan)
Katsutoshi Arai (Hokkaido University, Japan)

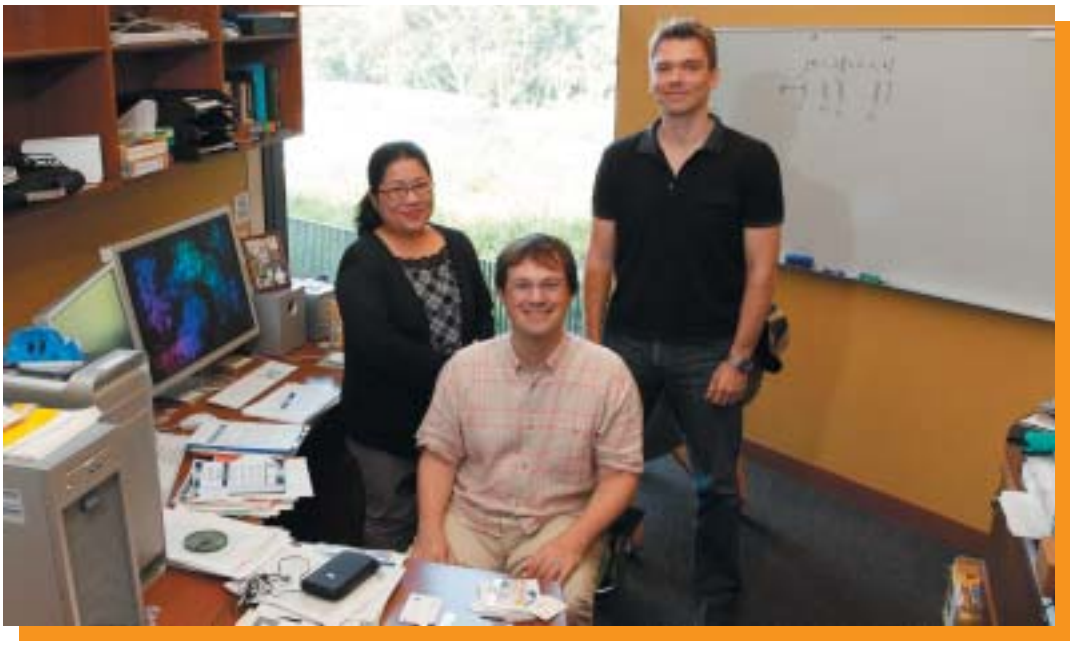
Norihiro Okada (Tokyo Institute of Technology, Japan)
Quanqi Zhang (Ocean University of China, China)
Shugo Watabe (The University of Tokyo, Japan)
Takehiro Kusakabe (Konan University, Japan)

Other remarks: Workshop: 33 other speakers.

Mathematical Biology Unit

Principal Investigator:
Dr. Robert Sinclair

Research Theme:
Mathematical Biology



Abstract

The long-term aim of this unit is to discover new mathematics through engagement with modern biology, and we continue to benefit from the uniquely interdisciplinary environment of OIST in our work. Our research has three themes: inference, discrete and formal methods, and collaborations. With regard to inference, we have developed new methods for the analysis of protein and whole genome sequences, and have made original steps towards a generalization of the standard error of the mean to small sample sizes. In discrete and formal methods, we have shown that the abstract notions of complexity and modularity are not always independent for large enough systems, and are making progress towards a method for the formal analysis of models of neuronal circuitry.

1. Staff

Researcher: Dr. Gunnar Wilken

Research Administrator / Secretary: Mrs. Shino Fibbs

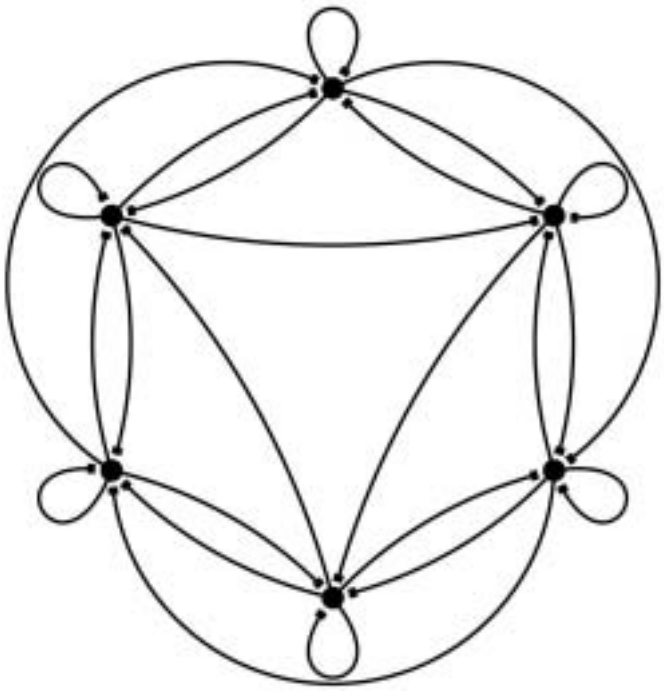
2. Partner Organizations

None.

3. Activities and Findings

3.1 Discrete Mathematics and Evolution

A recent suprising result of our work concerns the relationship between the abstract notions of complexity and modularity, both intuitive rather than well-defined. In some evolutionary contexts, it can appear as if modularity increases hand-in-hand with complexity. In order to better understand this on a theoretical level, we used a discrete network model as a theoretical model organism. The network model chosen was the simplest model for which both complexity and modularity can be clearly defined. What we found was that, for large enough networks, selection for complexity can lead directly to modularity, in the sense that the most complex networks are modular. We believe this has implications for our understanding of why complex organisms tend to have parts, often interchangeable ones. In continuing this work, we are attempting to see how general and robust the result is. The result already suggests new mathematical structures, since the optimal networks have unusual properties, far from those of random networks.



3.2 Small Sample Data Analysis

We believe that theory should not only exist for its own sake, but also be useful. It is clear that data analysis is an area in which theory is not yet fully developed. Most mathematical tools for data analysis (error estimation in particular) have been developed from considerations which only really make sense for extremely large data sets. For small data sets, the experimentalist is apparently expected to use a different set of tools. We have been attempting to establish a set of principles which make sense for all sample sizes, and to derive from them error estimates which can be applied to data sets of all sizes. It is important that these estimates also be easily computable (with a calculator, for example, rather than requiring sophisticated software). We have made progress in this direction, introducing a new principle based upon continuity, generalizing the standard error of the mean. The theory is not yet complete, but one can already see signs of internal coherence and ease of calculation.

3.3 Robust Phylogenetic Signatures

We are searching for phylogenetic signatures which should degrade only very slowly in the face of the types of changes bacterial genomes undergo. Our main result so far has been to show that comparing (i) the number of matches to a given ordering of motifs to (ii) the number of matches to the reverse ordering of the same motifs in bacterial coding sequences or whole genomes is likely to be such a type of phylogenetic signature. It is a measure of asymmetry, not unlike a skew. The idea is not new, since gene ordering has long been used as a phylogenetic marker, but it seems that it can be applied to sets of short motifs as well. We have so far applied it to the question of whether prokaryotes may have "lost their introns". We believe that the method may provide a new and quantitative approach to this old problem.

3.4 Stochastic Geometry and Dendritic Trees

We have been involved in a very stimulating collaboration with another OIST unit, that of Dr. De Schutter. The central issue (seen from a mathematical point of view) is the fact that it was observed long ago that local branchings in dendritic trees tend to be flat, but an important "control" was not done: no investigation was made as to whether local branchings in "random" trees would also tend to be flat. This is what we have done, and the result is positive. The main challenge, from a mathematical point of view, was to define "random" in a meaningful way, such that distributions could in fact be computed.

3.5 Formal Methods for Microcircuits

The long-term aim of this research project is the development of a new approach to modeling brain function realizing navigation and memory which is derived from an interdisciplinary background. Expertise in both mathematical logic and neuroscience is required for the intended adaptation of formal methods from mathematical logic and theoretical computer science, in particular temporal logics and model checking, to provide a flexible integrative framework accommodating rigorous specifications of anatomical and physiological properties of neural assemblies involved in mechanisms of navigation and memory.

3.6 Elementary Patterns of Resemblance

This project has its origin in the area of proof theory and is joint work with Prof. Timothy J. Carlson at the Ohio State University, USA. Patterns of resemblance (Carlson, 2001) provide a new approach to ordinal notation systems which in turn open the road for proofs of consistency of mathematical theories and the measurement and hence comparison of their strengths. Patterns are finite structures consisting of nested trees evolving along complex sequences of intriguing self-resembling repetition. An article with Carlson was submitted in June and is still under review, further articles are in preparation. The research on patterns of resemblance was presented in a plenary talk at the Spring meeting 2010 of the Mathematical Society of Japan.

3.7 Topology

has been applied before to questions of phylogeny, but it has often proven difficult to actually compute topological quantities from anatomical data. In collaborative work with Prof. Yasuhiko Kamiyama of the University of the Ryukyus, we have been exploring ways in which one could effectively compute coefficients related to the homology of certain spaces. We have been successful in computing such coefficients in a number of non-trivial cases, using a combination of traditional and computational techniques. Our goal is to compute these coefficients for fossil arachnids to see whether they are useful phylogenetic markers.

4. Publications

4.1 Journals

Bonnard, B., Caillau, J. B., Sinclair, R. & Tanaka, M. Conjugate and cut loci of a two-sphere of revolution with application to optimal control. *Annales De l'Institut Henri Poincaré - Analyse Non Linéaire* 26, 1081-1098, doi:DOI 10.1016/j.anihpc.2008.03.010 (2009).

Bruggeman, R. W. & Mühlenbruch, T. Eigenfunctions of transfer operators and cohomology. *J Number Theory* 129, 158-181, doi:DOI 10.1016/j.jnt.2008.08.003 (2009).

Enomoto, K., Itoh, J. & Sinclair, R. The Total Absolute Curvature of Open Curves in E^3. *Illinois J Math* 52, 47-76 (2008).

Wilken, G., Weiermann, A. Ordinal arithmetic with simultaneously defined theta-functions. *Mathematical Logic Quarterly* (in press).

Wilken, G., Weiermann, A. in *Typed Lambda Calculi and Applications Vol. 5608 Lecture Notes in Computer Science* 386-400 (Springer Berlin, 2009).

4.2 Book(s) and other one-time publications

Sinclair, R., Stiefel, K. *Multiscale Phenomena in Biology; Proceedings of the 2nd Conference on Mathematics and Biology*. Vol. 1167 (AIP, 2009).

4.3 Oral presentations

Sinclair, R. *The Geometry of Amoebae*, Differential Geometry Seminar Department of Mathematics, Tokai University, Japan, September 21, 2009

Sinclair, R. *Selection for Complexity can Induce Modularity*, The 5th Geometry Conference for Friendship of Japan and China, OIST, January 29, 2010

Wilken, G. *Normal Forms for Elementary Patterns*, Asian Logic Conference, National University of Singapore, June 22, 2009

Wilken, G., Weiermann, A. *Complexity of Gödel's T in Lambda Formulation*, Federated Conference on Rewriting, Deduction and Programming, University of Brasilia, July 1, 2009

Wilken, G. *Derivation Lengths Classification of Gödel's T extending Howard's Assignment*, Workshop Proof Theory, Keio University, March 18, 2010

Wilken, G. *Elementary Patterns of Resemblance. An Overview*, Spring Meeting of the Mathematical Society of Japan, Keio University, March 24, 2010

4.4 Posters

Yihwa, K., Sinclair, R., De Schutter, E. *Local planar dendritic structure: a uniquely biological phenomenon?*, CNS*2009 Berlin, Germany, July 18-23, 2009

5. Intellectual Property Rights and Other Specific Achievements

None to report.

6. Meetings and Events

6.1 On Hilbert's Ansatz

Date: August 25, 2009
Venue: University of the Ryukyus, Rikei Fukugouto
Co-organizers: Mathematical Sciences, University of the Ryukyus
Speakers: Prof. T. Arai, Graduate School of Science and Technology, Kobe University
Other remarks: Joint Mathematics Seminar

6.2 Hilbert, Gödel and Infinity

Date: September 14, 2009
Venue: University of the Ryukyus, Rikei Fukugouto
Co-organizers: Mathematical Sciences, University of the Ryukyus
Speakers: Prof. T. Carlson, Department of Mathematics,The Ohio State University
Other remarks: Joint Mathematics Seminar

6.3 DNA Topology Course 2009

Date: November 2-7, 2009
Venue: Seaside House, Okinawa, Japan
Co-organizers: Nafaa Chbili, UAE University, United Arab Emirates
Speakers: De Witt Sumners
Robert O. Lawton Distinguished Professor of Mathematics
and member of the Institute of Molecular Biophysics
Department of Mathematics
Florida State University, U.S.A.

Patrick Forterre
Biologie Moléculaire du Gène chez les Extrêmophiles (BMGE)
Institut Pasteur and Institut de Génétique et Microbiologie (IGM)
Université Paris-Sud, France

Jun O'Hara
Department of Mathematics and Information Sciences
Tokyo Metropolitan University, Japan

Javier Arsuaga
Mathematics Department
San Francisco State University, U.S.A.

Dorothy Buck
Applied Mathematics
Imperial College London, U.K.

Isabel Darcy
Department of Mathematics
University of Iowa, U.S.A.

Christian Laing
Chemistry Department
Courant Institute of Mathematical Sciences
New York University, U.S.A.

Jennifer K. Mann
Department of Mathematics
The University of Texas, U.S.A.

Mariel Vazquez
Department of Mathematics
San Francisco State University, U.S.A.

Koya Shimokawa
Department of Mathematics
Graduate School of Science and Engineering
Saitama University, Japan

Andrzej Stasiak
Center for Integrative Genomics
University of Lausanne, Switzerland

Lynn Zechiedrich
Department of Molecular Virology and Microbiology
Baylor College of Medicine, U.S.A.

Other remarks: This was a graduate course.

6.4 Impulsive control strategies for integrated pest management

Date: February 10, 2010
Venue: IRP Conference Room
Speakers: Dr. Paul Georgescu, Department of Mathematics, Technical University of Iasi, Romania

6.5 Biomedical Imaging of Function: Brain-Computer-Interface and Magnetic Nanomarkers

Date: March 10, 2010
Venue: Lab 1, Level D Meeting Room 1 (D36)
Speakers: Prof. M. Schilling, Institut für Elektrische Messtechnik und Grundlagen der Elektrotechnik, Technische Universität Carolo-Wilhelmina zu Braunschweig

Structural Cellular Biology Unit

Principal Investigator:
Ulf Skoglund

Research Theme:
Our goal is to understand the function of proteins in cells and tissues. The techniques of electron microscopy, including molecular electron tomography (MET), are the main tools in our unit, though we will draw upon well-established complementary techniques when called for.

Abstract

The ability to visualize individual macromolecules, whether in tissue or isolated, enables us to describe the dynamics of the different macromolecules and how they bind and interact with each other. Thus we believe that the MET technique will play an important role in the analysis of supra-molecular complexes such as elucidation of the mode of action of the cell's molecular machines. E.g. in a signal transduction case, by combining data from 3D reconstructed individual protein molecules with different conformations, we derived chemical equilibrium constants that govern the molecules conformational dependency on the concentration of calcium ions.

1. Staff

Researchesr: Lars-Goran Ofverstedt
Technical Staff: To be appointed.
Research Administrator / Secretary: Shizuka Kuda

2. Partner Organizations

Nothing to be reported due to the Skoglund Unit is under construction..

3. Activities and Findings

3.1 The Skoglund Unit was established Jan 2010. Currently, the equipment is being commissioned.

4. Publications

4.1 Journals

None.

4.2 Books and other one-time publications

None.

4.3 Oral presentations

Skoglund, U. *What is Moledcular Electron Tomography (MET)*, OIST Seminar, Okinawa Japan, July 15, 2009

4.4 Posters

Skoglund, U. *Structural Cellular Biology Uni*t, The OIST Opening Ceremony, Okinawa, Japan, March 28, 2010

5. Intellectual Property Rights and Other Specific Achievements

Nothing to be reported due to the Skoglund Unit is under construction.

6. Meetings and Events

Nothing to be reported due to the Skoglund Unit is under construction.

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. 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 aforementioned phenomena in cells subjected to these modulators. The theoretical approaches are biophysical simulations of neurons and the use of genetic algorithms.

This year, we continued our research into the dynamical systems behavior of neurons. Specifically, we are currently investigating stochastic resonance, the phenomenon in which a moderate level of background noise leads to improved signal processing in neurons. Furthermore, we are still investigating phase-reset curves of neurons, and have developed and published a novel method for determining this important measure of neural oscillatory behavior.

1. Staff

Researchers: Dr. Marylka Uusisaari,
Dr. Benjamin Torben-Nielsen
Research Administrator / Secretary: Ms. Ryoko Uchida

2. Partner Organizations

None.



3. Activities and Findings

3.1 Phase Reset Curves

We continued to investigate neurons as dynamical systems. In particular, we determined phase-response curves from neurons, which are an important measure of the oscillatory behavior of a neuron. These functions plot the phase shift experienced by neural spiking as a funtion of the phase of the perturbation and give valuable hints to the behavior of neurons in synaptcally connected networks. We developed, and published, a novel method for determining phase-response curves from fluctuating current injections. We combine slice recordings and biophysical simulations for this research project.

3.2 Stochastic resonance

Another aspect of non-linear neural behavior we investigate is stochastic resonance, the effect that a small amount of noise leads to an improved signal processing performance. we investigate this property in cortical pyramidal neurons, and measure how neuromodulation affects stochastic resonance. We also combine *in-vitro* experimental techniques and theoretical investigations for this project.

3.3 Dendritic morphologies

Dendrites are a neuron's cellular protrusions which collect synaptic inputs from other neurons. There is a multitude of dendrites in different animals and brain regions, but it is not known yet what computations many of these dendrites carry out. We investigate this with an inverse approach, where we use optimization algorithms to find dendrites optimized for certain computational functions. In the latest research project along these lines we investigate the function of wide-field motion integration, which VS cells of the fly visual system are also beleived to carry out. Striking similarities between the optimized neurons we find and real VS cells lead to novel insights about dendritic function - structure relationships.

4. Publications

4.1 Journals

Stiefel, K. M., Fellous, J. M., Thomas, P. J. & Sejnowski, T. J. Intrinsic subthreshold oscillations extend the influence of inhibitory synaptic inputs on cortical pyramidal neurons (vol 31, pg 1019, 2010). *Eur J Neurosci* 31, 1509-1509, doi:DOI 10.1111/j.1460-9568.2010.07245.x (2010).

Torben-Nielsen, B., Uusisaari, M., Stiefel, K. M. A comparison of methods to determine neuronal phase-response curves. *Frontiers in Neuroinformatics* 4, doi:doi:10.3389/fninf.2010.00006 (2010).

Torben-Nielsen, B. & Stiefel, K. M. Systematic mapping between dendritic function and structure. *Network-Comp Neural* 20, 69-105, doi:Doi 10.1080/09548980902984833 Pii 912832009 (2009).

Uusisaari, M. & Knopfel, T. GlyT2+Neurons in the Lateral Cerebellar Nucleus. *Cerebellum* 9, 42-55, doi:DOI 10.1007/s12311-009-0137-1 (2010).

Torben-Nielsen B., U., M., Stiefel K.M. A novel method for determining the phase-response curves of neurons based on minimizing spike-time prediction. *arxiv.org/abs/1001.0446* (2010).

4.2 Book(s) and other one-time publications

Torben-Nielsen B., U., M., Stiefel K.M. in *Multiscale phenomena in biology:Proceedings of the 2nd Conference on Mathematics.* 12-25 (American Institute of Physics).

4.3 Oral presentations

Torben-Nielsen, B. *VS cells are optimized wide-field motion detectors*, University College London, London, UK July 28, 2009

Torben-Nielsen, B. *VS cells are optimized wide-field motion detectors*, University of Hertforshire, UK, July 29, 2009

Stiefel, K. M. *Cholinergic neuromodulation of Phase Reset Curves*, RIKEN BSI, Saitama, Japan, September 3, 2009

Uusisaari, M. *Neuromodulation of information processing in cortical neural network*, OIST Internal Seminar, December 11, 2009

Torben-Nielsen, B. *The inverse approach: a framework to explore dendritic morphology-function relationships*, Hebrew University, Israel, March 23, 2010

Torben-Nielsen, B. *Morphological neuron models as Robot controllers*, University of Hertfordshire, UK, March 31, 2009

4.4 Posters

Uusisaari, M., Torben-Nielsen, B., Stiefel KM. *Neuromodulation of stochastic resonance in cortical neurons*, Australian Neuroscience Society Annual meeting, Sydney, Australia, February 2, 2010

Torben-Nielsen, B., Uusisaari, M., Stiefel, K. M. *Quanlitive comparison of phase response curve estimation methods using model and experimental data*, Australian Neuroscience Society Annual meeting, Sydney, Australia, February 1, 2010

5. Intellectual Property Rights and Other Specific Achievements

Nothing to be reported.

6. Meetings and Events

6.1 Okinawa Computational Neuroscience Course 2009

Date: June 15, 2009 – July 2, 2009
Venue: OIST Seaside House
Co-organizers: Dr.Erik De Shutter, Dr. Kenji Doya, Dr. Jeff Wickens,
Okinawa Institute of Science and Technology
Speakers:Bartos Marlene, Institute of Medical Sciences (IMS),
University of Aberdeen (UK)
Câteau Hideyuki, RIKEN BTCC
De Schutter Erik, OIST
Doya Kenji, OIST
Fukai Tomoki, RIKEN BSI
Gewaltig Marc-Oliver, Honda Research Institute Europe GmbH
Häusser Michael, University College London
Knöpfel Thomas, RIKEN BSI
Mainen Zachary, Instituto Gulbenkian de Ciência
Paninski Liam, Columbia University
Prescott Steven, McGill University
Redish David, University of Minnesota
Sinclair Robert, OIST
Stevens Charles, The Salk Institute
Stiefel Klaus, OIST
Stuart Gregory, The John Curtin School of Medical Research
Wickens Jeff, OIST
Wolpert Daniel, University of Cambridge

6.2 ANS/AuPS 2010 Australian Neuroscience Society Annual Meeting Symposia:"Breaking the code: the theory of spikes and axons"

Date: January 31, 2010 - February 3, 2010
Venue: Sydney Convention and Exhibition Center
Organizer: Dr. Dario Protti, University of Sydney
Co-chair: Dr. Klaus Stiefel
Speakers: G. Bard Ermentrout, University of Pittsburg, USA
Greg Stuart, Australian National University, ACT
Geoff Goodhill, Queensland Brain Institute, QLD
Klaus Stiefel, Okinawa Institute of Science and Technology, Japan

6.3 Seminar

Date: July 3, 2009
Venue: OIST IRP Conference Room
Speaker: Ping Wang (The Salk Institute)

6.4 Seminar

Date: August 6, 2009
Venue: OIST IRP Conference Room
Speaker: Bernard Englitz (Max Planck Institute for Brain Research)

6.5 Seminar

Date: November 6, 2009
Venue: OIST IRP Conference Room
Speaker: Andrzej Lewenstan (Abo Akadei University, Finland)

6.6 Seminar

Date: December 15, 2009
Venue: OIST IRP Conference Room
Speaker: Nicolangelo Iannella (RIKEN BSI)

6.7 Seminar

Date: March 25 & 26, 2010
Venue: Level D Meeting Room, New Campus
Speaker: John Jacobson (The Salk Institute)

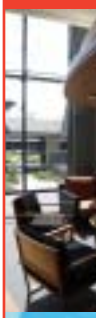
Cellular & Molecular Synaptic Function Unit

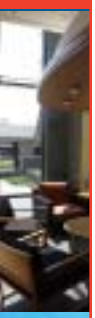
Principal Investigator:
Tomoyuki Takahashi
Research Theme:
Regulatory mechanisms for transmitter release

Abstract

In the neuronal system, dynamic changes of synaptic strength play critical roles in switching functional neuronal circuits. Regarding synaptic strength, compared with postsynaptic mechanisms much less is known for presynaptic mechanisms, primarily because of small nerve terminal structures preventing applications of electrophysiological and imaging techniques. The calyx of Held is a giant glutamatergic nerve terminal visually identified in the mammalian auditory brainstem slices. This fast relay synapse undergoes dramatic developmental changes in its structure, functional properties and molecular compositions during the second postnatal (P) week, when rodents start to hear sound at P10-12. By applying molecular, imaging and patch-clamp techniques to this synapse of developing rodents, we aim at elucidating presynaptic regulatory mechanisms underlying synaptic transmission. Our progress in the fiscal year 2009 is as follows.

(1) The II-III loop of the α subunit of voltage-gated Ca^{2+} channels comprises a binding region called synprint site, as it can bind to the exocytic machinery protein SNARE. Given that this binding is Ca^{2+} concentration-dependent, and that loading of synprint site fragment into presynaptic cells attenuates synaptic responses, it has been hypothesized that this site plays an essential role in exocytic release of neurotransmitter (synprint hypothesis). While screening for proteins, which can bind to the synprint site, Watanabe et al (*J Neurosci*, 2010) found a direct binding of the synprint with the adaptor protein for clathrin-coated vesicle endocytosis AP-2. In brain lysate, this binding occurred preferentially at low Ca^{2+} concentration, whereas at high Ca^{2+} concentration above 100 μM, synaptotagmin (Syt) competed off the AP-2-synprint binding. When the synprint site fragment was directly loaded into the calyx terminal via whole-cell recording pipette, it blocked vesicle endocytosis, assessed by capacitance measurements, without inhibiting exocytosis. This former finding is entirely new and the latter finding is contradictory to the ongoing synprint hypothesis. It is concluded that the voltage-gated Ca^{2+} channel structure plays essential





roles in vesicle endocytosis by binding of its synprint site to AP-2 and Syt in a Ca^{2+} concentration-dependent manner. Block of these interactions primarily blocks vesicle endocytosis, and will eventually attenuate exocytic release of neurotransmitter because of impaired vesicle recycling and replenishment.

(2) In the nerve terminal, in response to a presynaptic action potential, a high concentration Ca^{2+} domain of tens of nanometer in diameter is transiently formed around the site of Ca^{2+} entry. This Ca^{2+} nanodomain triggers exocytosis of synaptic vesicles for transmitter release. Yamashita et al (*Nature Neurosci*, 2010) discovered that this Ca^{2+} nanodomain plays a priming role for vesicle endocytosis at calyceal presynaptic terminals after hearing onset. At immature terminals of pre-hearing animals, It has been reported that endocytois depends upon Ca^{2+} outside of the Ca^{2+} nanodomain exclusively in a calmodulin (CaM)-dependent manner. At post-hearing terminals, however, the CaM-dependent mechanism no longer operated, but vesicle endocytosis after mild and massive exocytosis was still entirely dependent upon Ca^{2+} within and outside of nanodomain, respectively. These results suggest that both low and high affinity Ca^{2+} binding proteins are involved in vesicle endocytosis at mature presynaptic terminals. The present study has also clarified the indispensable role of G-proteins in endocytosis. Namely, at mature presynaptic terminals, endocytosis is exclusively GTP-dependent, despite the fact that GTP-independent mechanism partially underlies endocytosis at immature synapses. Essential role of Ca^{2+} nanodomain in vesicle endocytosis is consistent with our recent finding that Ca^{2+} channels, via its II-III structure, is involved in vesicle endocytosis (Watanabe et al, 2010, see above), and also with previous reports that the low affinity Ca^{2+} binding protein synaptotagmin plays dual roles in exocytosis and endocytosis of synaptic vesicles.

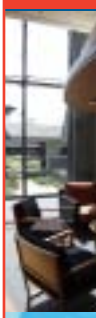
(3) Presynaptic Ca^{2+} imaging has so far been made only from whole terminal. Nakamura et al (Ms submitted) recorded Ca^{2+} transients in response to a single presynaptic action potential (AP) from a confocal spot at the calyx of Held presynaptic terminal of developing rats. Hot spots were found along the edge of the terminal facing synaptic cleft, almost ubiquitously at immature calyx (P7-8), but less frequently at more developed calyx of hearing rats (P14-15). Pharmacological mimics of post-hearing Ca^{2+} transients in pre-hearing calyces using Ca^{2+} channel blockers revealed that the reduction of functional Ca^{2+} channel density tightens Ca^{2+}-secretion coupling, as deduced from the effect of the presynaptically loaded slow Ca^{2+} -binding chelator EGTA on synaptic transmission. Given that the Ca^{2+} channel density decreases and AP duration becomes shorter during postnatal development, it is suggested that the developmental reduction of Ca^{2+} influx underlie establishment of fast synaptic transmission with a tight Ca^{2+}-secretion coupling. These results are also consistent with our previous findings that CaM having relatively low Ca^{2+} binding affinity operates only at prehearing synapse for Ca^{2+} channel inactivation (Nakamura et al, 2008) and for vesicle endocytosis (Yamashita et al, see above).

1. Staff

Group Leader: Takayuki Yamashita

Researchers: Kogaku Eguchi





Tetsuya Hori
Yukihiro Nakamura
Laurent Guillaud
Setsuko Nakanishi
Research Administrator / Secretary: Kaori Egashira

2. Partner Organizations

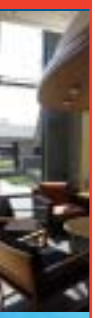
Doshisha University Faculty of Life and Medical Sciences
Type of partnership: Joint research
Name of principal researcher: Tomoyuki Takahashi
Name of researcher: Naoto Saitoh
Research theme: Regulatory mechanisms for transmitter release

Kyoto University Faculty of Engineering, Department of Biological Chemistry and Molecular Biology
Type of partnership: Scientific collaboration
Name of principal researcher: Yasuo Mori
Name of researchers: Yasuo Mori, Shigeki Kiyonaka
Research theme: Presynaptic roles of calcium binding proteins

University Paris V
Type of partnership: Scientific Collaboration
Name of principal researcher: David DiGregorio
Name of researchers: David DiGregorio
Research theme: Developmental changes in the presynaptic calcium transient profiles associated with transmitter release

Vollum Institute, Oregon Health & Science University
Type of partnership: Scientific Collaboration
Name of principal researcher: Henrique von Gersdorff
Name of researcher: Henrique von Gersdorff
Research theme: Developmental changes in the mechanisms underlying synaptic vesicle endocytosis

National Institute for Physiological Sciences, Department of Cerebral Research, Division of Cellular Structure
Type of partnership: Scientific Collaboration
Name of principal researcher: Ryuich Shigemoto
Name of researchers: Ryuich Shigemoto, Yugo Fukazawa
Research theme: Developmental changes in the presynaptic calcium binding proteins, and the distribution of P/Q type calcium channels



3. Activities and Findings

3.1 Direct interaction of AP-2 with Ca^{2+} channel synprint site involved in synaptic vesicle endocytosis (Watanabe *et al,* 2010).

In affinity column chromatography, clathrin-coated endocytosis adaptor protein AP-2 at its μ subunit was found to bind directly to synprint site of voltage-gated Ca^{2+} channel (VGCC) α1B subunit (Fig 1).

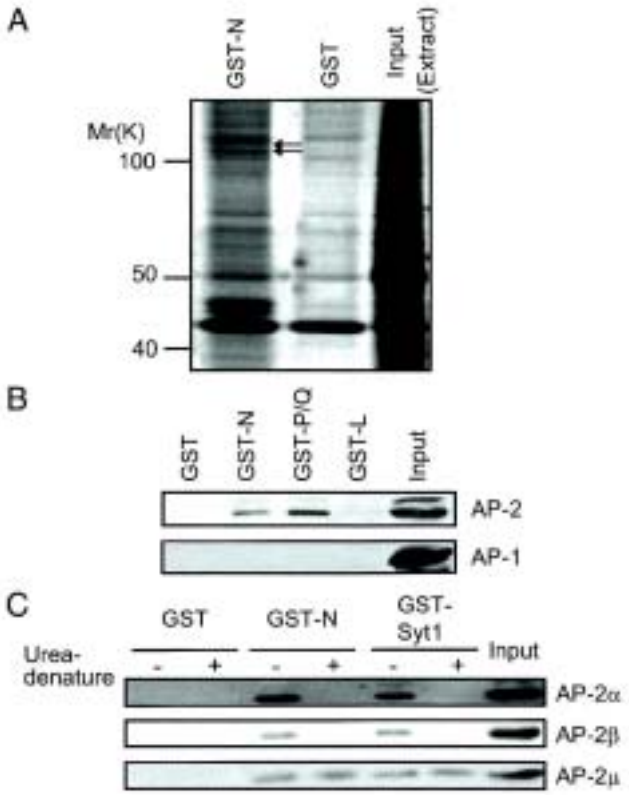


Figure 1

The binding affinity (K_d) estimated from scatchard plot was 31 nM (Fig 2).

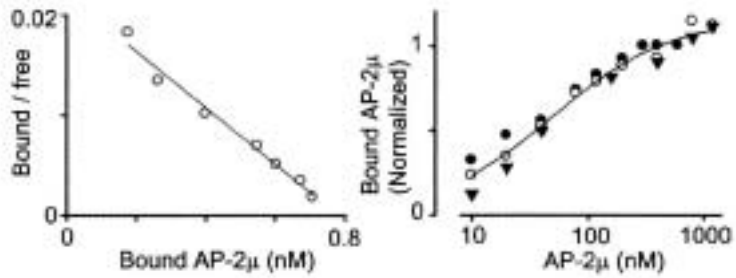


Figure 2

The AP-2μ binding region of synprint site coincided with the Syt 1 binding site (Fig 3A). The synprint site binding site of AP-2μ coincided with the Syt 1 binding site (Fig 3B).

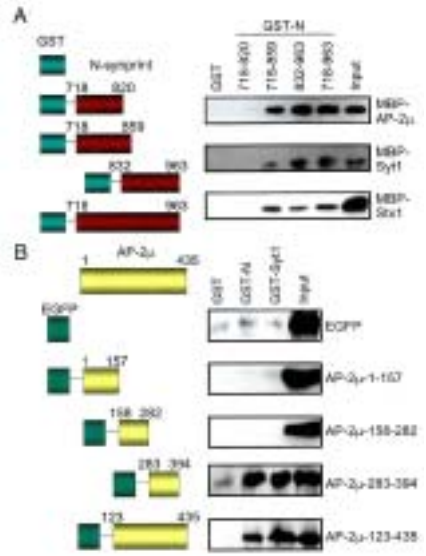


Figure 3

The AP-2m-synprint binding competed with the Syt 1-synprint binding, but not with the syntaxin-synprint binding (Fig 4).

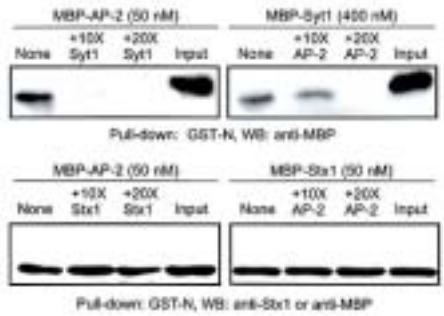


Figure 4

This binding was apparently Ca^{2+} concentration-dependent in brain lysate, in competition to the interaction between synprint and Syt 1 (Fig 5).

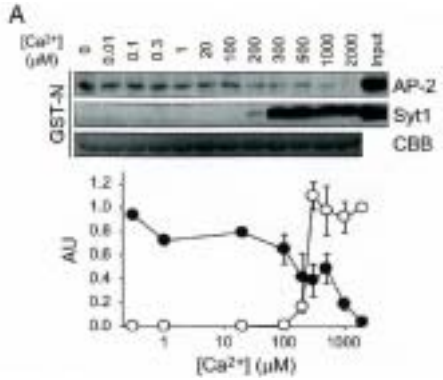


Figure 5

Intra-terminal loading of synprint site peptide fragment, as a dominant negative construct, at the calyx of Held markedly attenuated vesicle endocytosis (Fig 6).

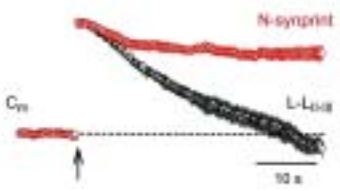


Figure 6

The synprint peptide fragment slightly enhanced Ca^{2+} current amplitude, thereby enhancing exocytic capacitance change (Fig 7).

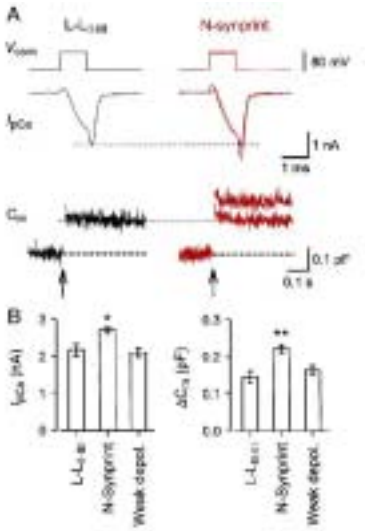


Figure 7

These results suggest that Ca^{2+} channel synprint site contributes to clathrin-coated vesicle endocytososis by interacting with AP-2 and synaptotagmin in a Ca^{2+} concentration-dependent manner. Previous reports that presynaptically loaded synprint peptide attenuated synaptic transmission must result secondarily from the block of vesicle endocytosis. Thus these results are contradictory to the previously postulated synprint hypothesis.

3.2 Involvement of Ca^{2+} nanodomain in vesicle endocytosis at a mature central synapse and developmental changes in the mechanism underlying Ca^{2+} -dependent vesicle endocytosis (Yamashita *et al,* 2010).

Ca^{2+} currents evoked by a single or a short train depolarizing pulse induced vesicle exocytosis followed by endocytosis, expressed as membrane capacitance changes. At immature calyx nerve terminal before hearing onset, the slow binding Ca^{2+} chelator EGTA, when loaded at 10 mM into the calyx, markedly attenuated endocytosis. However, as animals matured, at P13-14 after hearing onset, 10 mM EGTA the effect of 10 mM EGTA became small, but the fast binding Ca^{2+} chelator BAPTA at 10 mM nearly abolished endocytosis (Fig 8).

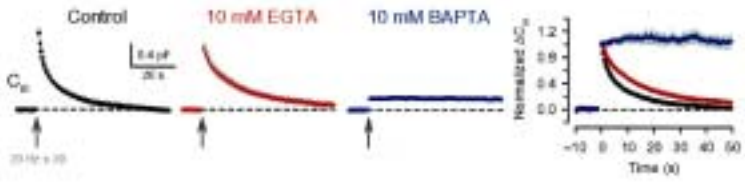


Figure 8

Given that 10 mM BAPTA can reach Ca^{2+} nanodomain having a diameter of tens of nanometer, whereas 10 mM EGTA can reach only Ca^{2+} microdomain having diameter of hundreds of nanometer, it is suggested that endocytosis is primed in the Ca^{2+} nanodomain at post-hearing calyx presynaptic terminals. This is an entirely novel finding as it is generally thought that Ca^{2+}-dependent endocytosis occur at periactive zone, being triggered at much lower Ca^{2+} concentration than that triggering exocytosis. Simultaneous trigger of exo- and endocytosis within a nanodomain is an ideal device for the exo-endocytic co-ordination, which is required for homeostatic balance of terminal membrane and maintenance of long-lasting synaptic transmission by vesicle recycling.

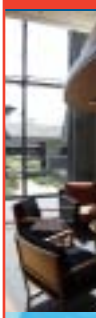
During massive vesicle exocytosis, endocytic rate increases by several to 10-fold to compensate for the loss of vesicles. After massive exocytosis, endocytosis slowly retrieved plasma membrane into vesicles to the original level. The fast and slow endocytosis during and after massive exocytosis, elicited by 1 Hz train of stimulation, are both dependent upon Ca^{2+}/CaM as shown by recent pharmacological studies, and calcineurin (CaN)-dependent as revealed in the present study, at immature calyx terminals. Surprisingly, however, after hearing onset, the Ca^{2+}/CaM/CaN dependence of the endocytosis became inoperative, despite the fact that the kinetics and magnitude of endocytosis in the same stimulation protocol remained similar.

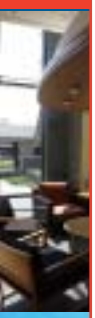
It is suggested that developmental reduction in Ca^{2+} influx in response to presynaptic action potential underlie this phenomenon. Ca^{2+} binding protein(s) having higher binding affinity than CaM may take over its role. Identification of such a protein would help clarifying molecular basis for the regulatory mechanism of vesicle endocytosis at mature synapses.

3.3 Developmental changes in the intraterminal profile of Ca^{2+} entry (Nakamura *et al*, submitted)

By applying the confocal spot Ca^{2+} detection method to the calyx of Held, we recorded fast Ca^{2+} transients in response to a single action potential. At prehearing calyces, these transients could unfailingly be observed along the edge of the terminal in apposition to the postsynaptic cell. As animals start to hear, however, hot spots of Ca^{2+} transients became less frequent and their amplitudes became smaller, despite the fact that their rise-time kinetics were faster compared with those in preheraing calyces. Given that total Ca^{2+} currents evoked by whole terminal stimulation remains unchanged during the development, these results suggest that Ca^{2+} channels in calyceal terminals undergo developmental decrease in their functional density.

Pharmacological tools can be used to test the effect of reducing Ca^{2+} entry on the Ca^{2+}-secretion coupling distance at immature preheraing calyx terminals. The Ca^{2+}-secretion coupling distance can be assessed by the effect of 10 mM EGTA loading into the nerve terminal (see above). At P8 calyces, EGTA loading markedly attenuates EPSC amplitude, whereas it had no effect at P14, suggesting developmental tightening in the Ca^{2+}-secretion coupling distance (Fig 9A, B). The N-type and R-type Ca^{2+} channel blockers in mixture attenuates EPSCs at P7 calyx. After blocking





these Ca^{2+} channel subtypes, 10 mM EGTA loading no longer attenuated the EPSC amplitude (Fig 9C). Likewise after attenuating P/Q type Ca^{2+} channels using ω-Agatoxin at low concentrations, 10 mM EGTA had no effect on the EPSC amplitude (Fig 9D). These results suggest that a reduction in Ca^{2+}entry by itself can tighten Ca^{2+}-secretion coupling distance.

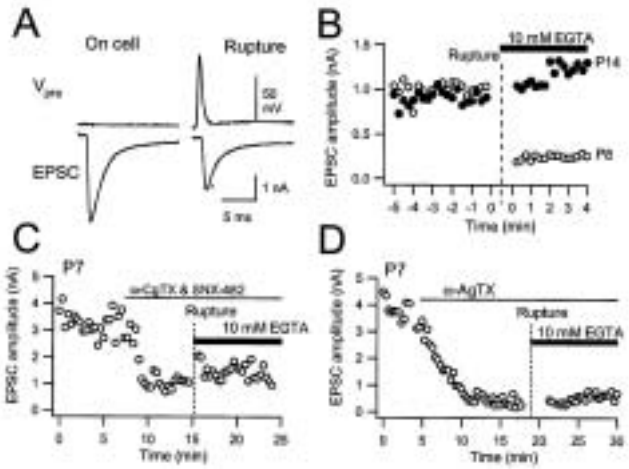


Figure 9

These pharmacological results taken together with Ca^{2+} imaging results suggest that developmental reduction in Ca^{2+} entry into the nerve terminal underlie developmental tightening of the Ca^{2+}-secretion coupling. These developmental changes also explain why transmitter release probability declines during development at this (Iwasaki & Takahashi, 2001, *J Physiol*) and many other synapses. Furthermore, they also explains why Ca^{2+}/CaM-dependent mechanisms for Ca^{2+} current inactivation (Nakamura *et al,* 2008, *J Physiol*) and vesicle endocytosis (see above 3.2) at immature presynaptic terminals become inoperative as animals mature.

4. Publications

4.1 Journals

Hori, T. & Takahashi, T. Mechanisms underlying short-term modulation of transmitter release by presynaptic depolarization. *Journal of Physiology-London* 587, 2987-3000, doi:DOI 10.1113/jphysiol.2009.168765 (2009).

Yamashita, T., Kanda, T., Eguchi, K. & Takahashi, T. Vesicular glutamate filling and AMPA receptor occupancy at the calyx of Held synapse of immature rats. *Journal of Physiology-London* 587, 2327-2339, doi:DOI 10.1113/jphysiol.2008.167759 (2009).

Watanabe, H., Yamashita, T., Saitoh, N., Kiyonaka, S., Iwamatsu, A., Campbell, K. P., Mori, Y. & Takahashi, T. Involvement of Ca^{2+} Channel Synprint Site in Synaptic Vesicle Endocytosis. *J Neurosci* 30, 655-660, doi:Doi 10.1523/Jneurosci.3214-09.2010 (2010).

Yamashita, T., Eguchi, K., Saitoh, N., Von Gersdorff, H., Takahashi, T. Developmental shift in the mechanism of synaptic vesicle endocytosis to require Ca^{2+} nanodomain. *Nature Neuroscience*, in press.

4.2 Book(s) and other one-time publications

None.

4.3 Oral presentations

Eguchi, K. *Developmental changes in AMPA receptor occupancy at the calyx of Held synapse*, OIST-IRP internal seminar, Uruma, Japan, April 10, 2009

Yamashita, T., Eguchi, K., von Gersdorff, H., Takahashi, T. *Developmental changes in the mechanisms underlying activity-dependent acceleration of synaptic vesicle endocytosis at the calyx of Held*, Japan Neuroscience Society Annual Meeting, Nagoya, Japan, September 17, 2009

Yamashita, T., Eguchi, K., Saitoh, N., von Gersdorff, H., Takahashi, T. *Developmental changes in the mechanisms underlying Ca^{2+}-dependent endocytosis of synaptic vesicles*, National Institute for Physiological Science Meeting, Okazaki, Japan, October 2, 2009

Yamashita, T. *Mechanisms underlying synaptic vesicle endocytosis at a fast central synapse,* Synapses & Circuits Seminar at EPFL, Lausanne, Switzerland, February 5, 2010

Nakamura Y., S., R.A., DiGregorio, D., Takahashi, T. *Developmental changes in Ca-secretion coupling at the calyx of Held presynaptic nerve terminal*, The NIPS internal meeting, Okazaki, Japan, November 11, 2009

4.4 Posters

Yamashita, T., Eguchi, K., von Gersdorff, H., Takahashi, T. *Developmental rearrangements of the mechanisms underlying vesicular endocytosis at the calyx of Held*, Society for Neuroscience Annual Meeting, Chicago, U.S.A., October 19, 2009

Nakamura, Y., Silver, R.A., Takahashi, T. *Developmental reduction in functional Ca^{2+} channel density tightens Ca^{2+}-secretion coupling at a central excitatory synapse*, The Annual Meeting of the Society for Neuroscience, Chicago, U.S.A., October 19, 2009

5. Intellectual Property Rights and Other Specific Achievements

Nothing to be reported

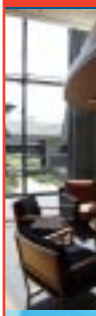
6. Meetings and Events

6.1 OIST Special Lecture

Date: 19 March 2010

Venue: Seaside House

Speaker: Angus R Silver, University College London



Electron Holography Unit

Principal Investigator:
Akira Tonomura

Research Theme:
Electron Microscope

Abstract

The aim of this project is to gain a better understanding of the microscopic behavior of materials through high-resolution electron phase measurements using our coherent electron wave techniques. The achievements this year were made mainly in nanoscale magnetism research using our OIST microscope. The two main achievements are as follows (Details in Section 3):

1. Colossal magnetoresistance (CMR), the drastic decrease in resistivity due to application of a magnetic field, has attracted considerable attention because of its potential applicability in advanced technologies related to magnetic data storage. We studied the microscopic mechanism of CMR in perovskite-type manganites using cryogenic Lorentz microscopy and electron holography. The magnetic observations revealed that the formation of a ferromagnetic domain network plays a crucial role in CMR manganite.

2. The magnetic properties of the soft underlayer (SUL) of perpendicular recording media significantly affect the recording performance. Using electron holography, we visualized and quantified the distribution and magnitude of the magnetic flux in the recording layer and SULs of perpendicular recording media. Pseudo SULs with different permeability values were deposited on the sliders of perpendicular recording heads. The SULs with higher permeability produced larger write magnetic flux than those with lower permeability, resulting in higher overwrite performance.

1. Staff

Research Advisor: H. Ezawa and F. Nagata
Researchers: H. Kasai, S. Mamishin, and M. Ikeda
Technical Staff: K. Yanagisawa
Research Assistant / Graduate Student: Y. Tamura
Research Administrator / Secretary: S. Deguchi

2. Partner Organizations

Tohoku University
Type of partnership: Joint Research
Name of principal researcher: D. Shindo
Name of researcher: Y. Murakami
Research theme: Study on the microscopic mechanism of colossal magnetoresistance in perovskite-type manganites

TDK, Ltd.
Type of partnership: Joint Research
Name of principal researcher: K. Yanagiuchi
Name of researchers: K. Hirata, Y. Ishida
Research theme: Electron holography observation of magnetization distribution in the pseudo soft underlayer of perpendicular magnetic recording media

3. Activities and Findings

3.1 Study of microscopic mechanism of colossal magnetoresistance in perovskite-type manganite

Colossal magnetoresistance (CMR), the drastic decrease in resistivity due to application of a magnetic field, has attracted considerable attention in the last decade because of its potential applicability in advanced technologies related to magnetic data storage [1-3]. It is believed that the key underlying mechanism of CMR is magnetic/thermal assistance in the formation of macroscopic conduction paths from ferromagnetic (FM) metallic domains [3]. However, it is so far unclear how the FM phase prevails in the crystal despite severe competition with the charge-ordered (CO) insulator phase [2,3]. We investigated this by performing cryogenic Lorentz microscopy and electron holography on a prototypical CMR manganite, $La_{0.25}Pr_{0.375}Ca_{0.375}MnO_3$, with a 300-kV transmission electron microscope (HF-3300X, Hitachi) at OIST.

This manganite undergoes a structural phase transition at approximately 55 K [4]. The mother phase (high-temperature phase) is nonmagnetic due to the characteristics of charge ordering, which represents a regular array of Mn^{3+} and Mn^{4+}. Although researchers are highly interested in the nucleation and growth of the FM phase (low-temperature phase), the mechanism remains unclear due to the lack of direct observations by electron microscopy. Our Lorentz microscopy observations have elucidated this essential feature[5]. As shown in Fig. 1a, the FM phase initially appears in a spherical single domain, as demonstrated by the asymmetric patterns in the greyscale image, in a negligible magnetic field (i.e., under the condition of zero-field cooling

(ZFC)). The FM phase changed to a double domain when the volume increased owning to cooling, which reduced the stray field energy (Figs. 1b, c)—notice the distinct magnetic contrast where the phase boundary (outer frame) is bright and the internal magnetic domain wall is dark. In all these stages of nucleation and growth, a clear interface separated the FM phase from the mother phase. Observations indicated that this magnetic phase transition proceeds in a style typical of a first-order phase transition. In other words, this phase transition is apparently affected by external fields, such as magnetic fields, which reduces the potential barrier between the two phases. A plot of FM phase domain size as a function of temperature reveals only a gradual volume increase in ZFC, as shown by the closed circles in Fig. 2.

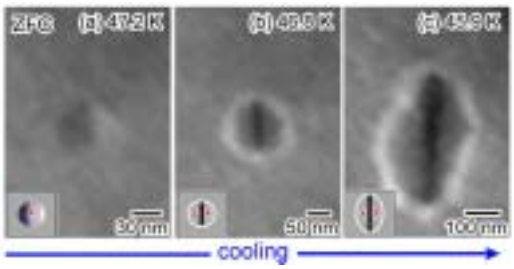


Figure 1: Lorentz microscope images of FM phase, observed under ZFC condition. Insets show schematic representation of domain structure and magnetization vectors in (red arrows).

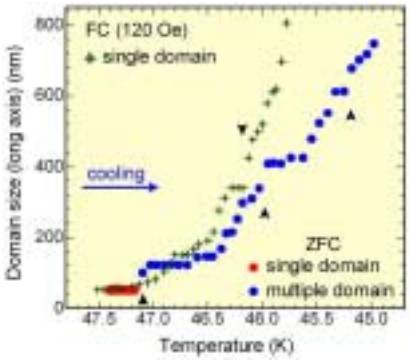


Figure 2: Growth curves of FM phase, observed under ZFC and FC conditions.

As shown by the plus symbols in Fig. 2, the growth rate was increased by applying a magnetic field, i.e., under the field-cooling (FC) condition. For example, the ZFC experiment required supercooling by 2.3 K to attain a domain size of 700 nm, while the FC experiment required supercooling by only 1.5 K to achieve the same domain size. The stepwise growth curves in Fig. 2 imply that the motion of the phase boundary must have been impeded by obstacles such as pinning centers. Another observation supporting this conclusion is the characteristic boundary structure in the FM phase. As shown in Fig. 3, zigzag boundaries separate the FM phase from the mother phase. The width of the dips protruding into the mother phase is the same step size measured from the growth curves shown in Fig. 2. We assert that the pinning force originates from the structural competition [3] between the FM phase regions and the CO phase regions that were preformed in the mother phase: i.e., propagation of the FM phase boundary was impeded by the CO portions in the untransformed mother phase. Thus, further supercooling is required to generate a strong driving force that can cause motion of the phase boundary. As illustrated in Fig. 2, the applied magnetic field appears to assist this boundary motion, in addition to the thermal driving force.

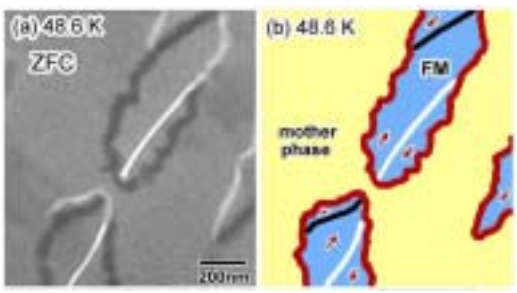


Figure 3: (a) Lorentz microscope image showing zigzag interface separating FM phase and mother phase. (b) Schematic illustration of mixed-phase state shown in (a). Red arrows indicate magnetization vectors in each domain.

It is argued that a percolative connection of FM domains is responsible for the dramatic reduction in the resistivity [3,4]. This mechanism is supported by a microscopic observation that provides evidence of both the formation of a network from FM domains and the high conductivity in those domains. We performed electron holography studies to clarify those two aspects. The results for ZFC are presented in Fig. 4, in which the contour lines represent lines of magnetic flux (in-plane component). With respect to the formation of an FM domain network, the images shown in Fig. 4 reveal the coalescence of FM regions (portions having sepia contour lines) under the specimen cooling condition. The domain coalescence occurs preferentially in portions where a large magnetic field leaks into the mother phase, as evidenced by the narrow spacing of the contour lines indicated by the circles [5]. This result reveals a close relationship between the CMR effect and the delicate nature of the magnetic microstructure of the mixed-phase state, which is highly sensitive to magnetic fields. The net magnetization is a good measure of the conductivity in the manganites since the alignment of the manganese spins dominates the transfer of conduction electrons [2]. Our holography observations revealed that the magnetic flux density in the FM phase is 0.74 T (average for the three points indicated by asterisks in Fig. 4b). This value agrees with the theoretical value of 0.73 T deduced for the state in which the manganese spins are fully aligned. We conclude that the FM regions are highly conductive immediately after their formation and that their coalescence plays an important role in the CMR effect.

To summarize, our magnetic observations have revealed that highly conductive FM domains were produced by nucleation and growth, both of which were assisted by a magnetic field, despite the impedance by the preformed CO regions. The observations indicate the crucial role played by a FM domain network formation in the CMR effect for $La_{0.25}Pr_{0.375}Ca_{0.375}MnO_3$. We are certain that our observations will provide critical information for a conceptual advance in the understanding of the phase competition mechanism, which is of particular importance in both the fundamentals of solid state physics and the device application of hole-doped manganites.

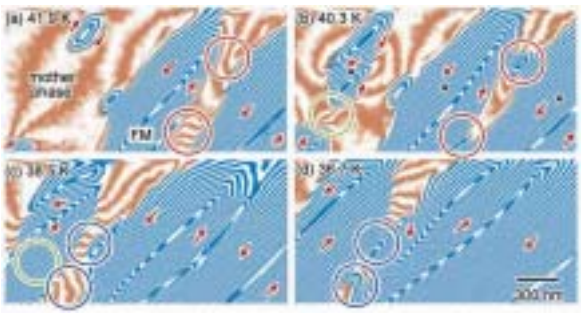


Figure 4: Changes in reconstructed phase image (result of magnetic flux mapping), observed under ZFC condition. Red arrows indicate direction of magnetic flux.

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[5] Y. Murakami, H. Kasai, J.J. Kim, S. Mamishin, D. Shindo, S. Mori and A. Tonomura, Nature Nanotechnology, 5, No. 1, 37-41 (2010).

3.2 Electron holography observation of magnetization distribution in pseudo soft underlayer of perpendicular magnetic recording media

The recent implementation of perpendicular magnetic recording systems has greatly enhanced the recording density of hard disk drives [6]. The soft underlayer (SUL) of perpendicular magnetic recording media plays an important role in the write performance because the SUL works as part of the write head [7]. The magnetic flux distribution in the off-track direction in the SUL is an important determination factor of the magnetic write width. Moreover, the magnetic flux density in the SUL reflects the write ability of overwrite performance [8,9]. We have shown that electron holography is very useful for visualizing the magnetic flux in the nano-sized writer pole tip of a recording head [10,11]. The direct visualization of the magnetic flux and the quantification of its magnitude in SULs will help us to understand the contribution of SUL permeability to overwrite and adjacent track erasure (ATE) performance. In this study, we compared the magnitude and distribution of magnetic flux in SULs with different values of permeability.

A multilayer comprising an Al_2O_3 layer, a pseudo SUL, and a Ti/NiFe layer was deposited by sputtering on the slider of a perpendicular magnetic recording head. We prepared two types of pseudo SULs (SUL-A and SUL-B) in order to study the effect of permeability on the magnitude and distribution of magnetic flux in SULs. The structure of SUL-A was CoFeB (27.5 nm)/CoFe (0.5 nm)/Ru (0.8 nm)/CoFe (0.5 nm)/CoFeB (27.5 nm), and that of SUL-B was simply CoFeB (60 nm). Saturation induction Bs of each SUL was approximately 1.25 T, and initial values of permeability μ' (= Bs/Hk) of SUL-A and SUL-B were 16.2 and 500, respectively. The SUL-A had antiferromagnetic coupling between the upper and lower CoFeB/CoFe layers via the Ru layer by RKKY interaction. The writer pole was made of $Fe_{90}Ni_{10}$; its Bs was 2.0 T and pole tip width was approximately 80 nm. The focused ion beam micro-sampling technique was used to form the writer pole tip and to fabricate thin-foil pseudo SUL specimens with a thickness of approximately 80 nm [12]. A 300-kV transmission electron microscope (TEM, Hitachi HF-3300X) with electron biprisms was used for the electron holography, which was carried out by applying an in-plane dc external field of 80 Oe along the y-axis as a write field at the writer pole tip.

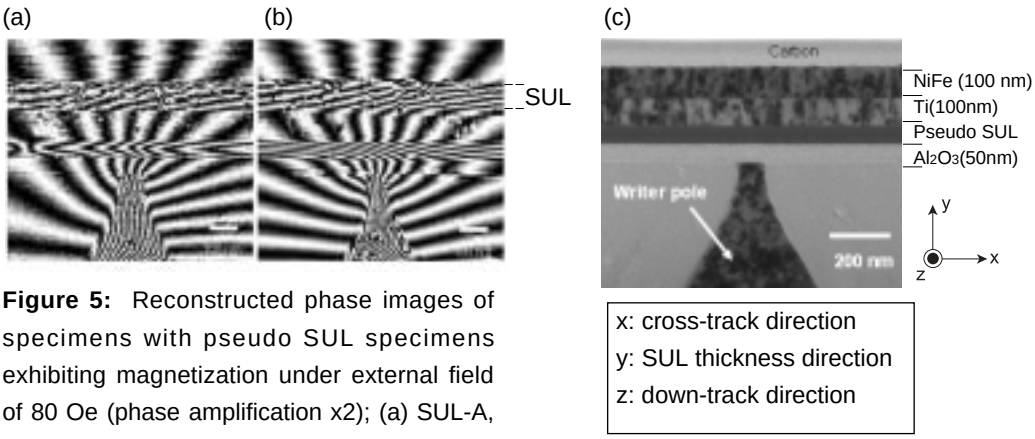

Figure 5: Reconstructed phase images of specimens with pseudo SUL specimens exhibiting magnetization under external field of 80 Oe (phase amplification x2); (a) SUL-A, (b) SUL-B, and (c) a TEM image of the sample specimen.

Figure 5 shows the reconstructed phase images and a TEM image of the specimens. The fringes indicate that the projected magnetic lines were quantized in magnetic flux units of h/e. The magnetic flux emerging from the writer pole tip passed through the Al_2O_3 layer, SULs, and Ti/NiFe layer. The magnetic flux was refracted at the center of SUL-A because the magnetization of the

magnetic layer had an antiparallel alignment with SUL-A through the Ru layer. In contrast, the magnetic flux in SUL-B was not refracted, and the magnetic flux emerging from the writer pole tip passed through SUL-B.

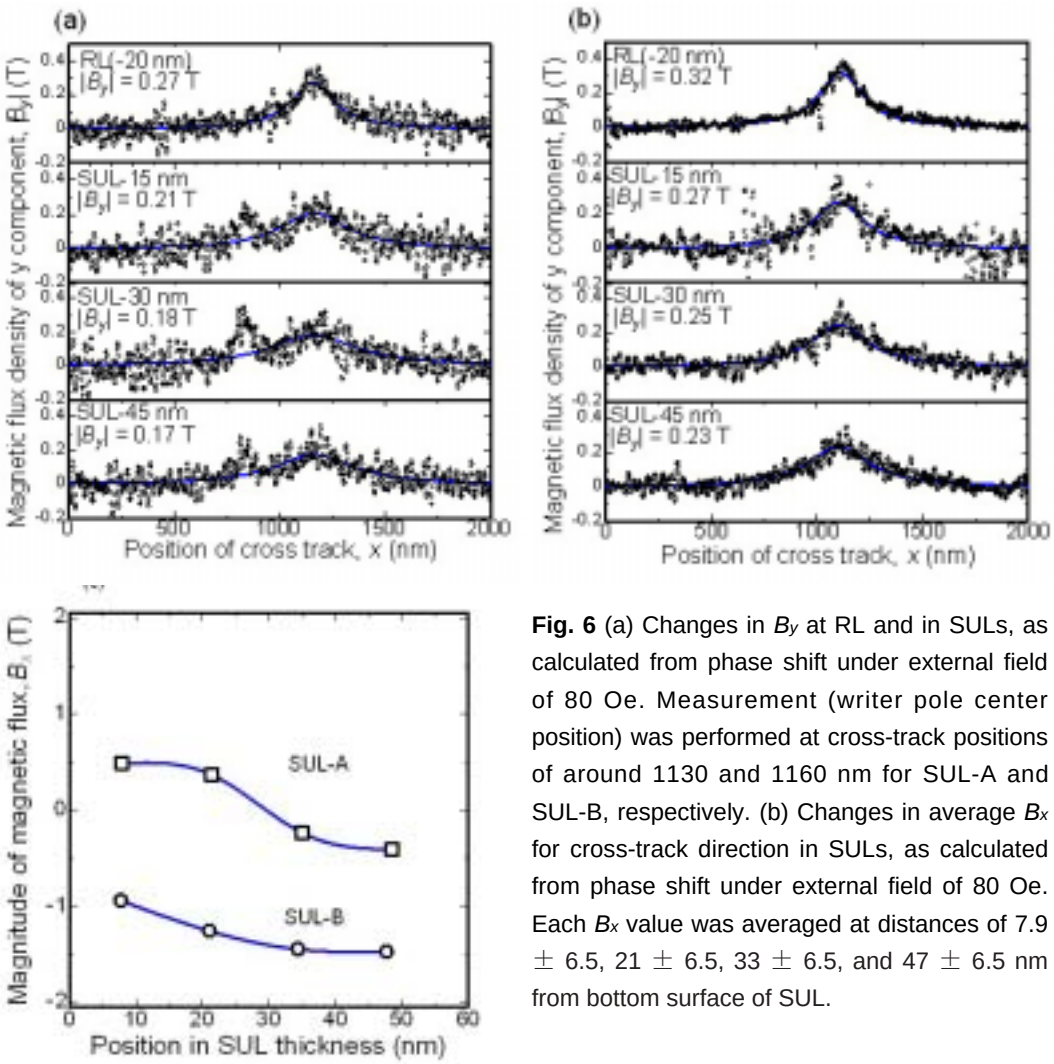

Fig. 6 (a) Changes in B_y at RL and in SULs, as calculated from phase shift under external field of 80 Oe. Measurement (writer pole center position) was performed at cross-track positions of around 1130 and 1160 nm for SUL-A and SUL-B, respectively. (b) Changes in average B_x for cross-track direction in SULs, as calculated from phase shift under external field of 80 Oe. Each B_x value was averaged at distances of 7.9 $\pm$ 6.5, 21 $\pm$ 6.5, 33 $\pm$ 6.5, and 47 $\pm$ 6.5 nm from bottom surface of SUL.

Figure 6 (a) shows the changes in magnetic flux By in the recording layer (RL) and SULs. The changes in By were calculated from the phase shift in the flux passing through the SULs, and the magnitude of B(x,y) was calculated using [13]

$$B_{(x,y)} = \frac{\hbar}{e \cdot l \cdot t_z} \times \frac{\partial \phi_{(x,y)}}{\partial x, \partial y} \quad (1)$$

where ø is the phase intensity, l is the pixel size of the image, and t_Z is the sample thickness (approximately 80 nm). The magnitude of B_y in the recording layer was measured at a distance of 20 nm from the surface of the writer pole tip, and the magnitude of B_y in the SULs was measured at distances of approximately 15, 30, and 45 nm from the bottom surface of the SULs. As shown by the solid lines in Fig. 6 (a), the changes in B_y showed good agreement with the Lorentzian fitting. The magnitude of B_y in SUL-B was greater than that in SUL-A at each measurement point

because SUL-B produced a higher write field than SUL-A due to the higher permeability (low Hs) of SUL-B, which resulted in better overwrite performance. Figure 6 (b) shows the changes in the average magnitude of B_x along the thickness direction of the SULs. To reduce the B_x value variation, we averaged the B_x values at distances of 7.9 ± 6.5, 21 ± 6.5, 33 ± 6.5, and 47 ± 6.5 nm from the bottom surface of the SULs. The magnitude of B_x in SUL-A showed a symmetric change with both positive and negative values, and the point at which the magnitude of B_x was zero closely corresponded to the position of the Ru layer. This change in the magnitude profile of B_x reflects the magnetization of the CoFeB/CoFe layers with antiparallel alignment through the Ru layer even under a high write field of 0.27 T (B_y) at RL. On the other hand, the magnitude of B_x in SUL-B showed only negative values because this SUL did not have an antiparallel magnetic alignment. The SUL-B had a uniform remanent magnetization of about -0.3 T along the cross-track direction in a 0-Oe external field. The absolute value of B_x increased with the increase in distance from the writer pole tip for an external field of 80 Oe. This suggests that SUL-B was magnetized at the SUL surface. We assumed that the magnetization behavior of the SUL-B caused the ATE. We found small magnetic flux leakage from the sample in the reference wave region (outside the specimen) in spite of the presence of the NiFe shield layer, and this leakage may have affected the reconstructed phase images. We estimated the effects of reference wave modulations on the reconstructed phase images and on the magnetic flux distribution, and investigated the accuracy of flux density measurement by electron holography. The magnetic flux densities inside the SUL were quantitatively evaluated.

Figures 7 (a) and (b) show the distributions of magnetic flux density inside the upper and lower SULs, along the lines a–a' and b–b', respectively. The red profiles in the figures indicate the smoothed experimental results and correspond to Fig. 5 (a). (These profiles are smoothed results from the experimental points, which had some dispersion). The green and black profiles indicate calculated magnetic flux densities in the object wave region and in the reference wave region, respectively, using the finite element method. The blue profiles indicate the results obtained by considering the modulation of the reference wave region. The calculated blue profiles should be compared with the experimental red profiles. We can see that in both positions, the green-line results give larger values than the blue-line results because of the effect of reference wave modulation. The peaks of the blue profiles show similar behavior to those of the red profiles. From these results, it was determined that the magnetic fluxes outside the specimen at the a–a'

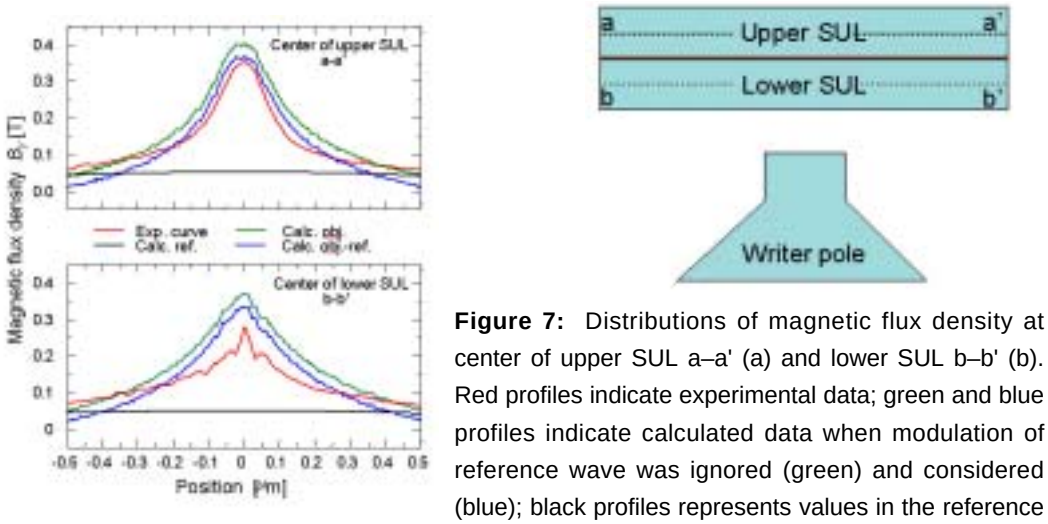

Figure 7: Distributions of magnetic flux density at center of upper SUL a–a' (a) and lower SUL b–b' (b). Red profiles indicate experimental data; green and blue profiles indicate calculated data when modulation of reference wave was ignored (green) and considered (blue); black profiles represents values in the reference wave region.

and b–b' positions were about 18.9% and 16.5% of the respective inner values. This indicates that large components of the outside magnetic fluxes were in the overall magnetic flux.

In summary, we measured the magnitudes and distribution of magnetic flux in SULs with different permeability values. We found that the magnitudes of B_y at RL and in the SUL were larger for SUL-B with larger permeability values. As a result, SUL-B had better overwrite performance than SUL-A.

Changes in the magnitude of B_x along the SUL thickness direction determined the SUL magnetic properties. The magnitude of B_x in SUL-A changed symmetrically through the Ru layer. The surface near the writer pole tip appeared to be magnetized for SUL-B; this may be related to the occurrence of ATE.

Magnetic flux density distributions were calculated by using the finite element method. The flux density was underestimated in the electron holography experiments probably caused by reference wave modulation. The measured magnetic flux was larger than the calculated magnetic flux inside the specimen due to the stray field outside the specimen.

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4. Publications

4.1 Journals

Batelaan, H. & Tonomura, A. The Aharonov-Bohm effects: Variations on a subtle theme. *Phys Today* 62, 38-43 (2009).

Hirata, K., Ishida, Y., Kim, J., Akashi, T., Kasai, H., Shindo, D., Tonomura, A. Electron holography observation of magnetization distribution in the pseudo soft underlayer of perpendicular magnetic recording media. *IEEE Magnetic Letters* (in press).

Hirata, K., Ishida, Y., Kasai, H., Akashi, T., Kim, J. J., Yanagisawa, K., Shindo, D. & Tonomura, A. Observing the Magnetization Distribution in the Pseudo Soft Underlayer of Perpendicular Magnetic Recording Media by Electron Holography. *IEEE T Magn* 46, 925-927, doi:Doi 10.1109/Tmag.2009.2032178 (2010).

Mamishin, S., Kasai, H., Xia, W., Murakami, Y., Shindo, D., Mori, S., Tonomura, A. Lorentz microscopy study on magnetization reversal in nanometer-sized ferromagnetic regions in manganite. *Japanese Journal of Applied Physics* (submitted).

Murakami, Y., Kasai, H., Kim, J. J., Mamishin, S., Shindo, D., Mori, S. & Tonomura, A. Ferromagnetic domain nucleation and growth in colossal magnetoresistive manganite. *Nat Nanotechnol* 5, 37-41, doi:Doi 10.1038/Nnano.2009.342 (2010).

Tonomura, A. in *Proceedings of the 9th International Symposium on Foundations of Quantum Mechanics in the Light of New Technology* (ed Ishioka, S. Fujikawa, K.) 301-306 (World Scientific, 2009).

Xia, W., Hirata, K., Ishida, Y., Yanagisawa, K., Kasai, H., Shindo, D., Tonomura, A. Quantitative evaluation of magnetic flux density in a magnetic recording head and pseudo soft underlayer by electron holography. *Journal of Electron Microscopy* (submitted).

4.2 Book(s) and other one-time publications

None.

4.3 Oral presentations

Hirata, K., Ishida, Y., Yanagisawa, K., Kasai, H., Shindo, D., Tonomura, A. *Observation of magnetization distribution in the pseudo soft underlayer of perpendicular magnetic recording media by electron holography*, Tokyo Institute of Technology, Tokyo, Japan, January 13, 2009

Tonomura, A. *Observation of magnetic fields based on AB effect, OIST Workshop Fundamentals of Quantum Mechanics and Its Applications*, Okinawa, Japan, May 13, 2009

Hirata, K., Ishida, Y., Kasai, H., Akashi, T., Kim, J., Tonomura, A. *Electron holography observation of magnetization distribution in the pseudo soft underlayer of perpendicular magnetic recording media*, Nagasaki University, Nagasaki, Japan, September 15, 2009

Murakami, Y., Kasai, H., Kim, J.J., Mamishin, S., Shindo, D., Mori, S., Tonomura, A. *Nucleation and Growth Process of the Ferromagnetic Metal Phase in a CMR Manganite*, Fall Meeting of the Japan Institute of Metals, Kyoto, September 17, 2009

Tonomura, A. *Observation of magnetic fields based on the AB effect*, The 20th Meeting of Frontiers of Electron Microscopy in Materials Science, Sasebo, October 1, 2009

Tonomura, A. *The AB Effect and Its Expanding Applications*, 50 years of the Aharonov-Bohm Effect: Concepts and Applications, Tel Aviv University, Tel Aviv, Israel, October 14, 2009

Tonomura, A. *Direct Observation of Microscopic Magnetic Structures in Materials by Electron Phase Microscopy*, ICMR2009AKITA The Final Circular, The Sixth International Conference on Materials Engineering for Resources, Akita View Hotel, Akita, Japan, October 21, 2009

Tonomura, A. *The Quantum World Observed using Electron Waves*, Aharonov Bohm Effect and Berry Phase Anniversary 50/25, University of Bristol, Bristol, United Kingdom, December 14, 2009

Hirata, K., Ishida, Y., Yanagisawa, K., Kasai, H., Shindo, D., Tonomura, A. *Electron holography observation of magnetization distribution in the soft underlayer of perpendicular magnetic recording media*, 11th Joint MMM-Intermag conference, Marriott Wardman Park Hotel, Washington D.C., U.S.A., January 19, 2010

Tonomura, A. *Observation of Microscopic Distribution of Magnetic Fields by Electron Waves*, 11th Joint MMM-Intermag Conference, Washington, D.C., U.S.A., January 20, 2010

Murakami, Y., Kasai, H., Kim, J.J., Mamishin, S., Shindo, D., Mori, S., Tonomura, A. *Lorentz Microscopy Studies on the Magnetic Phase Separation in a Manganite*, Spring Meeting of the Japan Institute of Metals, Tsukuba, March 27, 2010

4.4 Posters

Murakami, Y., Kasai, H., Kim, J.J., Mamishin, S., Shindo, D., Tonomura, A., Mori, S. *Magnetic Domain Observations in CMR Manganite La_0.25Pr_0.375Ca_0.375MnO_3*, OIST Workshop, Fundamentals of Quantum Mechanics and Its Applications, May 13, 2009

Hirata, K., Ishida, Y., Kasai, H., Akashi, T., Kim, J. J., Tonomura, A. *Electron holography observation of fine magnetization distribution in the pseudo soft underlayer of perpendicular magnetic recording media*, OIST Workshop, Fundamentals of Quantum Mechanics and Its Applications, Okinawa, May 13, 2009

Murakami, Y., Kasai, H., Kim, J.J., Mamishin, S., Shindo, D., Mori, S., Tonomura, A. *In Situ Observation of the Nucleation and Growth of Ferromagnetic Domains in CMR Manganite La_0.25Pr_0.375Ca_0.375MnO_3*, The 20th Meeting of Frontiers of Electron Microscopy in Materials Science, Sasebo, September 28, 2009

Kasai, H., Sugawara, A., Fukunaga, K., Tonomura, A. *OIST 300-kV Holography Electron Microscope for Nano-Magnetism*, The 20th Meeting of Frontiers of Electron Microscopy in Materials Science, Sasebo, September 28, 2009

Hirata, K., Ishida, Y., Yanagisawa, K., Kasai, H., Shindo, D., Tonomura, A. *Electron holography observation of magnetization distribution in the pseudo soft underlayer of perpendicular magnetic recording media*, The 20th Meeting of Frontiers of Electron Microscopy in Materials Science, Nagasaki Huis Ten Bosh, Sasebo, September 28, 2009

Mamishin, S., Kasai, H., Murakami, Y., Shindo, D., Mori, S., Tonomura, A. *Dynamic observation of magnetization reversal in tiny single-domains in CMR manganite*, The 20th Meeting of Frontiers of Electron Microscopy in Materials Science, Sasebo, September 29, 2009

5. Intellectual Property Rights and Other Specific Achievements

Nothing to be reported

6. Meetings and Events

6.1 OIST Workshop: "Fundamentals of Quantum Mechanics and Its Applications"

Date: May 13-15, 2009

Venue: OIST Seaside House

Co-organizers: Sumio Iijima (Meijo University, AIST, NEC)
Daisuke Shindo (Tohoku University)
Masahito Ueda (University of Tokyo)

Speakers: Chen Ning Yang (Tsinghua University: Nobel Laureate)
Peter Grünberg (Forschungszentrum Jülich GmbH: Nobel Laureate)
Klaus von Klitzing (Max-Planck Institute: Nobel Laureate)
Jun Kondo (The Japan Academy)
Anton Zeilinger (University of Vienna)
Federico Capasso (Harvard University)
Sumio Iijima (Meijo University)
Herman Batelaan (University of Nebraska-Lincoln)
Jaw-Shen Tsai (NEC)
Boris Altshuler (Columbia University)
Franco Nori (University of Michigan)
Andreas J. Heinrich (IBM)
Yoshichika Otani (University of Tokyo)
Masahito Ueda (University of Tokyo)
Kazu Suenaga (AIST)
Akira Tonomura (OIST)

Three Nobel Laureates attending the Okinawa Workshop (May 13, 2009)

Human Developmental Neurobiology Unit

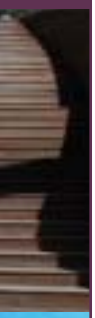
Principal Investigator:
Dr Gail Tripp
Research Theme:
Reward mechanisms in human behaviour and neuropsychiatric disorders

Abstract

The current research goals of the Human Developmental Neurobiology Unit are two fold. We wish to clarify the nature and extent of altered sensitivity to reinforcement in children whose behavior is consistent with a diagnosis of ADHD. In collaboration with the Wickens Neurobiology Unit we wish to elucidate the neurobiological basis underlying the altered sensitivity to reinforcement observed in children with ADHD. Our international research partnerships will facilitate identification of any additional contribution of environmental factors to the manner in which children with and without ADHD process reinforcement. The results from this work will make an important contribution to understanding the mechanisms underlying altered sensitivity to reinforcement and to the underlying causes of ADHD. We believe this knowledge can be translated into improved behavioral and pharmacological management for ADHD. Additional benefits arising from conducting our research in Japan include the opportunity to contribute to increased knowledge and awareness of ADHD in a country in which it has received relatively little research attention. In addition we are able to establish, test, and refine assessment procedures for local use.

1. Staff

Researchers: Dr Emi Furukawa, Dr Keiko Ito (departed April 2010)
Technical staff: Ms Yuko Hokama (departed March 2010),
Mr Naoya Miyagi (departed March 2010), Ms Waka Teruya (part time),
Ms Aska Matsudo (part time)
Research Administrator / Secretary: Ms Mika Matsuda (departed March 2010),
Ms Mayko Kurk
Student Volunteers: Ms Shirreka Mackay
Ms Jamilah Tetterton
Ms Amy Lucas



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 State of Rio de Janeiro

Type of partnership: Joint research agreement

Name of principal researcher: Dr Gail Tripp

Name of researchers: Dr Egas Caparelli-Dáquer, Dr Emi Furukawa

Research theme: Cross-national continuity of altered reward sensitivity in children with ADHD.

3. Activities and Findings

3.1 Research Activities

3.1.1 Participant Recruitment: We continue to engage actively with the community to recruit clinical and control participants for our research. This has included visits with the Mayor of Onna and local Education Offices (Yomitan, Onna); meetings with school Principals/Vice Principals of Onna-son; collaboration with several schools; visits to local medical clinics (Onna Clinic), attendance at parent groups, and development of a family friendly information brochure in both Japanese and English. We have been working with our colleagues in Brazil to extend the number of clinical recruitment sites for our data collection there.

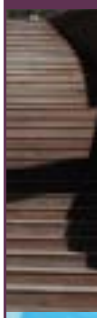
3.1.2 Data Collection: Here in Okinawa we are beginning to receive a steady number of referrals for clinical participants and have successfully carried out control group data collection in two schools. We will be presenting some preliminary findings from our control group data collection in Amsterdam in May 2010. Clinical participant data collection is continuing in Brazil and we hope to begin control group data collection there in the near future.

3.1.3 Training Activities/Ongoing Education: Unit technical staff engaged in a variety of data collection training exercises throughout the year, increasing their skill levels and allowing them to take a more active role in data collection and data management. The Unit PI and post doctoral researchers attended relevant national and international meetings (e.g., 20th meeting of the European Network for Hyperkinetic Disorders (EUNETHYDIS), 50th Annual Meeting of the Japanese Society for Child and Adolescent Psychiatry, International Symposium on Epilepsy in Autism Spectrum Disorders and Related Conditions, 8th Biennial Conference of the Asian Association of Social Psychology, 4th International Symposium on Cognition, Stress and Mental Health). We have been fortunate to have three graduate students from the Bowie State University Counseling Psychology Program, University of Maryland University College join our Unit as volunteers/student interns. They are training to assist with ongoing data collection.

3.2 Outreach Activities

3.2.1 International Research Meeting: To increase national and international awareness of OIST and the work of the Human Developmental Neurobiology Unit we hosted an international





workshop “Reinforcement and Attention Deficit Hyperactivity Disorder Workshop”. The workshop provided a forum for discussion between researchers studying the neural basis of reinforcement and those using behavioral and neuro-imaging methods to investigate reinforcement sensitivity in individuals with ADHD. Over the three day meeting 21 researchers at different stages in their careers (students, post docs, junior, senior and emeritus faculty) presented research relevant to the ongoing activities of the Human Developmental Neurobiology Research Unit. The meeting was truly international one with speakers from New Zealand, Japan, UK, Germany, Netherlands, USA, Brazil and South Africa.

3.2.2 Community Outreach Activities: To increase local awareness of ADHD and the research of the Human Developmental Neurobiology Unit we have made presentations to local schools and continued to develop community resources. For example, we provided a workshop on “Childhood Disorders that Affect Learning and Classroom Behavior” for teachers at the Miyazato Elementary School. This provided an opportunity for teachers to understand the relevance of our research to the community and facilitate our understanding of the issues faced by local teachers, which will lead to more effective data collection.

3.3 Collaborative Research Activities

3.3.1 Existing Collaborations: We have continued our externally funded research (Health Research Council of New Zealand) collaboration with Dr Brent Alsop at the University of Otago in New Zealand. Dr Alsop and Dr Sowerby (Research Fellow) presented results from our joint work in New Zealand at the “Reinforcement and Attention Deficit Hyperactivity Disorder Workshop”.

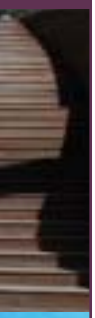
Our joint research project with Dr Egas Caparelli-Dáquer and the University of State of Rio de Janeiro (Cross-national continuity of altered reward sensitivity in children with ADHD) has successfully moved from the pilot testing to data collection phase.

3.3.2 New Research Collaborations: During her time in Brazil Dr Furukawa established two further collaborative relationships with research groups in Rio de Janeiro and São Paulo. The first with Dr Jorge Moll and the Neuroscience Center, LABS-D’Or Hospital Network (Functional imaging of altered reward sensitivity and its relation to human behavior), and the second with Dr Erasmo Barbante Casella and the University of São Paulo School of Medicine (Experimental study of altered reward sensitivity: Examining cross-national continuity and environmental influences). These collaborations build on the principal goals of our unit and examine the neurobiological and environmental contributions to reward sensitivity by incorporating multiple levels of measurements and developing an international, interdisciplinary team.

4. Publications

4.1 Journals

Galland, B. C., Tripp, E.G., & Taylor, B.J. The sleep of children with ADHD on and off methylphenidate: A matched case control study. *Journal of Sleep Research* (in press).



Luman, M., Tripp, G. & Scheres, A. Identifying the neurobiology of altered reinforcement sensitivity in ADHD: A review and research agenda. *Neuroscience and Biobehavioral Reviews* 34, 744-754, (2010).

Sowerby, P., Seal, S., & Tripp, G. Working memory deficits in ADHD: The contribution of age, learning/language difficulties and task parameters. *Journal of Attention Disorders* (in press).

Sutherland, K. R., Alsop, B., McNaughton, N., Hyland, B. I., Tripp, G. & Wickens, J. R. Sensitivity to delay of reinforcement in two animal models of attention deficit hyperactivity disorder (ADHD). *Behavioural Brain Research* 205, 372-376, (2009).

Tripp, G. & Wickens, J. R. Neurobiology of ADHD. *Neuropharmacology* 57, 579-589, (2009).

4.2 Book(s) and other one-time publications

Furukawa, E. & Hunt, J. *Therapy with refugees and immigrants experiencing shame: A multicultural perspective*. In R. L. Dearing and J. P. Tangney (Ed.), Shame in therapy hour. Washington D.C.: APA (in press).

4.3 Oral presentations

Tripp, G. *Dopamine Reinforcement and ADHD*, Kochi-Prefecture ADHD Study Group, June 2009.

Tripp, G. *Evidence for altered sensitivity to reinforcer frequency in boys with attention deficit hyperactivity disorde*r, OIST, Okinawa, July 2009.

Tripp, G. *Altered sensitivity to reinforcement as an explanation for ADHD*, Reinforcement and ADHD Workshop, Seaside House, OIST, Okinawa, September 2009.

Furukawa, E. *What's Wrong? How Can I Help?!: Addressing ADHD and Other Behavioral, Emotional and Learning Difficulties in the Classroom*, Presentation at the Institute Brasil Estados Unidos, Rio de Janeiro, Brazil, April 2009.

Furukawa, E. *Addressing ADHD and Other Developmental and Mental Health Issues in Childhood*, Presentation at the AmerAsian School, Ginowan, Okinawa, February 2010.

Furukawa, E. *学習や学校生活に影響を及ぼす小児期の障害について (Childhood Disorders that Influence Learning and School Activities)*, Presentation at 沖縄市立宮里小学校 (Miyazato Elementary School), Okinawa-shi, Okinawa, March 2010.

4.4 Posters

Robinson, T. Tripp, G. *Neuropsychological functioning in children with ADHD: A four year longitudinal study*, 20th Meeting EUNETHYDIS, Winchester, England, September 2009.

Tripp, G., Galland, B., Taylor, B., & Phillips, A. *Methylphenidate effects on sleep in children with ADHD*, 20th Meeting EUNETHYDIS, Winchester, England, September 2009.

5. Intellectual Property Rights and Other Specific Achievements

Nothing to be reported.

6. Meetings and Events

6.1 Reinforcement and Attention Deficit Hyperactivity Disorder Workshop

Date: September 8 - 10

Venue: OIST Seaside House

Co-organizer: Dr Jeff Wickens

Speakers: Eric Taylor

Department of Child and Adolescent Psychiatry, Institute of Psychiatry, Kings College London, UK.

Vivienne A. Russell
Department of Human Biology, Faculty of Health Sciences, University of Cape Town, South Africa.

Brian Hyland
Department of Physiology, University of Otago, New Zealand.

Shunsuke Kobayashi
Department of Physiology, Development and Neuroscience, University of Cambridge, UK.

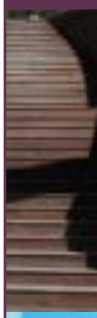
Paul E. M. Phillips
Department of Psychiatry and Behavioural Sciences, University of Washington, USA.

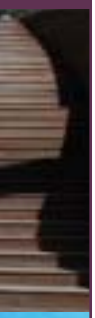
Jeff Wickens
Neurobiology Research Unit, Okinawa Institute of Science and Technology, Okinawa, Japan.

Chris Perk
Departments of Anatomy and Physiology, University of Otago, New Zealand.

Gail Tripp
Human Developmental Neurobiology Unit, Okinawa Institute of Science and Technology, Okinawa, Japan.

Paula Sowerby
ADHD Research Clinic, Department of Psychology, University of Otago, New Zealand.





Brent Alsop
Department of Psychology, University of Otago, New Zealand.

Taiji Masunami
University of Tsukuba, Japan.

Marjolein Luman
Department of Clinical Neuropsychology, Vrije Universiteit, Netherlands.

Greg G. Gerhardt
Department of Anatomy and Neurobiology, University of Kentucky, Chalndler Medical Center, USA.

Ronald Kuczenski
Psychiatry Department, University of California, USA.

Jim Swanson
Child Development Center, UCI School of Medicine, USA.

Patrick de Zeeuw
Rudolf Magnus Institute of Neuroscience, University Medical Centre, Netherlands.

Patricia Bado
Cognitive & Behavioral Neuroscience Unit, LABS-D'OR Hospital Network Neuroscience Centre, Brazil.

Michael M. Plichta
Department of Psychiatry, Psychosomatics and Psychotherapy, University of Würzburg, Germany.

Catharina S. Van Meel
Department of Clinical Neuopsychology, Vrije Universiteit, Netherlands.

Samantha Broyd
Institute for Disorders of Impulse and Attention (IDIA), University of Southampton, UK.

Jonathan Williams
National Health Service, Institute of Psychiatry, UK.

Other remarks: Research meeting

Neurobiology Research Unit

Principal Investigator:
Dr 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. Research activity has focused on experimental studies of synaptic plasticity in the corticostriatal pathway, and theoretical studies of striatal network dynamics and reinforcement mechanisms important in learning. 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. We are undertaking 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) precise timing requirements for synaptic plasticity (ii) cell-type specific differences in synaptic plasticity, and (iii) dendritic mechanisms underlying 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. We are also undertaking theoretical studies at cellular, network and systems levels, which aim to integrate the experimental findings with network level activity and examine their implications for understanding and treating neuropsychiatric disorders.

1. Staff

Researchers: Dr Tomomi Shindou, Dr Catherine Vickers, Dr Mayumi Ochi-Shindou,
Dr Adam Ponzi, Dr Luca Aquili
Technical Staff: Dr Saori Miura, Mr. Kavinda Liyanagama,
Mr. Prageeth Saraka Wimalaweera (departed October 2009),
Ms Michelle Callahan (departed 15 January 2010),
Mr. Kiyoshi Baba (departed 31 January 2010)
Research Assistant / Graduate Student: Mr. Takashi Nakano (based in Doya Unit)
Mr. Yu-Ting Li, Ms Yi-Ling Huang
Research Administrator / Secretary: Ms Yukako Suzuki

2. Partner Organizations

University of Otago, New Zealand
Type of partnership: Joint Research
Name 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
Name of researchers: Dr Jason Chen, 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 year of operation, the laboratory interior was completed and the equipment was commissioned. Recruitment has continued and in the past year the Unit grew to its final size when two new researchers joined us. Research activity has focused 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 timing requirements for induction of synaptic plasticity.

Learning mechanisms are thought to operate at the level of individual synapses. Based on findings in other systems, the timing of presynaptic relative to postsynaptic activity is likely to be crucial for plasticity in the corticostriatal system. In addition, our previous work suggests that modulation by dopamine is important. We hypothesize that the selection of appropriate corticostriatal synapses for modification during learning involves activity-dependent plasticity with precise timing requirements, which may be modulated by dopamine.

We have extended our studies of dendritic mechanisms associated with spike-timing dependent plasticity (STDP) in the corticostriatal pathway. Using whole-cell recording from striatal projection neurons in adult mice we have measured STDP over a range of different timing and excitation

conditions. We found that long-term depression (LTD) of corticostriatal inputs was maximally induced by pairing protocols in which the cortically evoked excitatory post-synaptic potential (EPSP) preceded postsynaptic action potentials by 10 ms (pre-post protocol). In contrast, post-pre protocols, or stimulation of the pre- or post-synaptic sites alone, induced no change in the cortical EPSP. The LTD was blocked by the intracellular calcium chelators, BAPTA or EGTA, suggesting a requirement for elevated calcium in induction of LTD. However, this form of LTD was not blocked by the NMDA-receptor antagonist, APV, indicating a non-NMDA channel source of the calcium signal. On the other hand, LTD was not observed in the presence of a CB1 receptor antagonist, indicating the involvement of endocannabinoid signaling pathways.

Since intracellular Ca^{2+} levels play a key role in the induction of synaptic plasticity in the corticostriatal pathway, they may - as in other systems - be the basis of the temporal requirements observed. To investigate the mechanism underlying the spike-timing dependence of LTD, we are using 2-photon microscopy to measure intracellular Ca^{2+} concentration in individual dendritic spines, after uncaging of glutamate adjacent to the spine. Dendritic spine Ca^{2+} signals have been measured during STDP induction protocols. Pre-post protocols that induced LTD were associated with larger spine Ca^{2+} transients than protocols that produced no LTD, see Figure 1. These findings suggest a Ca^{2+} threshold for induction of LTD in the corticostriatal pathway and map out the way the timing of pre- relative to post-synaptic activity is translated into local Ca^{2+} signals. Current work is investigating modulation of these timing requirements by dopamine.

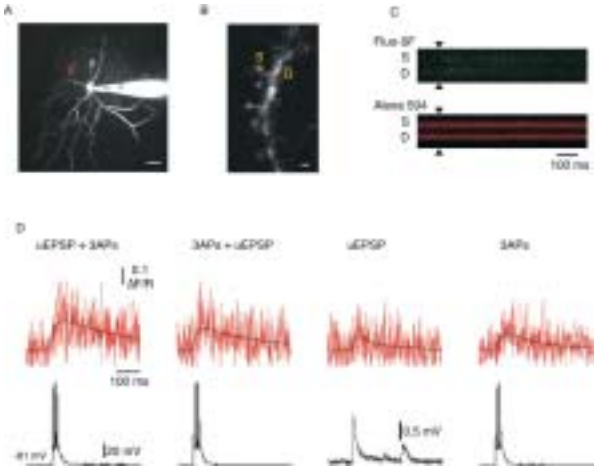


Figure 1: Pre-post protocols were associated with larger spine Ca^{2+} transients than protocols that produced no LTD. A, Two-photon scan of a spiny neuron, including the recording pipette at the soma. The region indicated by the box is shown on an expanded scale in B. B, Fluorescence image of an active synaptic spine. The dashed line indicates the position of the line scan. Scale bars: A, 50 µm; B, 1 µm. C, An example line scan image for the Ca^{2+}-sensitive indicator Fluo-5F (green, top) and the Ca^{2+}-insensitive indicator Alexa Fluor 488 (red, bottom) in response to uncaging of MNI-glutamate (uEPSP, arrowheads) combined with three postsynaptic action potentials (Δt = 10 ms). D. The Ca^{2+} transients (top) and the corresponding somatic voltage recordings (bottom) for a uEPSP and three APs at Δt = 10 ms, three APs and uEPSP at Δt = -30ms, uEPSP, and three APs. Traces are averages of three responses. The Ca transients add supralinearly when the uEPSP precedes the spike time by 10 ms.

3.2 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 from 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 developed plasticity-inducing stimulation protocols for mouse brain slices. Our ability to definitively identify morphologically similar cells by their expression of GFP is a powerful new technique that is adding important new understanding. We have tested the hypothesis that dopamine differentially regulates synaptic plasticity in dopamine D1a 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. We found that STDP protocols produced LTD in dopamine D1a receptor-expressing neurons but not in dopamine D2 cells, as shown in Figure 2. However, LTD was not blocked by a D1 receptor antagonist, indicating that although the capacity for LTD is associated with expression of the receptor, activation of the receptor is not required for LTD induction.

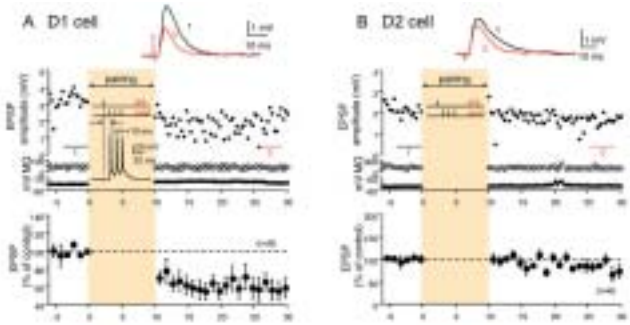


Figure 2. Cell-type specific difference in LTD. A. Pre- before post protocols induced LTD in D1a-receptor positive cells. B. The same protocols produced no LTD in D2-receptor positive cells.

Induction of plasticity using high frequency cortical stimulation (100 Hz) produced a different pattern of results in which potentiation predominates and D1a and D2 cells display cell specific responses. D2 cells potentiate to a significantly higher degree that the D1a cells, suggesting that afferent information integration and processing is different in the two cell types.

3.3 Actions of dopamine on STDP

One of the earliest issues in the theory of reinforcement learning, dating back to Thorndike, was the effectively retroactive action of reward on decision-making substrates. One idea to solve this issue was the concept of an eligibility trace, localized to synapses that were active prior to the actions that led to the reward. Subsequent reward may then act on eligible synapses, thus automatically assigning credit to neural activity that preceded the decision. Eligibility traces have been widely employed in this form in computational models of learning. We tested a synaptic eligibility trace hypothesis in corticostriatal synapses, using spike-time dependent plasticity induction protocols combined with photolytic uncaging of dopamine. In contrast to predictions, dopamine was most effective when uncaged immediately (30 ms) prior to conjunction of presynaptic and postsynaptic spikes, and had no effect when uncaged after a delay. These experiments led us to reject the eligibility trace hypothesis in the dorsal striatum. However, they do not rule out eligibility traces in other brain regions. Also, uncaging of dopamine does not necessarily produce the same effect as the synaptic release of dopamine.

To address the actions of synaptic release of dopamine we have developed the ability to express in selected neurons the algal protein Channelrhodopsin-2 (ChR-2), a rapidly gated light-sensitive cation channel, by using lentiviral gene delivery. We have expressed the protein in cultured striatal and cortical neurons and demonstrated light-induced activation. As shown in Figure 3, high expression of ChR-2 permits repetitive and reliable light driven spiking of neurons. This technique enables temporally precise, non-invasive control of activity in well-defined neuronal populations. In ongoing work we aim to use this method to cause dopamine release by optical stimulation, which will enable us to investigate the effects of endogenously released dopamine on synaptic plasticity. To date we have been able to express ChR-2 in dopaminergic neurons of intact mice and evoke dopamine release by optical stimulation of striatal slices prepared from these mice.

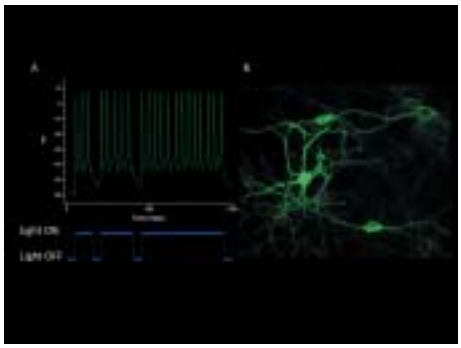


Figure 3: High expression of ChR-2 permits light driven spiking of neurons. A. Electrophysiological response of cortical neurons (top trace) to light pulses (bottom trace). B. Neurons expressing green fluorescent protein, indicating successful expression of channel rhodopsin 2 FCK GFP.

3.4 Measurement of dopamine levels in awake animals

We have previously shown that dopamine acts at the cellular level to strengthen synapses in the striatum of the forebrain. These actions of dopamine on synaptic strength provide a cellular mechanism for reward-related learning. In this mechanism, precisely timed and pulsatile increases in dopamine concentration are critical for correct processing of reward. To complement these ongoing studies of dopamine modulation of plasticity, we aim to measure dopamine release in awake animals on a precise, subsecond timescale. We have developed the ability to use fast-scan cyclic voltammetry (FSCV) to measure dopamine release in awake-behaving rats using carbon fibre microelectrodes. We have developed hardware and software to conduct FSCV and perform principal components regression to better identify the chemicals detected. We are now using this approach to measure the effects of methylphenidate on the phasic dopamine signal. Our longer-term aim is to use this method to test a theory concerning the therapeutic actions of methylphenidate in the treatment of attention-deficit hyperactivity disorder (ADHD).

3.5 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). We are extending this work to investigate the effects of active dendrites on the back-propagation of action potentials from the cell soma to dendritic spines.

In addition to analysis of biophysical requrements we have been developing computer simulations of network activity in striatal inhibitory networks. We found that realistic connectivity patterns led to spontaneous generation of assemblies that fire in sequence in response to unstructured input. This suggests an important functional role for sparse lateral inhibition in the striatum that may be relevant to neural activity sequences encoding behavior.

In addition to cellular and network modelling, we have also contributed to the development of a neurobiological hypothesis of altered reinforcement mechanisms in ADHD (in collaboration with the Tripp Unit). We proposed that a number of symptoms of the disorder could be interpreted as due to a failure of dopaminergic responses to established reinforcers to transfer to new predictors of reinforcement. This model makes a number of specific behavioural and neural predictions that are testable by experiment. Our theory has been widely cited in experimental tests of the model. We aim to test further predictions of the model by measuring phasic release of dopamine in awake, behaving animals during learning of tasks involving delay of reinforcement.

4. Publications

4.1 Journals

Johansen, E. B., Killeen, P. R., Russell, V. A., Tripp, G., Wickens, J. R., Tannock, R., Williams, J. & Sagvolden, T. Origins of altered reinforcement effects in ADHD. *Behav Brain Funct* 5, 7 (2009).

Joshua, M., Adler, A., Prut, Y., Vaadia, E., Wickens, J. R. & Bergman, H. Synchronization of midbrain dopaminergic neurons is enhanced by rewarding events. *Neuron* 62, 695-704 (2009).

Ponzi, A. & Wickens, J. Cell assemblies in large sparse biologically realistic networks of spiking neurons. *Advances in Neural Information Processing Systems* 21, 1273-1281 (2009).

Ponzi, A. & Wickens, J. Sequentially switching cell assemblies in random inhibitory networks of spiking neurons in the striatum. *J Neurosci* 30, 5894-5911 (2010).

Schulz, J. M., Redgrave, P., Mehring, C., Aertsen, A., Clements, K. M., Wickens, J. R. & Reynolds, J. N. Short-latency activation of striatal spiny neurons via subcortical visual pathways. *J Neurosci* 29, 6336-6347 (2009).

Sutherland, K. R., Alsop, B., McNaughton, N., Hyland, B. I., Tripp, G. & Wickens, J. R. Sensitivity to delay of reinforcement in two animal models of attention deficit hyperactivity disorder (ADHD). *Behavioural Brain Research* 205, 372-376 (2009).

Tripp, G. & Wickens, J. R. Neurobiology of ADHD. Neuropharmacology 57, 579-589 (2009).

Voon, V., Fernagut, P. O., Wickens, J., Baunez, C., Rodriguez, M., Pavon, N., Juncos, J. L., Obeso, J. A. & Bezard, E. Chronic dopaminergic stimulation in Parkinson's disease: from dyskinesias to impulse control disorders. *Lancet Neurol* 8, 1140-1149 (2009).

Wickens, J. R. Synaptic plasticity in the basal ganglia. *Behavioural Brain Research* 199, 119-128 (2009).

4.2 Books and other one-time publications

Plenz, D. & Wickens, J. R. The striatal skeleton: Medium spiny projection neurons and their lateral connections, in *Handbook of Basal Ganglia Structure and Function* (eds H. Steiner & K.Y. Tseng) 99-109, (AP, 2009).

Shindou, T., Arbuthnott, G. W. & Wickens, J. R. Neuromodulation and neurodynamics of striatal inhibitory networks: implications for Parkinson's disease, in *Cortico-subcortical dynamics in Parkinson's Disease, Contemporary Neuroscience Series* (ed Kuei Y. Tsen) 233-243, (Humana Press & Springer Editorials, 2009).

Shindou, T. & Wickens, J. Drugs for motor disorders, in *Encyclopedia of Neuroscience Vol. 4* (ed Nobutaka Hirokawa Marc D. Binder, Uwe Windhorst) (Springer, Berlin, Heidelberg, 2009). DOI 10.1007/978-3-540-29678-2_1625

Wickens, J. R. & Arbuthnott, G. W. Gating of cortical input to the striatum, in *Handbook of Basal Ganglia Structure and Function* eds H. Steiner & K.Y. Tseng) 341-352, (AP, 2009).

4.3 Oral presentations

Wickens, J. R. *Neurobiological challenges for theoreticians*, Okinawa Computational Neuroscience Course 2009, OIST Seaside House, Okinawa, Japan, June 15-July 2, 2009

Wickens, J. R., Ochi-Shindou, M., Shindou, T. *Dopamine dependent synaptic plasticity in the corticostriatal pathway*, Multidisciplinary approaches to basal ganglia functions, XXXVI International Congress of Physiological Sciences, Kyoto, Japan, July 27-August 1, 2009

Wickens, J. R. *Reinforcement and attention deficit hyperactivity disorder*, Reinforcement and Attention Deficit Hyperactivity Disorder Workshop, OIST Seaside House, Okinawa, Japan, September 8-10, 2009

Wickens, J. R., Shindou, T., Ochi-Shindou, M. *So long eligibility trace, come on eligibility state*, Betsheva Seminar on Reward and Decision Making in the Brain, Jerusalem, Israel, February 16-19, 2010

4.4 Posters

Ochi-Shindou, M., Shindou, T., Wickens, J. R. *Dopamine modulates spike-timing-dependent synaptic plasticity in the D1 receptor-positive spiny neurons in the neostriatum of adult mice*, XXXVI International Congress of Physiological Sciences, Kyoto, Japan, July 27-August 1, 2009

Ponzi, A., Wickens, J.R. *Irregular bursting assemblies emerge in a striatal network model*, Annual Meeting of Japan Neuroscience Society 2009, Nagoya, Japan, September 16-18, 2009

Ponzi, A., Wickens, J.R. *Irregular bursting assemblies emerge in a striatal network model,* The Basal Ganglia in Health and Disease Symposium, Okazaki, Japan, September 14-15, 2009

Ponzi, A., Wickens, J.R. *Irregular bursting assemblies emerge in a striatal network model,* The 4th Molecular and Cellular Cognition Society (MCCS)-Asia Symposium, Nagoya, Japan, September 15, 2009

Ponzi, A., Wickens, J.R. *A balanced striatal network model*, Society for Neuroscience 2009 Annual Meeting, Chicago, USA, October 17-21, 2009

Ponzi, A., Wickens, J.R. *Investigation of striatal network dynamics by computational modeling*, The 10th Winter Workshop, Rusutsu, Hokkaido, Japan, January 12-14, 2010

Shindou, T., Ochi-Shindou, M., Wickens, J. R. *A Ca^{2+} threshold for induction of spike-timing dependent depression in the striatum?*, Gordon Research Conference on Dendrites: Molecules, Structure & Function, Il Ciocco Hotel and Resort in Lucca (Barga), May 17-22, 2009

Shindou, T., Ochi-Shindou, M., Wickens, J. R. *Dendritic spine Ca^{2+} signals associated with spike-timing dependent synaptic plasticity in medium spiny neurons in the mouse neostriatum*, XXXVI International Congress of Physiological Sciences, Kyoto, Japan, July 27-August 1, 2009

Shindou, T., Ochi-Shindou, M., Wickens, J. R. *Dopaminergic modulation and dendritic calcium signals in spike-timing dependent synaptic plasticity in the striatum*, Gordon Research Conference on Catecholamines, University of New England, Maine, USA, August 9-14, 2009

Vickers, C., Arbuthnott, G.W., Wickens, J. R. *Dopamine D2 receptor expressing striatal projection neurons display long term potentiation after high frequency stimulation of cortical afferents*, Australian Neuroscience Society 2010, Sydney, Australia, January 31-February 3, 2010

5. Intellectual Property Rights and Other Specific Achievements

Nothing to report

6. Meetings and Events

6.1 OIST Seminar

Date: July 22, 2009

Venue: Uruma City Laboratory, OISTPC

Speakesr: Mr. Luca Aquili

Other remarks: University of St. Andrews, PhD Student

6.2 OIST Internal Seminar

Date: December 11, 2009

Venue: Uruma City Laboratory, OISTPC

Speakesr: Mr. Yu-Ting Li

Other remarks: Institute of Biomedical Engineering, National Cheng Kung University, Graduate Student Research Assistant

6.3 Okinawa Computational Neuroscience Course 2009

Date: June 15 – July 2, 2009

Venue: OIST Seaside House, Onna-son, Okinawa

Co-organizers: Dr Erik De Schutter, Dr Kenji Doya, Dr Klaus Stiefel, Dr Jeff Wickens, Okinawa Institute of Science Technology

Co-sponsors: Nara Institute of Science and Technology, Japanese Neural Network Society

Speaker: Invited faculty

G0 Cell Unit

Principal Investigator:
Mitsuhiro Yanagida

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

Abstract

The regulation of cellular proliferation and quiescence (G0 phase) in response to changes in the environment, such as the availability of nutrients, is a central issue in biology and medicine. Our research goal is to understand the molecular mechanisms by which cell division and cell cycle arrest in the G0 phase are regulated in response to available nutrients. To this end, we have adopted the fission yeast *Schizosaccharomyces pombe,* a simple unicellular eukaryote, as a model organism. This year, we continued to study G0 phase arrest induced by nitrogen starvation, and published three reports regarding the mechanisms of maintenance of G0 phase cells and metabolomic changes in G0 phase. First, we identified 33 genes required for G0 phase entry/maintenance by screening a library of temperature-sensitive (ts) mutants. We found that stress-activated MAP kinase pathways, actin-regulated endocytosis, and vacuole fusion regulation are involved in cell entry into the G0 phase. Second, we analyzed previously identified genes required for G0 phase maintenance. The ubiquitin/proteasome system and autophagy cooperatively maintain mitochondria and prevent the lethal accumulation of reactive oxygen species (ROS). Finally, we established a cell metabolomic analysis method using mass spectrometry. Based on a metabolomic comparison between cells in the G0 and proliferation phases, we identified several compounds that are drastically increased or decreased in the G0 phase. This method is also applicable for clarifying the metabolomic aspects of certain mutants. Furthermore, we initiated new studies, such as more comprehensive screening of mutant and gene-knock-out libraries, physiologic adaptation of cells upon shifting to low glucose conditions, and a comprehensive analysis of the library of temperature-sensitive mutants using the next generation sequencer. Based on these and previous studies, we published a review article in Trends in Cell Biology on the genetic conservation mechanism of cellular quiescence.



1. Staff

Okinawa

Group leader: Mizuki Shimanuki (until December 2009)

Researchers: Koji Nagao (until July 2009) , Kojiro Takeda, Kenichi Sajiki, Takahiro Nakamura (from January 2010), Kumiko Ohta (from January 2010)

Technical staff: Sakura Kikuchi, Aya Kokubu (until July 2009), Ayaka Mori, Risa Uehara, Tomas Pluskal, Bryan Mathis (until June 2009), Alejandro Villar-Briones.

Research assistant/graduate student: Yuria Tahara (From March 2009)

Research Administrator / Secretary: Tomomi Teruya

Kyoto

Researchers: Takeshi Hayashi, Kumiko Ohta (until December 2009)

Research Administrator / Secretary: Takako Shiono (from November 2009)

2. Partner Organizations

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 of Schizosaccharomyces pombe

Institute for Protein Research, Osaka University

Type of partnership: Collaboration

Name of researcher: Junko Kanoh

Research theme: Analysis of TOR complexes by specific and common subunits

Department of Molecular Biotechnology, Graduate School of Advanced Science of Matter, Hiroshima University

Type of partnership: Collaboration

Name of principal researcher: Masaru Ueno

Research theme: Metabolite structure determination using NMR

Institute of Life Science, Kurume University

Type of partnership: Collaboration

Name of principal researcher: Shigeaki Saitoh

Research theme: Identification of fission yeast mutants, which show growth defect under low glucose condition

Division of Metabolism and Nutrition, Department of Biochemistry and Molecular Biology, Nippon Medical School
Type of partnership: Collaboration
Name of principal researcher: Hideo Orimo
Name of researcher: Asako Kounosu
Research theme: Metabolomic analysis of human cultured cells under low-glucose condition

3. Activities and Findings

3.1 Identification of genes essential for G0 entry and/or maintenance under nitrogen starvation

A search for genes required for both proliferation and quiescence was performed using a previous method with 610 ts mutants, and approximately a quarter of the strains examined were defective at quiescence entry and/or maintenance. Among them, 33 core genes crucial for entry into and maintenance of quiescence were identified (Figure 1).

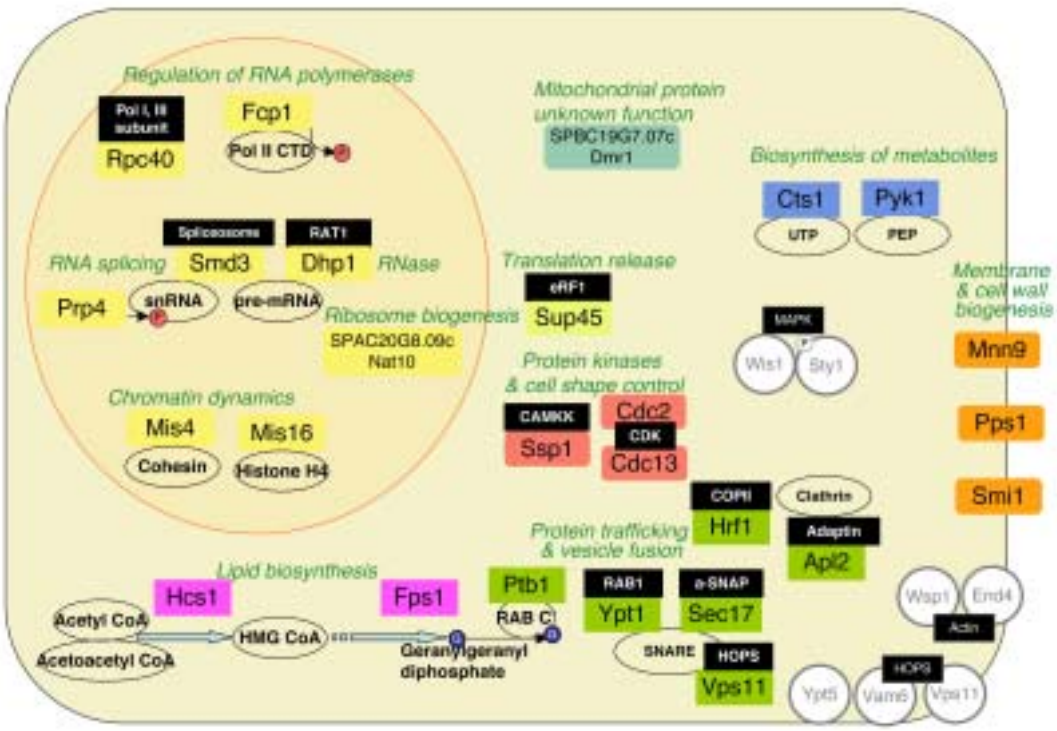


Figure 1: Identified genes involved in the regulation of G0
Twenty-six genes required for the maintenance of S. *pombe* G0, and seven genes for the entry (gray circles).

Because these genes are essential for both cell proliferation and the G0 phase, we designated them ‘super house-keeping genes’. These genes cover a broad range of cellular functions in the cytoplasm, membrane, and nucleus. They encode proteins for stress-activated and cell-cycle

kinase signaling pathways, actin-bound and osmo-controlling endosome formation, RNA transcription, splicing and ribosome biogenesis, chromatin silencing, lipid and ATP biosynthesis, cell-wall and membrane morphogenesis, protein trafficking, vesicle fusion. We examined the morphology of the temperature-sensitive mutant strains of these genes under nitrogen starvation by DAPI staining (Figure 2). Eight strains defective for G0 entry had mutations of the stress-activated MAP kinase Sty1 (also known as MAP kinase Spc1 and MAPK); the activator Wis1 MAP kinase kinase (MAPKK); Ypt5 (RAB5 in mammals), Vam6 (VPS39 in mammals), and SPAC823.12 (also known as Vps11 and PEP5), which are involved in vacuole (lysosome in higher eukaryotes) fusion; Wsp1 (also known as LAS17 and WASP) and End4 (also known as SLA2), both of which interact with actin. Among these strains, MAP kinase mutants had a rod-like shape similar to proliferating cells, while Ypt5, Vam6, and SPAC823.12 mutants (vacuole-fusion related) had a pear-like shape. We found that these vacuole fusion-related mutants accumulated small vesicles in their cytoplasm after nitrogen starvation (data not shown).

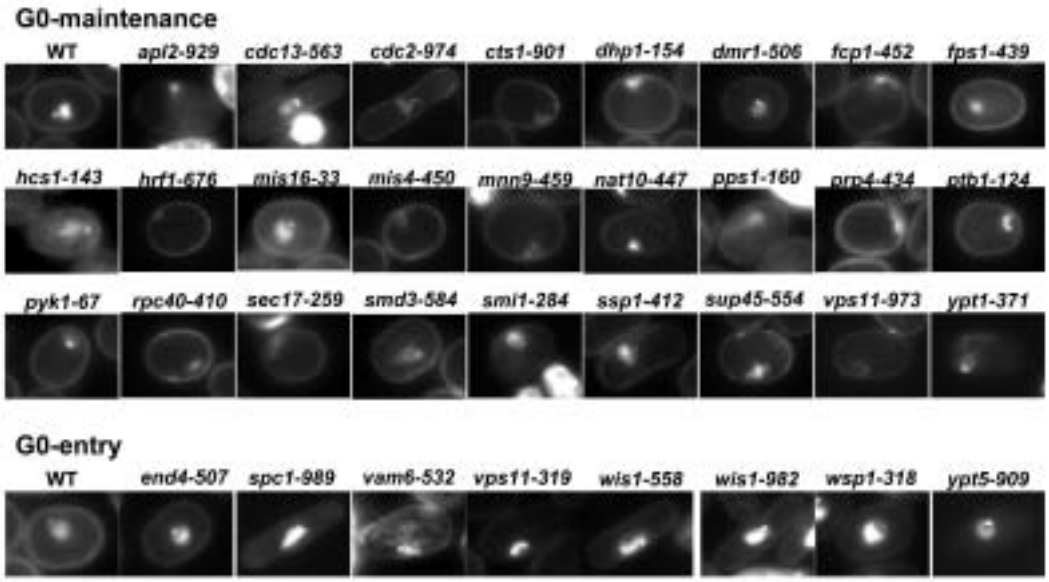


Figure 2: DAPI images of mutants identified as deficient for G0 entry and/or maintenance.

On the other hand, 26 strains were viable after nitrogen starvation, but lost their viabilities in G0 at the restrictive temperature (37°C). Therefore these strains were defective for G0 maintenance and their mutation sites were identified. Among these strains, *cdc2-974, cdc13-563,* and *ssp1-412* had a rod shape, even after nitrogen starvation. Ssp1 and Cdc2-Cdc13 might cooperate with Sty1-Wis1 to change cell shape upon nitrogen starvation.

After shifting the wild-type cells to the minus nitrogen source (–N) medium, growth was immediately arrested and the cells divided twice to produce small and round G0-phase cells. MAP kinase mutants, however, continued to grow and divide like proliferating cells (Figure 3A). Because no nitrogen was available, the protein concentration was decreased in the MAP kinase mutants (Figure 3B) and cells began to die within 12 hours after switching to the –N medium (data not shown).

To understand the cell-cycle regulation in *sty1* and *wis1* mutants after shifting them to the –N medium, we used immunoblotting to examine the levels of some cell-cycle regulators (Fig. 3C). In the wild-type cells, the levels of mitotic cyclin (Cdc13) decreased after 6 hours in the –N medium, and the levels of the CDK inhibitor Rum1 sharply increased at around 6 hours, suggesting that

most CDK is inactivated. By sharp contrast, the Rum1 levels did not increase at all in the *sty1-wis1* mutants, even 24 hours after the shift to –N medium, whereas the Cdc13 levels were relatively high.

Electron microscopy of these mutants under nitrogen starvation revealed an unusually large nucleus (Fig. 3D). These data indicate that the stress-activated MAP kinase pathways are necessary for growth arrest, normal cell cycle regulation, and nuclear structure upon nitrogen starvation.

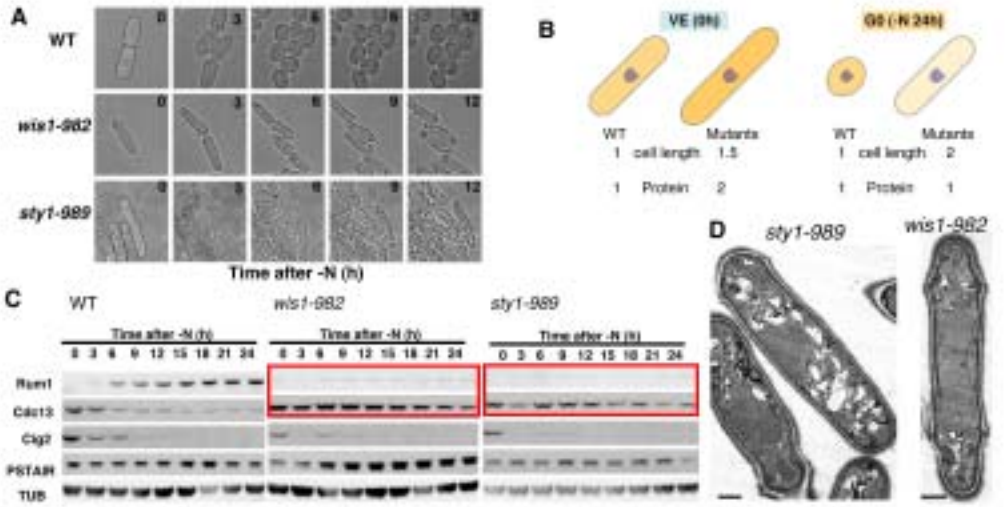


Figure 3: MAP kinase mutants cannot stop growing after -N.
A. After -N, WT divided twice to make small round cells. However, MAP kinase mutants showed growth arrest defect. **B.** Growth arrest defect may cause the reduction of protein concentration. Number showed the ratio of cell length or protein amount against WT. **C.** The protein expressions of cell cycle regulators in MAP kinase mutants were abnormal after -N. **D.** The electron micrograph of MAP kinase mutants 24h after -N showed elongated cell shapes and nuclei.

3.2 Cooperative roles of the proteasome and autophagy in mitochondrial maintenance and life-span in the G0 phase

Although we identified 42 genes required for G0 phase entry/maintenance (Sajiki et al., 2009 and Shimanuki et al., 2006), several important factors remain unidentified. One such candidate is the ubiquitin/proteasome pathway. The ubiquitin/proteasome system is involved in various important pathways in the cell, such as cell division, protein quality control, transcriptional regulation, etc. In fission yeast, the essential role of the proteasome in proliferation is to degrade mitotic regulators, such as Cdc13/cyclin and Cut2/Securin. In the G0 phase, however, the essential roles of the proteasome are not well understood. In this fiscal year, we reported that the ubiquitin/proteasome system and autophagy cooperatively contribute to lifespan during the G0 phase by reducing the lethal accumulation of ROS. This cooperation is G0-specific and is not observed during proliferation (Takeda et al., 2010).

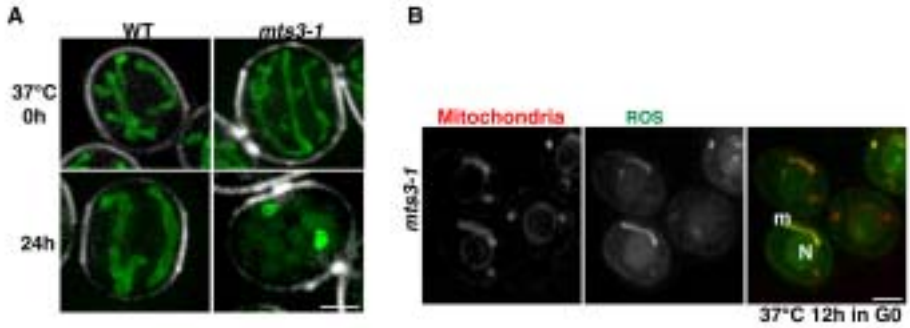


Figure 4:G0-specific phenotypes of the proteasome mutant *mts3-1*

A. Mitochondria were largely diminished in the proteasome mutant in G0 phase. Mitochondria were visualized by GFP-tagging to a mitochondrial protein (Green). **B.** ROS was accumulated in the nucleus and mitochondria in the proteasome mutant in G0 phase. The accumulation of ROS was stained by specific dye, H_2DCFDA (green). Mitochondria was also stained by Mitotracker dye (red). N; the nucleus. m; mitochondria.

Using the proteasome mutant *mts3-1* (the ts mutant of the Rpn12 subunit of the 19S regulatory particle of the proteasome), we found that inactivation of the proteasome in the G0 phase induces cell death along with several specific phenotypes that are not observed during cell proliferation. One phenotype is the massive decrease in the number of mitochondria and another is the huge accumulation of ROS in the mitochondria (Figure 4). Mitochondrial degradation was dependent on autophagy (mitophagy) and contributed to sustaining viability in the G0 phase when the proteasome is inactivated. The proteasome and autophagy double mutant had a severe loss of viability with hyper-accumulation of ROS (Figure 5). Quenching ROS with the anti-oxidant N-acetyl cysteine reduced the severe lethality of the double mutation, indicating that autophagy contributes to cellular survival by preventing the lethal accumulation of ROS induced by mitochondrial degradation (Figure 5).

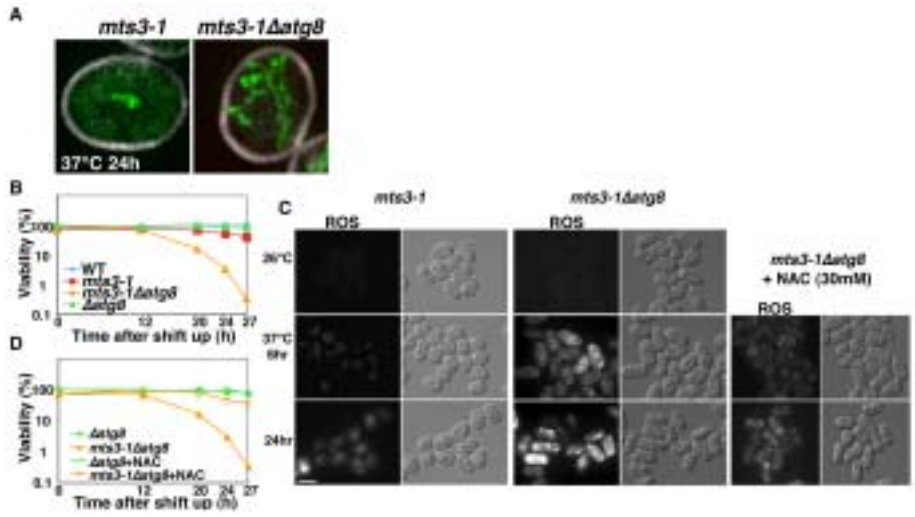


Figure 5: Cooperation of the proteasome and autophagy

A. Mitochondrial degradation was inhibited by autophagy mutant, Δ*atg8.* **B.** The double mutant of the proteasome and autophagy (*mts3-1*Δ*atg8,* orange) showed much severe lethality than single mutants. **C.** ROS was hyper-accumulated in the double mutant, *mts3*Δ*atg8*. N-acetyl cysteine (NAC) was used to quench hyper-accumulation of ROS. **D.** NAC alleviated the severe lethality of the double mutant.

Taken together, our results suggest that the ubiquitin/proteasome system directly or indirectly contributes to maintaining mitochondrial function and autophagy occurs to clear the damaged mitochondria that increase ROS production (Figure 6). This cooperation is G0 phase-specific and is not observed during proliferation.

ROS are mainly generated in mitochondria as an inevitable byproduct of respiration. Therefore, maintenance of mitochondrial homeostasis is crucially related to the health and lifespan of cells and individuals. For example, age-related diseases like neurodegeneration (such as Parkinson's disease) are profoundly associated with ROS and mitochondria. Neurons are in a post-mitotic state (thus in G0 phase). Our current study on the cooperation of the proteasome and autophagy for mitochondrial maintenance and cellular lifespan in the G0 phase might serve as a good model system for understanding the pathologic mechanisms of these human diseases.

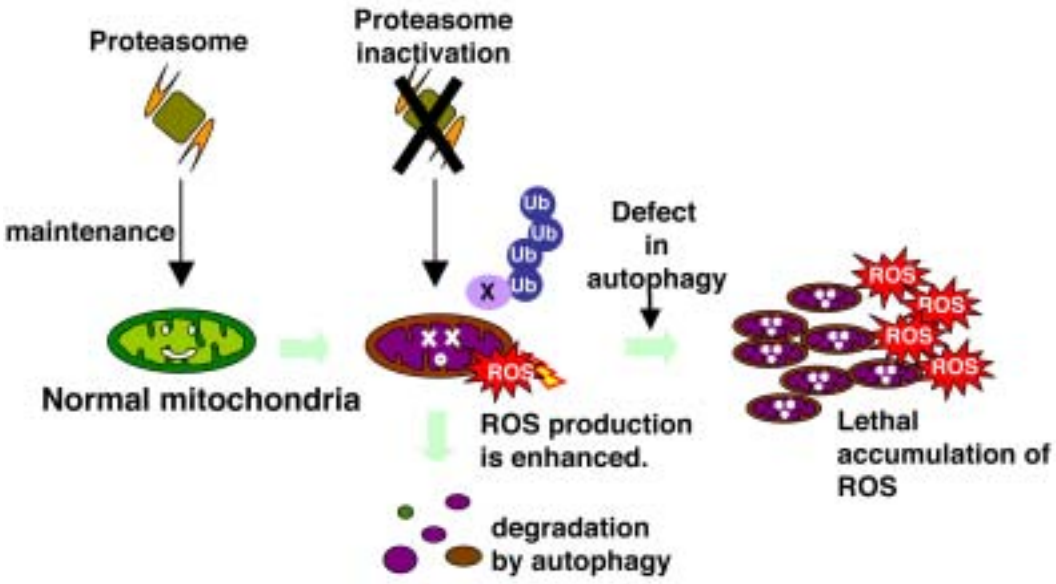


Figure 6: The model of cooperation of the proteasome and autophagy in G0

3.3 Metabolomic profiling of *S. pombe*

In the 2009 fiscal year, we reported our previously developed metabolic profiling method in the first publication demonstrating a global analysis of the *S. pombe* metabolome using liquid chromatography-mass spectrometry (Pluskal et al., 2010). Furthermore, using this method, we confirmed the increase in two anti-oxidative metabolites (glutathione and ergothioneine) in the *mts3* proteasome mutant, which is oxidatively stressed by defective mitochondria at a restrictive temperature (Takeda et al., 2010).

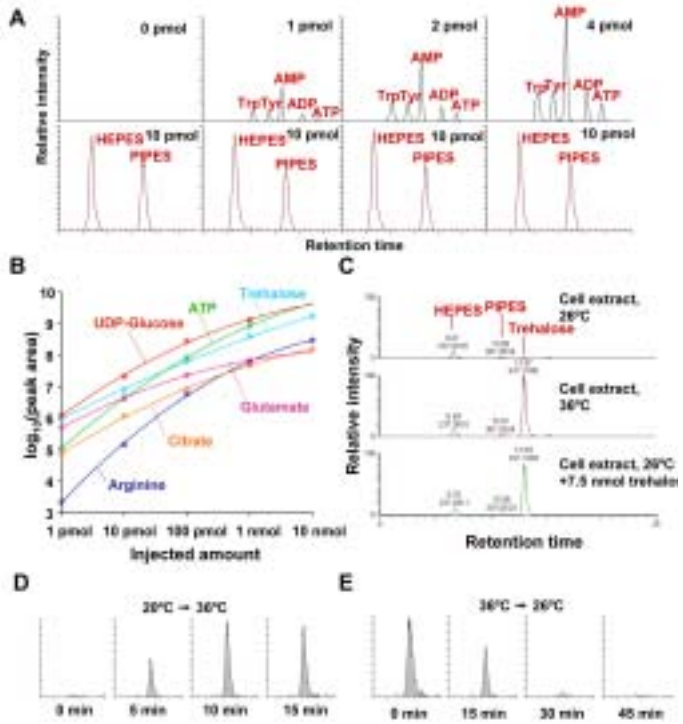


Figure 7: Quantification of compounds by LC-MS using pure standards

A. Four mixture samples containing AMP, ADP, ATP, tyrosine, and tryptophan were injected and their extracted ion chromatograms obtained (upper panel) using a constant amount of standard PIPES and HEPES (lower panel). **B.** The amount of pure compounds and resulting size of peak area in LC-MS were plotted. **C.** Trehalose increased about 200-fold after the temperature shift from 26°C to 36°C. **D.** Trehalose increased rapidly and became saturated within 10 min after a temperature up-shift. E. After decreasing the cultivation temperature, the trehalose level was restored to the original within 30 to 45 min.

First, we investigated the quantification aspects of the obtained liquid chromatography-mass spectrometry data. Samples containing known amounts of pure metabolites were measured (Figure 7A). To further examine the validity of the quantification, we tested pure compounds injected in a wide range of amounts (Figure 7B). There was a good correlation between the peak areas and the injected amounts, although the shape of the calibration curves depended on the individual compounds. Trehalose gave the largest peak in the extracts from cells cultivated at 36ºC. We estimated its amount by spiking an extract from cells cultivated at 26°C with a known amount of trehalose (Figure 7C). Time-course experiments revealed that a high amount of trehalose was produced within 10 min after the temperature shift to 36ºC (Figure 7D), suggesting that the enzymatic activation of trehalose synthesis was rapid. The trehalose concentration was reduced back to the original level within 30 to 45 min after decreasing the temperature to 26°C (Figure 7E).

Metabolic profiling of cells grown at 26°C and 36°C was performed using three independently prepared cell cultures at each temperature. As shown in the scatter plot in Figure 8A, 96% of the metabolites in cells independently cultured at the same temperature (26°C) changed 2-fold or less; therefore, the reproducibility of the extraction and detection of these identified compounds was very good. Only 69% of the peaks changed 2-fold or less when comparing the metabolites obtained from cells cultured at 26ºC and 36ºC (Figure 8B, left and right panels).

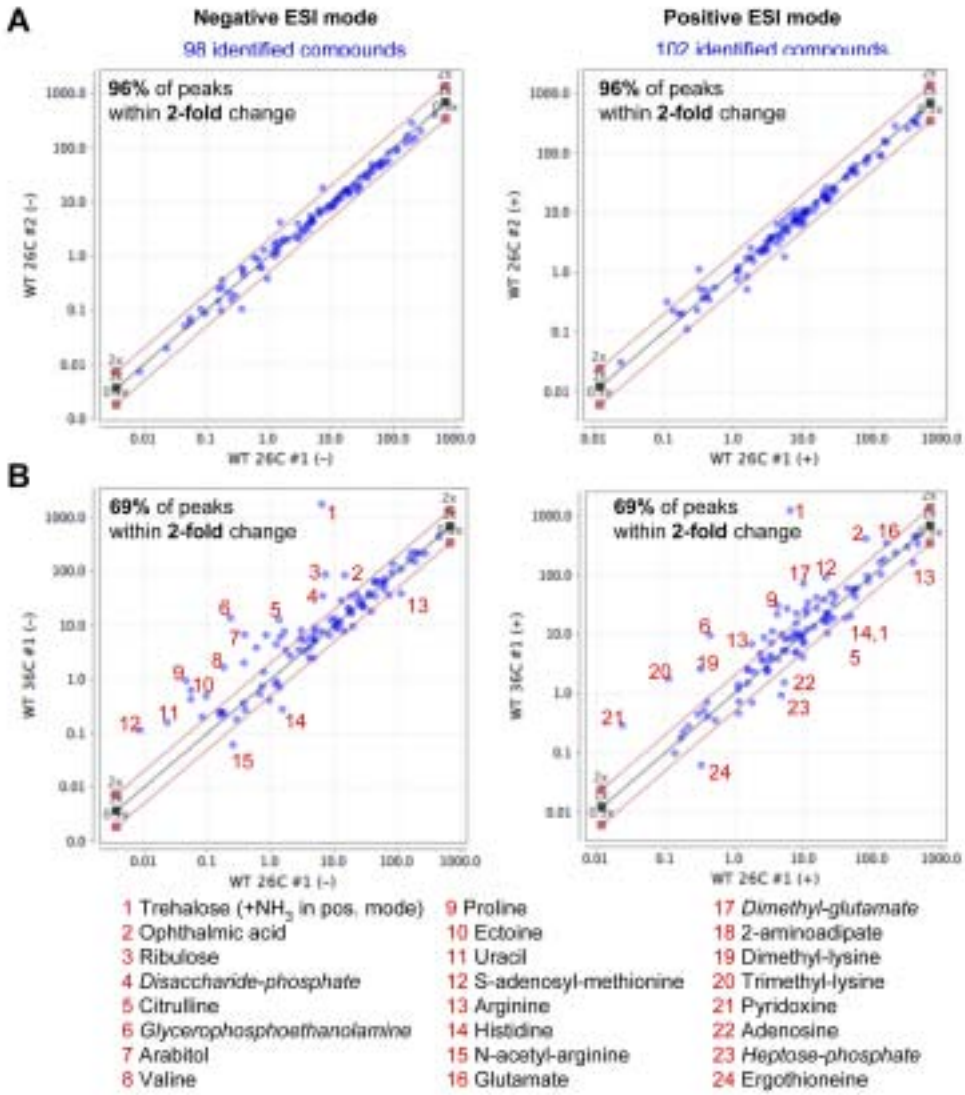


Figure 8: Comparison of 123 identified metabolites in cells grown at 26°C and 36°C, respectively **A.** Two scatter plots showing normalized peak areas of the 123 identified metabolites are compared in two independently obtained extracts from cells grown under the identical culture conditions (EMM2 at 26°C). **B.** Scatter plots of the 123 identified metabolites in extracts from cells grown in the same medium but at two different temperatures (26°C vs 36°C). The most changed peaks are annotated according to the table.

We are currently focused on performing a thorough metabolomic comparison of proliferating and non-proliferating cells in various nutritional environments (Figure 9) to identify the key metabolic biomarkers of different nutritional conditions, as well as the metabolites important for the maintenance of a long-term non-dividing state as part of our research of lifespan and aging.

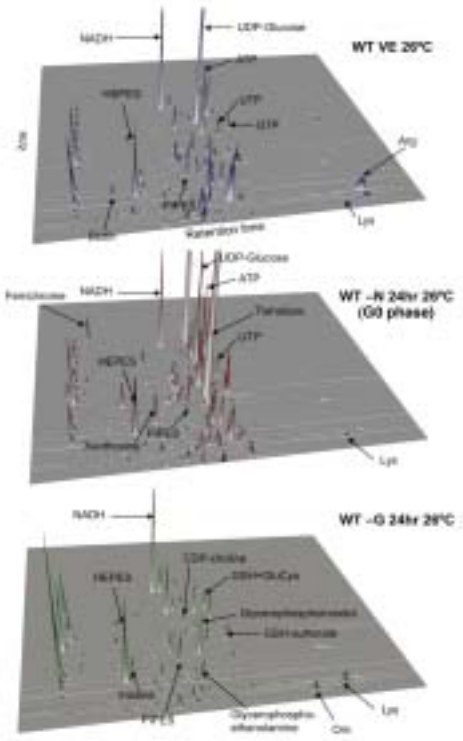


Figure 9: LC-MS profiles of vegetative (VE), nitrogen-starved (-N; G0) and glucose-starved (-G) cells in negative ionization mode. Several important identified metabolites are annotated in each condition. Each sample includes HEPES and PIPES as internal standards.

3.4 Other activities in progress

Screening for mutants with G0 phase defects is not yet complete. For more comprehensive screening, we adopted a fission yeast gene-knock-out library (2800 strains). This large-scale screening was facilitated by automated robotic systems (Biomek FX and Singer ROTOR). As several mutants had defects in both nitrogen- and glucose- limited environments, physiologic adaptations of fission yeast to low-glucose conditions were analyzed. Mutants with growth defects in a low-glucose condition were isolated (in collaboration with Kurume University). We also analyzed genes previously identified to be required for G0 phase maintenance or growth in low-glucose conditions. Among the 1015 strains in our temperature-sensitive library, the responsible genes of 851 strains were difficult to identify by classical methods. Thus, a next generation sequencer was applied to identify the responsible genes. To date, we have read the whole genomes of 232 mutants and identified the responsible genes of 160 strains. These activities will be continued in this year.

4. Publications

4.1 Journals

Hanyu, Y., Imai, K. K., Kawasaki, Y., Nakamura, T., Nakaseko, Y., Nagao, K., Kokubu, A., Ebe, M., Fujisawa, A., Hayashi, T., Obuse, C. & Yanagida, M. Schizosaccharomyces pombe cell division cycle under limited glucose requires Ssp1 kinase, the putative CaMKK, and Sds23, a PP2A-related phosphatase inhibitor. *Genes Cells* 14, 539-554, doi:DOI 10.1111/j.1365-2443.2009.01290.x (2009).

Pluskal, T., Nakamura, T., Villar-Briones, A. & Yanagida, M. Metabolic profiling of the fission yeast S. pombe: quantification of compounds under different temperatures and genetic perturbation. *Mol Biosyst* 6, 182-198, doi:Doi 10.1039/B908784b (2010).

Sajiki, K., Hatanaka, M., Nakamura, T., Takeda, K., Shimanuki, M., Yoshida, T., Hanyu, Y., Hayashi, T., Nakaseko, Y. & Yanagida, M. Genetic control of cellular quiescence in S-pombe. *J Cell Sci* 122, 1418-1429, doi:Doi 10.1242/Jcs.046466 (2009).

Takeda, K., Yoshida, T., Kikuchi, S., Nagao, K., Kokubu, A., Pluskal, T., Villar-Briones, A., Nakamura, T. & Yanagida, M. Synergistic roles of the proteasome and autophagy for mitochondrial maintenance and chronological lifespan in fission yeast. *P Natl Acad Sci* USA 107, 3540-3545, doi:DOI 10.1073/pnas.0911055107 (2010).

Yanagida, M. Clearing the way for mitosis: is cohesin a target? *Nat Rev Mol Cell Bio* 10, 489-496, doi:Doi 10.1038/Nrm2712 (2009).

Yanagida, M. Cellular quiescence: are controlling genes conserved? *Trends Cell Biol* 19, 705-715, doi:DOI 10.1016/j.tcb.2009.09.006 (2009).

4.2 Book(s) and other one-time publications

Yanagida, M., Nishida, E., Noda, R. *Molecular Biology (in Japanese)*. 2 edn, (Tokyokagakudojin, 2009).

Yanagida, M., et al. *Inquiry to ethics and mission of University (in Japanese).* (Kyoto University Press, 2010).

4.3 Oral presentations

Hayashi, T., Hatanaka, M., Kanoh, J., Ikai, N., Yanagida, M. *Tel2-Tti1 complex regulates TOR pathways in fission yeast*, The 32nd annual Meeting of the Molecular Biology Society of Japan, Yokohama, Japan, December 9-12, 2009

Takeda, K., Nagao, K., Kokubu, A., Yoshida, T., Nakamura, T., Kikuchi, S., Pluskal, T., Villar-Briones, A., and Yanagida, M. *The proteasome and autophagy system cooperatively contributes to the maintenance of G0 phase by reducing cellular oxidative stress,* The 5th International Fission Yeast Meeting, Tokyo, Japan, October 26-31, 2009

Yanagida, M. *How life is inherited?* : *Control of chromosome segregation*, National Institute of Biological Sciences, Beijing, China, April 9, 2009

Yanagida, M. *Clearing the Way for Mitosis : Is Cohesin the Target?*, Peking University, Beijing, China, April 10, 2009

Yanagida, M. *How life is inherited ? : Control of Chromosome Segregation*, Institute of Biochemistry and Cell Biology, Shanghai, China, April 13, 2009

Yanagida, M. *Clearing the way for mitosis*, Biozentrum University, Basel, Switzerland, May 13, 2009

Yanagida, M. *Control of Chromosome/Chromatin Dynamics in Fission Yeast (Plenary Lecture)*, Switzerland-Japan Joint Meeting on the Molecular Mechanisms Regulating Chromosome Dynamics and Genome Stability, Villars-sur-ollon, Switzerland, May 14, 2009

Yanagida, M. *Requirement of CTD Phosphatase Fcp1 and type 2A-like phosphatase inhibitor Sds23 for nutritional control of quiescence and proliferation in S. pombe*, EMBO Conference Protein Phosphatases in Development & Disease, Egmond aan Zee, The Netherlands, July 14-18, 2009

Yanagida, M. *Biorientation Road from Innercentromere to Kinetochore Microtubule*, FASEB Summer Research Conferences, Mitosis: Spindle Assembly and Function, Il Ciocco Resort, Lucca, Tuscany, Italy, August 30- September 4, 2009

Yanagida, M. *Nutoritional Control of Chromosome Segregation*, Cell Cycle Regulation and Tumorigenesis Symposium, Aspiration Theatrette, Singapore, September 6-8, 2009

Yanagida, M. *Quiescent Fission Yeast, Diabetic S. pombe*, The 5th International Fission Yeast Meeting, Tokyo, Japan, October 26-31, 2009

Yanagida, M. *Systems for cellular proliferation and quiescence* : *Novel progress of molecular biology research under limited glucose and nitrogen starvation conditions*, University of Tokyo, Tokyo, Japan, December 15, 2009

4.4 Posters

Ohta, K., Hanyu, Y., Fujisawa, A., Ebe, M., Hayashi, T., Shimanuki, M., Sajiki, K., Yanagida, M. *Ssp1 kinase, the putative CaMKK, and Sds23, a PP2A-related phosphatase inhibitor are required to use limited glucose under proliferation and quiescence*, The 5th International Fission Yeast Meeting Pombe 2009, Tokyo, Japan, October 26-31, 2009

Pluskal, T., Nakamura, T., Villar-Briones, A., Yanagida, M. *Metabolomic analysis of the fission yeast Schizosaccharomyces pombe*, The 34th FEBS Congress, Prague, Czech Republic, July 4-9, 2009

Pluskal T., N. T., Villar-Briones A., Yanagida M. *Metabolomic analysis of cell cycle arrest*, The 5th International Fission Yeast Meeting, Tokyo, Japan, October 26-31, 2009

Sajiki, K., Hatanaka, M., Nakamura, T., Takeda, K., Shimanuki, M., Yoshida, T., Hanyu, Y., Hayashi, T., Nakaseko, Y., Yanagida, M. *Determination and analysis of essential genes for G0 entry and maintenance in S.pombe*, 8th Nuclear Dynamics Meeting, Shizuoka, June 18-20, 2009

Sajiki, K., Hatanaka, M., Nakamura, T., Takeda, K., Shimanuki, M., Yoshida, T., Hanyu, Y., Hayashi, T., Nakaseko, Y., Yanagida, M. *Control of proliferation and quiescence by RNA polymerase Ⅱ CTD phosphatase*, Fcp1, 24th Naito Conference, Sapporo, Japan, June 23-26, 2009

Sajiki, K., Hatanaka, M., Nakamura, T., Takeda, K., Shimanuki, M., Yoshida, T., Hanyu, Y., Hayashi, T., Nakaseko, Y., Yanagida, M. *Genetic control of cellular quiescence in S.pombe*, IMCB Cell Cycle Regulation & Tumorigenesis Symposium, Singapore, September 7-8, 2009

Sajiki, K., Hatanaka, M., Nakamura, T., Takeda, K., Shimanuki, M., Yoshida, T., Hanyu, Y., Hayashi, T., Nakaseko, Y., Yanagida, M. *The proteasome and autophagy system cooperatively contributes to the maintenance of G0 phase by reducing cellular oxidative stress*, The 5th International Fission Yeast Meeting, Tokyo, Japan, October 26-31, 2009

Takeda, K. *Mitochondrial regulations and the maintenance of S.pombe cells under limited nutrient condition*, The 3rd Workshop on transcriptional and signaling network of lifestyle related diseases, Kamakura, Japan, February 20, 2010

Takeda, K., Nagao, K., Kokubu, A., Yoshida, T., Nakamura, T., Kikuchi, S., Pliuskal, T., Villar-Briones, A., Yanagida, M. *The proteasome and autophagy system cooperatively contributes to the maintenance of G0 phase by reducing cellular oxidative stress*, The 5th International Fission Yeast Meeting, Tokyo, Japan, October 26-31, 2009

5. Intellectual Property Rights and Other Specific Achievements

Nothing to be reported.

6. Meetings and Events

6.1 The 4th International Workshop on Cell Regulations in Division and Arrest

Date: November 29-December 3, 2009

Venue: OIST seaside House

Speakers: Kiyokazu Agata (Kyoto University)
Charlie Boone (University of Toronto)
Dana Branzei (IFOM-IEO Campus)
Marc Bühler (Friedrich Miescher Institute)
Rafal Ciosk (Friedrich Miescher Institute)
Renaud Dentin (Cochin Institute)
Dale Dorsett (St. Louis University of Medicine)
Li-Lin Du (National Institute of Biological Science)
Robert Fisher (Mount Sinai School of Medicine)
Atsushi Hirao (Kanazawa University)
Shinichiro Imai (Washington University School of Medicine)
Shunsuke Ishii (RIKEN Tsukuba Institute)
Hideki Katagiri (Tohoku University)
Shigeaki Kato (University of Tokyo)

Tatsuhiko Kodama (University of Tokyo)
Issei Komuro (Osaka University)
Hiroshi Kondoh (Kyoto University)
Luis Lopez-Maury (University College London)
Tatsuya Maeda (University of Tokyo)
Masayuki Miura (University of Tokyo)
Noboru Mizushima (Tokyo Medical & Dental University)
Yusaku Nakabeppu (Kyushu University)
Keiichi Nakayama (Kyushu University)
Keiko Nakayama (Tohoku University)
Eisuke Nishida (Kyoto University)
Osamu Nureki (University of Tokyo)
Kunihiro Ohta (University of Tokyo)
Motomi Osato (Cancer Science Institute of Singapore)
Stephen Osmani (Ohio State University)
Markus Ruegg (University of Basel)
Paul Russell (Scripps Research Institute)
Satoshi Sawai (University of Tokyo)
Midori Shimada (Nagoya City University)
Haruhiko Siomi (Keio University School of Medicine)
Katsuhiko Shirahige (Tokyo Institute of Technology)
Stewart Shuman (Sloan-Kettering Institute)
Takashi Takeuchi (Tottori University)
Fuyuhiko Tamanoi (UCLS)
Yoichi Taya (Cancer Science Institute of Singapore)
Kazuya Yamagata (Kumamoto University)
Minoru Yoshida (RIKEN Wako Institute)
Mitsuhiro Yanagida (OIST, Kyoto University)

Other remarks: 22 posters were also presented by the participants

Education and Training Activities

During fiscal year 2009, 11 workshops either hosted or co-sponsored by OIST took place, bringing as many as 600 lecturers and participants together for vigorous scientific discussions.

Fundamental of Quantum Mechanics and its Applications

Outline: The purpose of this workshop was to bring together leading scientists and engineers from all over the world to explore future possibilities in fundamental issues of quantum mechanics unveiled by new advanced technologies. Thanks to the recent progress in such technologies, new possibilities of directly investigating fundamental issues in quantum mechanics have been opened up. The workshop focused on the interference phenomenon in quantum physics. Major topics included the Bose-Einstein condensate, the Aharonov-Bohm effect, Josephson devices, magnetic interference, and superconducting quantum interference. Other topics pursued were high-performance electron microscopy and scanning probe microscopy, which images and/or uses interference, as well as new phenomena investigated using further new technologies, such as aberration-free electron lenses and pulsed electron guns. The purpose of this workshop was to encourage young students and researchers by giving them a chance to speak with world-leading scientists, including Nobel Prize winners.

Date: May 13 – 15, 2009

Organizer: Dr. Akira Tonomura, OIST

Venue: OIST Seaside House

Participants: Lecturers 19, Participants 22

Okinawa Computational Neuroscience Course (OCNC) 2009

Outline: The aim of the Okinawa Computational Neuroscience Course was to provide opportunities for young researchers with theoretical backgrounds to learn the latest advances in neuroscience, and for those with experimental backgrounds to have hands-on experience in computational modeling.

Date: June15 – July 2, 2009

Organizers: Dr. Erik De Schutter, OIST
Dr. Kenji Doya, OIST
Dr. Klaus Stiefel, OIST
Dr. Jeff Wickens, OIST

Venue: OIST Seaside House

Participants: Lecturers 18, Tutors 8, Participants 29

Reinforcement and Attention Deficit Hyperactivity (ADHD) Disorder

Outline: The ADHD Workshop provided a forum for discussion between researchers studying the neural basis of reinforcement and those using behavioural and neuro-imaging methods to investigate reinforcement sensitivity in individuals diagnosed with, or displaying symptoms of, attention deficit hyperactivity disorder (ADHD). The meeting took the form of oral presentations and round table discussions. The first day of the three day meeting was devoted to the neurobiology of dopamine and reinforcement, day two focused on the behavioural assessment of reinforcement sensitivity in ADHD and the actions of Methylphenidate while the third day considered the role of Imaging techniques and computational modeling in the study of reinforcement and ADHD.

Date: September 8 – 10, 2009

Organizer: Dr. Gail Tripp, OIST

Venue: OIST Seaside House

Participants: Lecturers 20, Participants 13

DNA Topology Course

Outline: DNA topology is one of the areas in which cutting-edge pure mathematics and biology meet very directly, in the study of topoisomers. The point of the course was to give young and early-career biologists a chance to learn about this exciting and active research field from international experts. This was achieved through a combination of introductory lectures with coursework, and also a short series of conference-level research talks, to give the participants an understanding of both the basic theory and current research directions. DNA topology is a challenging area, requiring expertise in both mathematics and biology. Furthermore, topology is not typically taught to undergraduate biologists at all. It was therefore an outstanding topic for an international Ph.D.-level course at OIST.

Date: November 2 – 7, 2009

Organizer: Dr. Robert Sinclair, OIST

Venue: OIST Seaside House

Participants: Lecturers 14, Participants 23

The Retina: Neural Stem Cells and Photoreceptor Depeneraion

Outline: This small meeting, held at the ocean front seminar facilities of the OIST, included sessions on retinal development and genetic diseases. The aim of the meeting was to discuss the mechanisms underlying retinal neurogensis, retinal stem cell establishment and maintenance, photoreceptor differentiation and degeneration, and retinal repair.

Date: November 9 – 12, 2009

Organizer: Dr. Ichiro Masai, OIST

Venue: OIST Seaside House

Participants: Lecturers 15, Participants 42

Cell Regulations in Division and Arrest

Outline: This was the fourth year of cell division and arrest workshops organized by the Yanagida Unit. In 2009, the featured topic was human diseases with respect to the regulation of cell division and arrest.
Date: November 29 – December 2, 2009
Organizer: Dr. Mitsuhiro Yanagida, OIST
Venue: OIST Seaside House
Participants: Lecturers 41, Participants 31

Winter Course 2009 “Evolution of Complex System”

Outline:	The aim of the OIST Winter Course "Evolution of Complex Systems" (OWECS) was to provide opportunities for young researchers with biological backgrounds to meet each other and learn the latest advances in the field of evolutionary developmental biology.
Date:	December 7 – 12, 2009
Organizers:	Dr. Sydney Brenner, OIST Dr. Noriyuki Satoh, OIST
Venue:	OIST Seaside House
Participants:	Lecturers 10, Participants 36

Dr. Sydney Brenner, OIST President and 2002 Nobel Laureate in Physiology or Medicine

Garuda One Workshop

Outline: This Workshop was intended to bring together a group of researchers able to join as founding members of the project that aims to develop open and unified platform for systems biology driven healthcare research and services.
Date: February 23 – February 25, 2010
Organizer: Dr. Hiroaki Kitano, OIST
Venue: Seaside House
Participants: Lecturers 13, Participants 8

Co-sponsored Workshops

“Neural Computing Workshop” on May 25-27, 2009 at OIST Seaside House
“High Performance Computing and Bioscience” Workshop on June 5, 2009 at OIST Seaside House
"A Course on Physiome and Systems Biology" Workshop on December 14 – 28, 2009 at OIST Seaside House
“The 5th Geometry Conference for Friendship of Japan and China” on January 29- February 2, 2010 at OIST Seaside House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