The 10th Anniversary of Drosophila Genetic Resource Center

INTERNATIONAL BIORESOURCE SYMPOSIUM "Drosophila"

ショウジョウバエ遺伝資源を用いた
最先端生命科学研究と‘いのち’を考える

PROGRAM & ABSTRACTS

March 17 & 18, 2010

Hieizan Enryakuji
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Curator

Drosophila Genetic Resource Center
Kyoto Institute of Technology
http://www.DGRC.kit.ac.jp/

京都工芸繊維大学
KYOTO INSTITUTE OF TECHNOLOGY

NBRP

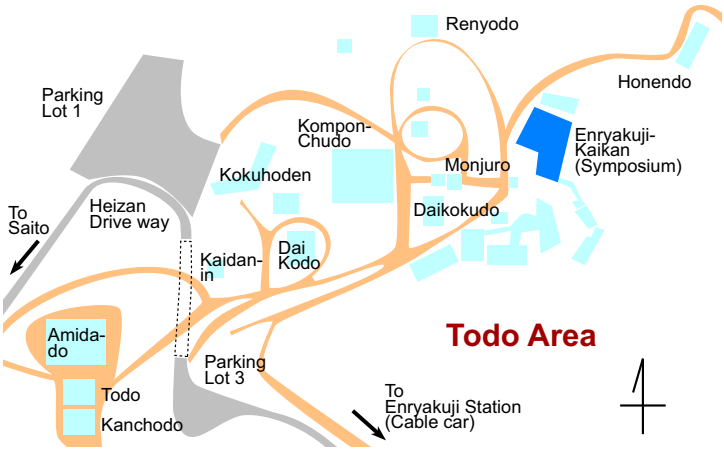
Greetings

The Drosophila Genetic Resource Center (DGRC) at the Kyoto Institute of Technology was founded in 1999 as a governmental research facility and the building was completed in 2000. DGRC began with my collection of about 2,400 stocks which was the largest collection in Japan at the time. Now the stock number 36,000 of DGRC and sub-institutes is the largest among stock centers in the world. This splendid achievement depends on the support of the National BioResource Project from the Ministry of Eduction, Culture, Sports, Science and Technology, and all members of DGRC who are involved in various tasks in collection, maintenance, and provision of the stocks. I sincerely thank to all members of DGRC and we should be proud of ourselves to this achievement.

It is a great pleasure that we all get together on this occasion and celebrate the 10th anniversary of the Drosophila Genetic Resource Center. For further support and service to the Drosophila research community all over the world, we share this opportunity to discuss Drosophila biology and to consider our common interests in life at the most spiritual place in Kyoto, Japan.

Masa-Toshi Yamamoto, Ph.D
Director & Professor
Drosophila Genetic Resource Center
Kyoto Institute of Technology

Area map



KOMPON CHU-DO

The three areas of Enryakuji "Todo, Saito and Yokawa" each have a central hall. The Halls are called "Chu-do", and Kompon Chu-do in Todo is the largest hall. After it was built by Dengyo Daishi in the seventh year of Enryaku (788), it met with many disasters, but each time it was rebuilt, the scale became larger and larger. The present hall was completed by Tokugawa Iemitsu in the 19th year of Kan-ei (1642).

The Inner Chamber and THE ETERNAL LIGHT
Since Kompon Chu-do was founded, the light has been kept burning for 1200 years.

From Website of Enryakuji http://www.hieizan.or.jp/

About Enryakuji & Mt. Hiei

A holy place of silent beauty and strict discipline, Enryakuji may at first glance seem uninviting. But Hieizan (Mt. Hiei) is grounded in the beliefs of people who are passionate, possess a warmness of spirit, and have a zest for life. The approach and essence of Hieizan Enryakuji (Mt. Hiei's Enryakuji) can be found in our strong conviction to face those pursuing life in a very straightforward way.

Mt. Hiei is the beautiful mountain that straddles Kyoto and Shiga Prefectures. It is the mother of Japanese Buddhism, in which breathes 1200 years of history.

From Website of Enryakuji
http://www.hieizan.or.jp/

Internet access

Wireless local area network (wireless LAN) system is installed in the symposium area. No LAN system is installed in hotel rooms.

Time Table

Wednesday, March 17

13:00 Registration

Opening Remarks

15:00 Masa-Toshi Yamamoto

Speeches Chair: Tetsuya Tabata

Yoshimichi Ejima, *President of Kyoto Institute of Technology*
Ministry of Education, Culture, Sports, Science and Technology of Japan

Keynote Speeches Chair: Masa-Toshi Yamamoto

15:30 Ven. Ryusho Kobayashi, *Senior Priest of Enryakuji-temple*
16:30 Yuji Kohara, *National Institute of Genetics, Japan*

18:00 Reception

Posters

20:00 Session begins

Thursday, March 18

Lectures

Session 1 Chair: Ryu Ueda

8:30 Norbert Perrimon, *Harvard Medical School, Howard Hughes Medical Institute, USA*
9:15 Kenji Matsuno, *Tokyo University of Science, Japan*
10:00 Utpal Banerjee, *University of California, Los Angels, USA*

Break

Session 2 Chair: Tadashi Uemura

11:00 Tetsuya Tabata, *University of Tokyo, Japan*
11:45 Leslie Griffith, *Brandeis University, USA*

Lunch

Session 3 Chair: Masa-Toshi Yamamoto

13:45 Kenneth B. Storey, *Carleton University, Canada*
14:30 Takashi Okuda, *National Institute of Agrobiological Sciences, Japan*
15:15 Shigeo Hayashi, *RIKEN Center for Developmental Biology, Kobe, Japan*

Break

Session 4 Chair: Tetsuya Tabata

16:15 Timothy Karr, *Arizona State University, USA*
17:00 Masa-Toshi Yamamoto, *Kyoto Institute of Technology, Japan*

Closing Remarks

17:45

Keynote Speech 1

The Buddhist View of Life
Ven. Ryusho Kobayashi, Senior Priest of Enryakuji-temple

The Buddhist View of the Universe

In Buddhism, the infinitely expanding universe, including the world we human beings live in, is called *trisāhasra-mahāsāhasra-loka-dhātu* (J., *sanzen daisen sekai*; literally, "three-thousandfold world system"). This explains the Buddhist view of the universe. The number of three thousand can also be said limitless, and the basis of three thousandfold is explained as following; Śākyamuni Buddha, born about two thousand and five hundred years ago in kapila, North India and left home at the age of twenty nine, came to an enlightenment at thirty five, then extended his teachings to many people all over in India for forty five years till he was eighty. The center location of his teaching activities was called Mt. Sumeru (Vulture Peak), which is known as the place where the sutras familiar to Japanese Buddhism were taught, like the Lotus sutra and Amida Sutra. Buddha considered this Mt. Sumeru as the center of the Universe, surrounded by four great continents, eight great seas, where humans and all sentient beings exist. Meaning this earth is thought as one small world. One thousand of these small worlds form a medium chiliocosm, and one thousand of these form a great chiliocosm. Further, three thousand great chiliocosm form the three thousandfold great world system, which is considered as the universe. In this three-thousandfold world system, all creatures exist, change, and are reborn in flux. This is the Buddhist view of the universe.

Time and Space

In Buddhism, a unit of time is called "Gô" and one Gô is calculated as five thousand six hundred seventy million years in a sutra by Indian calculation, and it is said that it takes ten Gô-training as Amida Buddha to become a Buddha in Buddhism. Setting half-eternal time as a premise, and the figure that infinitely keeps changing in flux in this half-eternal time was thought as "emptiness." The condition of limitless time and space functioning and moving lively was thought of as the universe.

About Engi (Dependent Origination)

When it is asked if someone or some rule dominates the action of limitless time and space, Buddhism gives an answer of Engi (dependent origination). This is a primary Buddhism teaching that places En (ties) right in the middle between causes and results, meaning all the happenings (results) can be traced to causes with En. Moreover, the results become the causes of the next results with the next En. Every thought, mind and act of people, all phenomena in nature, or even revolution of the universe change and function under the influence of En. Nothing in the universe exists apart from or without En.

Ichinen-Sanzen (One thought in the universe, the universe in one thought)

Chih-i (538-597), Tendai-Daishi in Sui period of China taught this idea; there is the whole universe in each of our thoughts, and each of our thoughts exists in the universe. This is Chih-i's view of the universe and he pointed the very basis of it as En. Neither the universe nor we exist without each other. This idea of "emptiness (J., Kû)" is taught in the Heart Sutra.

The Idea of the Three Worlds of the Past, Present, and Future (J., Sanse-kan)

In Buddhism, it is thought that the whole universe is operated by the idea of the three worlds. The three worlds of the past, present, and future are always together and never be apart. For example, "today" exists in the relation to "yesterday (past)" and "tomorrow (future)", and the universe and every single sentient beings and inorganic substances there function, in the relation to the three worlds under the influence of En. It is called "Engi teaching" in Buddhism, which explains that the present life exists with the former life and the after life.

基調講演 一

小林隆彰　延暦寺長臈

仏教の宇宙観

我々人間が住む世界を含めて無限に広がる大宇宙を三千大千世界と称している。この三千大千世界が仏教の宇宙観である。

三千という数字は無限とも言い替えることが出来るが、その数の根拠は古来次のように言われている。

釈迦仏が二千五百年余り昔、北インドのカピラで出生し、二十九歳出家、三十五歳で宇宙の実相を明らかに感得してより八十歳まで四十五年間、インド各地を巡錫して多くの人びとに教えを説かれた。

その中心地が法華経や阿弥陀経など日本仏教に縁の深い教えを説かれた霊鷲山（りょうじゅせん）である。

この霊鷲山を見立てて宇宙の中心、須弥山（しゅみせん）とし、その須弥山を中心に四大州があり、また、八大海などがあって、そこに人間をはじめ一切の動植物が生存する。即ち、この地球を一つの小世界と考えた。

我々の住む地球の如き小世界が千個集まったものを中千世界と称し、中千世界が千個集まって大千世界と呼び、大千世界が三千集まったものを三千大千世界と言い、この大宇宙と考えた。

その三千大千世界に一切の動植物などが生存し、生死流転している。即ち、すべてが変化し、生々流転、破壊再生しつつ存在している。とするのが仏教における宇宙観である。

時間と空間

仏教の時間単位を劫（こう）という、この一劫を仏典では、五十六億七千万年とインド人が計算し、これを十回重ねたものを十劫とし、阿弥陀仏が修行して仏になるまでの時間を十劫正覚としている。即ち従には半永遠の時間を考え、無限に転々変化する姿を「空」と考えた。

無限の時間と無限の空間が活動し躍動しているものを宇宙としたのである。

縁起について

無量の時間と空間の躍動は誰かの支配とか何かの法則によっているのであろうか、と問うとき、仏教は、縁起（えんぎ）を以って答えている。

縁起とは原因と結果のまん中に縁を置く仏教本来の教えであって、すべての事象は、原因に縁が加わって結果が生まれる。結果も亦次の縁によって原因となり結果となる。すべて、人びとの思考も、行為も、自然、天然の現象もすべて。この縁によって変化し実動する。それは、人の心も、物理的な現象も宇宙そのものの運行も縁によらないものは無い、天地万物、その一つとして縁を離れて存在しない。

一念三千

中国、隋代の天台大師智顗禅師（五三八～五九七）は、一念三千をもつて説いた。吾人の一念の中に宇宙全体を備え持っている。宇宙全体の中で吾人の一念は存在し、一念の中に全宇宙を含んでいる。とする大宇宙観で、その根源が縁にあると説いた。

吾人を離れて宇宙なし、宇宙を離れて吾人なし、これは、般若心経で説く。空の思想でもある。

三世観

この宇宙のすべては三世によって動いている、と考えた。三世とは過去、現在、未来のことであって、この「三世」は何時如何なる時も別離することは出来ない。

例えば、今日という日は昨日（過去）を含み、明日（未来）を持っている。宇宙も、その中に住む動植物も無機物もそのすべてが三世とかかわり、縁あって動いている。これを仏教の「縁起説」という。現世は、前世と来世を共に持っているとするのである。

About the Origin of Life (the Buddhist View of Life)

In Buddhism, the origin of life is considered to be everlasting (that is, neither beginning nor end is known), hence, its beginning is not explained. It's also said that neither no God nor Buddha created it either. Life comes out of darkness. It passes by quickly and has no constant form. Human life is as short as the time of pored dry sand staying on your fingernail. This is the basis of the Buddhist teaching. Therefore, it teaches the significance and purpose of our lives, cautioning us not to waste our lives, for being born as a human is the most difficult and precious thing. Śākyamuni Buddha came to the human realm from the pure land of Buddha to teach Buddhism to us, for humans are the most precious things to lead, according to his observation. Humans are fundamentally good but also depraved at the same time. Stopping depravation and working diligently for goodness make human souls purified through the realms of heaven, bodhisattva, and Buddha, and then humans become Buddhas with heart of compassion in the after life. In the next step, humans are reborn for compassionate conduct in the next life. This is why it is said that humans have unlimited ability.

Buddha

Buddha is the master who is enlightened about the principle of the true nature of the universe, and teaches the meanings and means of life. He was aroused to compassion in his infinite past and was reborn many times with the special power to support people in pain. He also has an ever-changing function and unlimited life, which make him a ray of hope in the human realm.

The Buddhist View of Human Beings

Though humans are animals, it is thought that they are the most evolved of all animals. In Buddhism, although the idea of transmigration (endless cycles of death and rebirth) is not a fixed concept, the human realm is thought to exist over the realms of hell (a world in which there is continuous suffering day and night), hungry ghosts (a world of hunger), animals (a world of unquenchable appetite and sexual desire), and asuras (a world of constant strife and bloodshed). To be born into this human realm is fortunate, for humans can enjoy both good and bad. Buddhism explains that being born into the human realm is more difficult than threading a needle with a thin string hung from a high mountain so that human life is precious.

However, human beings are capable of taking other lives for their own purposes, destroying nature, and killing each other in wars. At the same time, they are also capable of sacrificing themselves to save others, protect nature, and help plants and animals live. Consequently, if educated, human beings can strive to help and lead others. For this reason, it is taught that Śākyamuni Buddha went out into the human realm to teach people to devote themselves to helping people and all living beings, society, and humans. Buddhism explains that the present exists because the past, present, and future overlap. Thus, the idea of the three worlds of the past, present, and future, as well as the idea of karmic retribution-the principle of good action leading to good results and bad deeds reaping evil-was established in Buddhism. It teaches that those who selfishly ignore others will suffer the repercussions in future lives.

People today are egocentric, and we are becoming more self-centered as a society. This is called the period of mappô (the latter days of the Buddhist teachings). People today live in this age of decadence. Hence, good things are few and evils are many. However, Buddha taught that since humans stand above all other living beings, they must work at changing that egotism toward altruism.

生命の原初について（生命観）

仏教では生命の原初については説かれていない、無始無終としている。始めなく、終りを知らず、神や仏が作りあげたという説をとらない。

無明である。しかも、無常迅速である。

例えば人間の一生を、乾いた砂を爪の上に乗せてすべり落ちるその極めて短い時間であると説き、無常迅速、と言い表した。この無常迅速が教えの根本であり、得難い人間界に生を受けた者は、無為に過ごしてはならぬ、と人生の意義と目的を説いたのが仏教である。

釈迦牟尼仏は、人間こそ導くに足る最も大切な生き物であると観察して仏界から人間界に下生して仏教を説いた、と言われる。

即ち、人間は、基本的には性善であるが性悪も兼ね備えている。悪を止め、善に勤しむ。

それによって人間は、天上界、菩薩界、仏界へと魂が浄化し、来世、仏となって慈悲の心となり、再び転生して慈悲の行為をする。

故に人間は無限の可能性がある。

仏とは

仏とは天地本然の理を悟り、人間に対して生きる意味、生き方を教える教え主を言う。

無限の過去世に慈悲の心を起こし、何度も生まれ変わって苦しむ人びとを救済する仏力を持ち、自由自在な働きと無限の生命力で人間界の光明となっている。

仏教の人間観

人間も亦動物であるが、すべての動物の中で最も成長発展した生きものであると教えている。

輪廻転生（生まれかわり死にかわる）の思想は固定した観念ではないが、地獄界（日夜苦しみの連続）餓鬼界（空腹）畜生界（食欲と性欲のみの世界）修羅界（争い争って終日流血する世界）の上に人間界があると説く。

この人間界は善悪共に並べ行うことが出来るから幸せである。人間界に生まれるのは、高い山から細い糸をたらして、その糸を針の穴に通ずるよりむずかしい、と説いて、人間が貴重な存在であることを説諭されている。

しかし人間は、自己の為に他の生命を奪い自然を破壊し戦争をして殺し合いもするが、自己を犠牲にしてでも、他を助け、自然を守り、人間以外の動植物を生かせることもできる。故に人間を教育すれば自己以外の他の為に救い導くことが出来る。

この故に釈迦は人間界に出て説諭し、一切の生きもの、社会、人類の為に心身を捧げるように説諭された。

仏教では、過去、現在、未来が重なり合って現在が存在すると説く。故に過去世、現在世、来世の三世観からその教えは成り立っている。

故に因果応報の思想があり、善因善果、悪因悪果が原則である。自己中心に他を無視すれば必ずその報いとしての苦しみを受けねばならぬ、と説く。

現代の人間は自己欲が強く、自己中心的な世相になっている。これを末法時代と言い、現代人は末法の中に生存している、故に善が少なく悪が多い。しかし、人間は、万物の霊長であるから、自己愛を利他愛へと努力して生活せねばならぬ、と説かれた。

Keynote Speech 2

Yuji Kohara
National Institute of Genetics, Japan

Lectures

A *Drosophila* RNAi resource to study oogenesis and the maternal contribution

Norbert Perrimon

Department of Genetics, Harvard Medical School, Howard Hughes Medical Institute, USA

Advances in RNAi-based methodologies and tools in recent years have provided unprecedented opportunities to interrogate systematically the function of all genes relevant to a particular process. In *Drosophila*, arguably the best-understood multicellular organism and a proven model system for human diseases, conditional and tissue-specific expression of hairpin constructs using the Gal4/UAS system has emerged as the method of choice to interrogate the function of all genes in the soma. In this regard, genome-wide libraries of hairpin-based vectors that generate long double-stranded RNAs (dsRNAs) are now available, and have already been used to screen for new and novel components of signaling pathways, resistance or susceptibility to bacteria, and fat deposition. However, expression of long dsRNAs in the female germline are not effective at triggering gene knock down and thus cannot be used to study oogenesis or early embryonic development. Recently, we have been able to solve this problem and achieved excellent knock down of gene activity in the female germline by building an optimized vector that delivers siRNAs using the endogeneous microRNA pathway, referred to as small hairpin microRNAs (shmiRs). Using this method, it is now possible to use RNAi to investigate a myriad of fundamental biological questions in cell biology, signal transduction and developmental biology using the oogenesis and early embryogenesis *Drosophila* paradigms that have been used by hundreds of laboratories over the past 30 years. I will describe our progress at building in collaboration with Greg Hannon's laboratory at Cold Spring Harbor a genome scale collection of shmiRs.

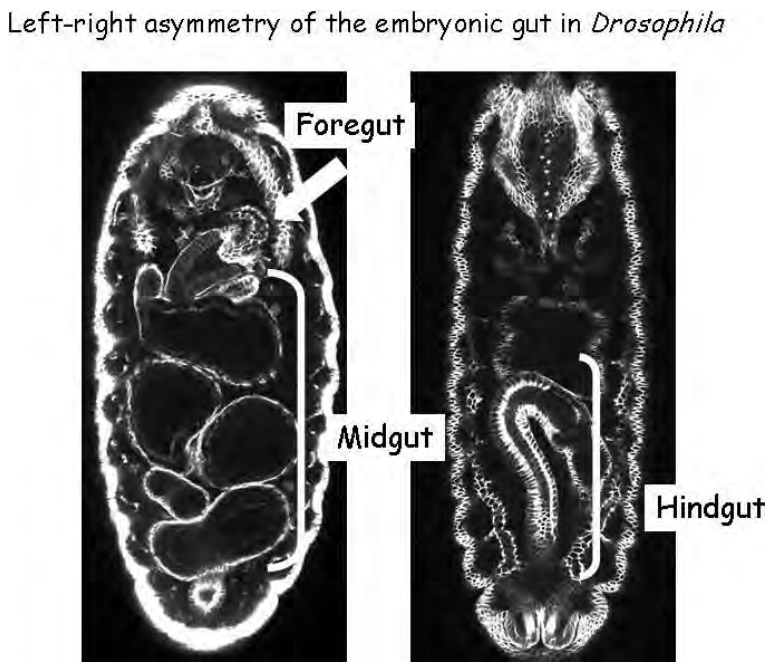
Planar Cell Chirality Contributes to the Left-right Asymmetric Morphogenesis of Epithelium in Drosophila

Kenji Matsuno

Department of Biological Science and Technology, Tokyo University of Science, Japan

The left-right (LR) asymmetric morphology of animal organs is formed according to the LR polarity information. Cellular basis of how LR asymmetric morphology is generated are still elusive, although the mechanisms of LR polarity formation began to be understood. To understand the genetic pathways controlling LR asymmetric development, we have been studying the LR asymmetric development in *Drosophila*. *Drosophila* shows LR asymmetry in various organs, such as the gut, brain, testes, and mail genitalia. Among them, the embryonic gut is the first organ that shows directional LR asymmetry during *Drosophila* development.

To identify genes involved in LR asymmetric development, we have conducted saturation screens for mutants on the second and third chromosome. We have identified about ten genes, which are required for the LR asymmetric development of the anterior part of the embryonic gut. In addition, seven genes affecting the LR asymmetry of the posterior part of the embryonic gut has been identified. We revealed that mutation in *Myosin31DF*, which encodes *Drosophila* Myosin ID (MyoID), results in the reversion of laterality in various organs. In addition, its functions depend on the interaction with actin filament. More recently, we found that in the mutants of *Drosophila epithelial-Cadherin* (*DE-Cad*) gene, the laterality of the embryonic gut was randomized. The functions of these two genes are required in the hindgut epithelium at the same stage.



During the LR asymmetric morphogenesis in the hindgut, cell proliferation does not occur and apoptosis is not detected. Therefore, rearrangement or shape change of epithelial cells is likely responsible for the LR asymmetric morphogenesis of the hindgut. Our detailed analysis of cell-shape change revealed that the hindgut epithelial cells show LR bias in their cell-shape before the LR asymmetric morphogenesis. This LR bias of cell-shape is referred to as "planar cell chirality" (PCC) in this study. Using *in silico* simulation model, we demonstrated that PCC was sufficient to induce the LR asymmetric morphogenesis of the hindgut epithelial tube. We found that PCC of the hindgut epithelium is reversed in *MyoID* mutants, in which laterality of the hindgut is also reversed. Thus, a "default" PCC, which is observed in *MyoID* mutants, is opposite to that of wild-type and reversed by the *MyoID* functions. In addition, PCC was not formed in the hindgut epithelium of *DE-Cad* mutants. Based on these results, we propose a novel mode for developing LR asymmetric organs that involves the PCC of epithelial cell morphology.

Drosophila as a genetic model for hematopoiesis and stress response

Cory Evans, Julian Martinez-Agosto, Lolitika Mandal, Tina Mukherjee, Edward Owusu-Ansah, Sergey Sinenko, Jiwon Shim and Utpal Banerjee

University of California, Los Angels, USA

Several important concepts underlying *Drosophila* blood development have allowed us to propose this system as an appropriate genetic model for vertebrate hematopoiesis. Three zones have been identified in the primary lymph gland lobe, the principal site of definitive hematopoiesis, one containing the differentiating cells (Cortical Zone, CZ), one containing the hematopoietic stem cells (Medullary Zone, MZ) and the third comprising the signaling niche (Posterior Signaling Center, PSC). The stemness of the MZ precursors is maintained through the combined action of a Niche Signal, generated by Hedgehog (Hh) and a proposed reverse signal from the differentiated cells of the CZ to the MZ that we have termed the Equilibrium Signal. This process is initiated by a receptor tyrosine kinase signal involving the *Drosophila* PDGF/VEGF-related receptor (Pvr) and its ligand Pvf1. This, in turn, controls Adenosine deaminase growth factor (Adgf), a central component of the Equilibrium Signal. The homeostatic balance in stem cell number is maintained by controlling the extracellular levels of the small molecule adenosine, a mechanism akin to "quorum sensing" in prokaryotes. Unpublished data on how this balance is achieved will be presented.

The *Drosophila* hematopoietic repertoire, comprising myeloid-like cells called hemocytes, is ideal for the study of various stress response systems. Hypoxia-related factors and ROS play a role both in hematopoietic development and in stress response. The emerging view from these studies is that basic developmental mechanisms are co-opted again for stress, injury and inflammatory responses by the myeloid hematopoietic system.

Cellular and molecular mechanisms underlying neural formation in Drosophila visual system development

Tetsuya Tabata, Institute of Molecular and Cellular Biosciences, University of Tokyo, Japan

In Drosophila, each of the approximately 750 ommatidial units comprising the compound eye contain six outer photoreceptors (R1-6) and two inner photoreceptors (R7-8). R1-6 cells send their axons to the first optic ganglion, the lamina, whereas R7-8 cells send axons through the lamina to the second ganglion, the medulla.

R1-6 cells in each ommatidium make stereotypic connections with particular lamina neurons and forms the ‘ lamina column ’ . We have found that *hibris* (*hbs*) is expressed in the lamina neuron precursors (LNPs) and required for the lamina column formation. Hbs belongs to the immunoglobulin superfamily and has a similarity to nephrin. LNPs fail to associate R axons in the *hbs* mutants. We next found a NEPH1 homolog, Roughest (Rst). In the *rst* mutant, R axons are not associated with LNPs as in *hbs* mutants. The *rst* mutation led to changes in Hbs localization, suggesting the interaction between Hbs and Rst (Figure 1).

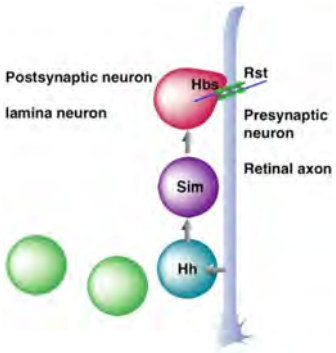


Figure 1. Interaction between LNPs and R axons. Lamina precursor cells receive Hh from R axons, which trigger Sim expression and differentiation to LNPs (green). Sim then induces Hbs expression and an Hbs-Rst interaction enables LNPs to associate with an R axon bundle (magenta).

The epithelial part of the optic lobe is composed of a single layer of neuroepithelial (NE) cells. NE cells are programmed to differentiate into neuroblasts at the medial edge of the developing optic lobe. The wave of differentiation progresses synchronously in a row of cells from medial to the lateral regions of the optic lobe; it is preceded by the transient expression of the proneural gene *lethal of scute* (*l(1)sc*) and thus we named it the proneural wave. *l(1)sc* is expressed transiently at the wave front and required for timely differentiation of NBs (Figure 2). We identified the EGFR signaling pathway as the key to promote proneural wave progression. EGFR activation is regulated by transient expression of Rhomboid (Rho), which is required for the maturation of the EGF ligand Spitz. Rho expression is also regulated by the EGFR signal.The transient and spacially restricted expression of Rho generates sequential activation of EGFR signaling and assures the directional progression of the differentiation wave.This study also provides new insights into the role of Notch signaling. Expression of the Notch ligand Delta is induced by EGFR, and Notch signaling prolongs the proneural state.Notch signaling activity is downregulated by its own feedback mechanism that permits cells at proneural states to subsequently develop into neuroblasts. Thus, coordinated sequential action of the EGFR and Notch signaling pathways cause the proneural wave to progress and induce neuroblasts formation in a precisely ordered manner (Figure 3).

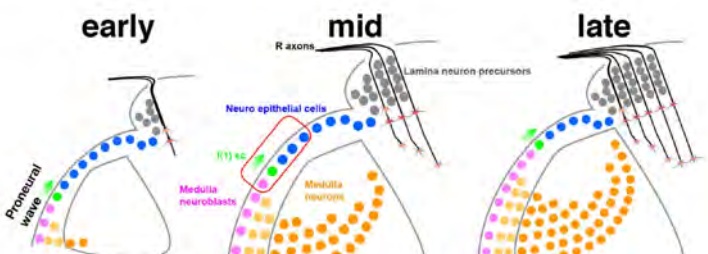


Figure 2. Proneural wave. The proneural wave progresses in a medial-to-lateral direction.

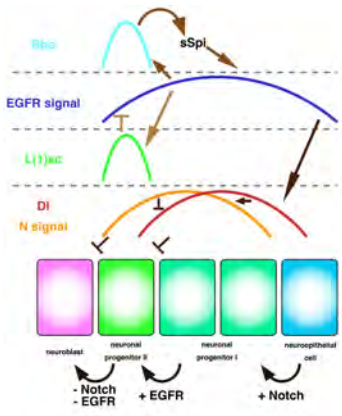


Figure 3. EGF and Notch coordinate proneural wave progression. (1) The EGFR signal induces Rho expression to stimulate ligand secretion and to move the EGFR activation site more laterally. (2) The EGFR signal induces L(1)sc expression and triggers a transition from neuroepithelial cells to neuroblasts. Proneural genes then turn off the EGFR activation. (3) The EGFR signal activates the Notch signal through regulation of Dl expression. The Notch signal causes neuroepithelial cells to differentiate into the neuronal progenitor I state and inhibit premature transition to neuronal progenitor II state and neuroblast. Dl expression decreases by Notch activation. Attenuation of EGFR and Notch signal allows neuronal progenitor II cells to differentiate into neuroblasts.

Neuronal circuits controlling sleep in *D. melanogaster*

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Why and how we sleep has been a matter of speculation and study for millennia. Every day our brains cycle between waking and sleeping states. Both of these brain states are highly active, but the nature of the activity and the connection of the brain to the outside world in each state are distinct. Primate and rodent model systems have provided great insights into sleep, but the circuitry in these organisms is quite complex. The recent finding that insects sleep suggests that *Drosophila melanogaster*, a simple and genetically tractable organism, can be used to study this process.

In recent years, work from my lab and others has exploited the new genetic and electrophysiological tools available in *Drosophila* to push forward our understanding of sleep by identification and manipulation of the underlying circuitry. In this talk I will discuss the evolutionary conservation of sleep at the behavioral and circuit levels in the fly and how dissection of the circuitry in this organism may allow us to understand the fundamental nature of sleep regulation.

As in mammals, sleep in flies is regulated by both circadian and homeostatic drives, and the onset of the sleep state correlates with changes in high frequency brain activity and an increase in arousal thresholds for acute sensory stimuli. The circuit that generates this behavior consists of GABAergic sleep-promoting neurons which make contacts with wake promoting neurons that are also part of the circadian clock circuit and receive light input. Other brain areas have been identified that may act to modulate this small core circuit. The ability to acutely and chronically control neuronal activity in specific parts of this circuit will allow a detailed understanding of the fly sleep switch. Pressing questions such as how integration over multiple time scales can occur in the switch can be addressed using these tools and will have implications for studies of mammalian switch function. Identification of new circuit components that feed into the integrator, and the ongoing efforts in gene discovery which are providing new molecules that regulate sleep, will make flies true contributors to our understanding of this human behavior.

Insect cold hardiness – the secret is in the genes

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Carleton University, Canada

Winter survival for many species of insects requires strategies for sustaining viability at temperatures below 0°C. Typically, this means either freeze tolerance (a high percentage of body water converted to extracellular ice) or freeze avoidance (deep supercooling to maintain the liquid state). Two species of gall insects that overwinter as larvae on goldenrod have been extensively used by our lab and others as models of each strategy - the freeze tolerant gall fly, *Eurosta solidaginis*, and the freeze avoiding gall moth, *Epiblema scudderiana*. Recent studies in our lab have explored novel aspects of metabolic regulation and cell preservation that underlie natural cold hardiness in these species. Long term winter survival is enhanced by minimizing and prioritizing ATP use and analysis of energy-expensive cell functions such as ion pumping, transcription, translation, and energy metabolism has revealed active suppression of multiple enzymes mediated by protein kinases. Mitochondrial activity is also suppressed over the winter including reduced activities of enzymes such as cytochrome c oxidase. By contrast, survival of freeze-induced anoxia/ischemia by freeze tolerant species appears to be aided by up-regulation of the hypoxia inducible transcription factor, HIF-1, to coordinate the gene expression responses that provide hypoxia/ischemia protection. Long term viability over the winter is also aided by increased expression of a variety of chaperone proteins (heat shock proteins, glucose-regulated proteins, crystallins) that stabilize protein structure/function in cytoplasmic, endoplasmic reticulum and mitochondrial compartments. Multiple signal transduction cascades are involved on both a seasonal and a temperature-responsive basis; these include actions by cAMP-dependent protein kinase (PKA), mitogen-activated protein kinases, and several protein phosphatases. Overall, our studies reveal that cold hardy insect species use a suite of dynamic responses to modulate cellular metabolism in response to seasonal, low temperature, and freeze/thaw cues. For more information, visit www.carleton.ca/∼kbstorey. (Funded by NSERC Canada)

Molecular mechanism for tolerance to complete desiccation in the Sleeping Chironomid, *Polypedilum vanderplanki*

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Life and death are mutually exclusive states. But some organisms showing no sign of living due to complete desiccation are nevertheless able to resume active life after rehydration. Organisms in this peculiar biological state are highly resistant to dehydration in a condition referred to as "cryptobiosis" or "anhydrobiosis". Larvae of the Sleeping Chironomid *Polypedilum vanderplanki*, living in temporary rock pools in semi-arid areas on the African continent is able to achieve anhydrobiosis. When the rock pools dry up, the larvae become dehydrated but can revive within about an hour after water becomes available upon the next rain (Fig.1-A). Many anhydrobiotic animals accumulate large amounts of the disaccharide trehalose, which serves as a compatible solute thought to protect desiccating tissues by replacing the primary water of hydration and possibly through sugar glass formation. Anhydrobiotic *P. vanderplanki* larvae indeed accumulate trehalose to levels of about 20% of their dry weight (40 μg/individual). Using a classical insect endocrine technique we also demonstrated that even larvae without a brain and thorasic ganglia could synthesize trehalose and enter anhydrobiosis successfully (Fig.1-D). Those observations indicated that induction of anhydrobiosis in *P. vanderplanki* is not under cerebral control, in spite of the fact that the brain has a significant role in regulating both induction and termination of insect diapause, in general. LEA (late embryogenesis abundant) proteins (initially isolated from plant seeds) also occurred in *P. vanderplanki*. Physico-chemical analysis confirmed that the anhydrobiotic larvae enter a vitrified state which is certainly stabilizing molecules and cell membranes of the larvae under complete dehydrartion, and both hydrophilic molecules (trehalose and LEA proteins) were importantly contributing to the glass formation. Other protective factors such as heat-shock proteins (HSPs) and anti-oxidative enzymes were also expressed for self-protection. Nevertheless, to our surprise DNA was damaged during anhydrobiosis. The mending of the damage should take place after rehydration.

The anhydrobiotic larvae can stand another extreme conditions such as exposure to 100°C, -270°C, 100% ethanol, 7kGy gamma-rays and vacuum. As space environment is a typical extreme condition, we propose that the sleeping chironomid could be a potential experimental animal for space-related study, and indeed such experiments are rightly underway at International Space Station (ISS).

Recently we isolated a trehalose transporter, *tret1* from *P. vanderplanki*. This gene has a great potential for application such as anhydro- and cryo-technology.

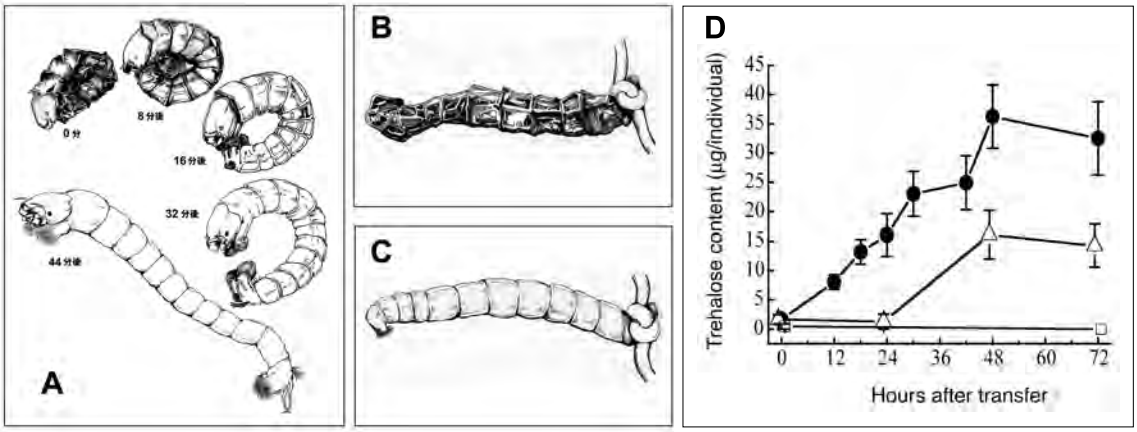

Fig. 1. *Polypedilum vanderplanki* larvae during recovering from anhydrobiosis. A, a larva recovering from anhydrobiosis at 0, 8, 16, 32 and 44 min after rehydration. B, decapitated anhydrobiotic larva. C, decapitated larva on the 2nd day after rehydration. D, changes of trehalose level in the following larvae. ●: intact larvae (dry); △: decapitated larvae (dry); □: decapitated larvae (hydrated).

Cell and ECM morphogenesis in leg segmentation

Shigeo Hayashi

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Segmented limbs of insects have been used as versatile tools for locomotion and feeding. Evolutional history that has lead to their morphological variation and cellular mechanisms underlying limb morphogenesis have been of great interest for developmental biologists. This talk will start from discussion on ancestral forms of insect limbs based on our recent findings on prototypical limbs of basal insects bristletail and mayfly, and polychaete annelide. I will then introduce our studies on insect leg segmentation with special focus on the morphogenesis of the non-cellular part of the body, extracellular matrix (ECM).

Tarsal segments of Drosophila leg.

Ball-and-socket type joint tarsus.

SURPRISES FROM THE EMERGING FIELD OF SPERM PROTEOMICS

Timothy Karr

Arizona State University

As a central player in the reproductive success of sexually reproducing organisms, sperm clearly represent a focal point in providing organismal fitness through its role in fertilization. Both human fertility and infertility pose significant global health problems. In developing countries human fertility is the biological engine that drives population explosions thus producing associated societal and public policy issues impacting the human condition. Likewise, human infertility negatively impacts both individuals who desire children, and globally, where some countries are struggling with population-wide reductions in birthrates as often reported in the popular press and the subject of intense study by demographers, statisticians and sociologists. Thus, both sides of the "fertility coin" will benefit significantly from a deeper understanding of the molecular basis of variation in fertility. In the diverse landscape of eukaryote cellular function, spermatozoa are biochemically 'simple' and therefore ideally suited for study at the systems level. Using high-throughput tandem mass spectrometry in conjunction with bioinformatics, genomics and molecular evolutionary methodologies, I will present a second reiteration of the Drosophila melanogaster sperm proteome ($\sim$750 proteins) along with our initial analysis of sperm proteomes from the 5 remaining members of the melanogaster subgroup. A detailed molecular evolutionary and functional genomic analysis has revealed a surprising level of diversity in protein composition including at least two large families of recently duplicated genes of unknown function. The presentation will also include analysis of the mouse ($\sim$1000 proteins) sperm proteome and a comparative analysis of both mouse and Drosophila sperm proteomes. As expected, both proteomes contain highly conserved elements including proteins involved in energetics and motility, but we find also a surprising array of previously unidentified sperm proteins, a significant proportion ($\sim$25%) with no functional annotation. A survey of these classes of proteins will be presented and discussed in the context of sperm function and evolution. The signature of sexual selection in the mouse is apparent, with a robust evolutionary acceleration of sperm cell membrane and acrosomal gene compartments. The selective forces driving the accelerated evolution of these membrane proteins may occur at a number of locations during sperm development, maturation and transit through the female reproductive tract where the sperm cell membrane and eventually the acrosome are exposed to the extracellular milieu and available for direct cell-cell interactions.

Studies on the male reproductive system of *Drosophila melanogaster*

Masa-Toshi Yamamoto

Drosophila Genetic Resource Center, Kyoto Institute of Technology, Japan

A brief introduction of the National BioResource Project "Drosophila" will be presented. The project was commenced in 2002 as a national project promoted by the Ministry of Education, Culture, Sports, Science and Technology Japan and "Drosophila" is one of 27 bioresources. The number of Drosophila resources of living culture is now reached to about 36,000 with the help of sub-institutes, National Institute of Genetics, Ehime University and Kyorin University. This extremely large collection of Drosophila strains will be continued in the future and further support international Drosophila community in collaboration with the Bloomington stock center by maintaining and providing high quality strains. In order to support *Drosophila* research, we must be professionals to culture flies and characterize their specific features. Thus my research group has been working on the reproductive system of Drosophila and recently we are working on proteomics of the male reproductive system.

Among the male sterile mutations collected so far, several mutants fail to produce functional sperm in fertilization and sperm storage in the female sperm storage organs. They are roughly classified in two types, and each type of mutation, *misfire*, *n55*, and a combined type *wasted* will be described. We are also interested in the proteome dynamics in the process of spermatogenesis, and processing of sperm fluid and sperm storage in the female after copulation.

The internal male reproductive system of *Drosophila melanogaster*.

It consists of five organs, pair of testes, seminal vesicles, and accessary glands, an ejaculatory duct and an ejaculatory bulb. A transgenic strain of HisH2Av-EGFP was observed by the Confocal stereomicroscope Nikon AZ-C1.

Green indicates nuclei. Blue is intrinsic fluorescence. Note that double nuclei are clearly visible in the accessary glands.

Posters

P-1 **Programmed Cell Death Contributes to the Spacing Pattern Formation in *Drosophila***

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During vertebrate and invertebrate development, organs and tissues must be precisely patterned. Spatial control of neural gene expression is an early and essential event in neuronal patterning. The bristles on the thorax of the adult fly, called microchaete, are the external sensory organs and regularly spaced. The development of microchaete is well studied as the simple model to investigate the neural pattern formation. The bristle pattern is mainly mediated by the expression of *achaete-scute* proneural genes and the Notch-mediated lateral inhibition. Epidermis with strong expression of *achaete* become sensory organ precursor cells (SOP) and start to suppress the surrounding epidermal cells to become SOPs via Notch/Delta signaling. By using the live-imaging analysis, we observed that SOPs emerge asynchronously and most of them develop properly. However about 20% of the SOPs did not differentiate to sensory organs. These SOPs tend to locate in the ectopic positions, and the time-course of cell division is different compared with normal SOPs. They stop to differentiate after the expression of neural gene, *neuralized* and most of these ectopic SOPs are eliminated by caspase-dependent cell death, and finally proper patterning is achieved. Our results suggest that in the process of spacing pattern formation, programmed cell death functions to eliminate the SOPs that appear ectopically and contributes to the proper pattern formation.

P-2 **Systematic identification of higher-order auditory neurons in a fruitfly brain**

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To understand how animals control their behavior in response to external auditory cues, a comprehensive knowledge about the auditory neural pathways from the ear down to the motor control center is required. Towards the identification of the complete auditory neural circuits in a fruitfly brain, we had established a comprehensive map of the primary auditory projections and subsequently identified four types of secondary auditory neurons in the brain (Kamikouchi et al, 2006; Kamikouchi, Inagaki et al., 2009). To reveal a more complete map of the higher-order auditory neural circuits, we have started a systematic screening of the secondary auditory neurons. By examining ~4,000 GAL4 strains, we identified 214 candidate lines that may label the higher-order auditory neurons. These neurons were anatomically categorized into at least two types: (1) neurons that arborize broadly in the ventrolateral protocerebrum (VLP) and (2) those that send projections only to confined areas in it. Considering that VLP receives input not only from the auditory pathway but also from the olfactory and visual pathways (Tanaka et al., 2004; Otsuna & Ito, 2006), these higher neurons might participate in different aspects of sensory processing and integration.

References:

Kamikouchi A, Shimada T & Ito K. (2006) J Comp Neurol. 499:317-356
Kamikouchi A, Inagaki HK et al. (2009) Nature 458:165-171
Tanaka NK, Awasaki T, Shimada T & Ito K. (2004) Curr Biol 14:449-457
Otsuna H & Ito K. (2006) J Comp Neurol 497:928-958

P-3 **Introgression of *Drosophila simulans Nup160* (*nuclear pore protein 160*) in *Drosophila melanogaster* alone does not cause inviability but does cause female sterility**

Sawamura K[1], Maehara K[2], Mashino S[2], Kagesawa T[3], Kajiwara M[3], Matsuno K[3], Takahashi A[4,5], Takano-Shimizu T[4,5,6,7]
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We have been analyzing genes for reproductive isolation by replacing *Drosophila melanogaster* genes with homologs from *Drosophila simulans* by interspecific backcrossing. Among the introgressions established, we found that a segment of the left arm of chromosome 2, *Int(2L)S*, carried recessive genes for hybrid sterility and inviability. As Tang and Presgraves (2009) reported, *Nup160* (*nuclear pore protein 160*), which is located in the introgression region, is involved in hybrid inviability. Male hybrids carrying an X chromosome of *D. melanogaster* were not rescued by the *Lethal hybrid rescue* (*Lhr*) mutation when the *D. simulans Nup160* allele was made homozygous or hemizygous. Furthermore, we uniquely found that *Nup160* is also responsible for hybrid sterility. Females were sterile when *D. simulans Nup160* was made homozygous or hemizygous in the *D. melanogaster* genetic background. Genetic analyses indicated that the *D. simulans Nup160* introgression into *D. melanogaster* was sufficient to cause female sterility but that other autosomal genes of *D. simulans* were also necessary to cause lethality. The involvement of *Nup160* in hybrid inviability and female sterility was confirmed by transgene experiments. Our results suggest a genetic model in which three proteins of the nuclear pore complex, when derived from different species and expressed in certain specific combinations, are incompatible and lead to hybrid inviability and sterility.

P-4 **Linking Global Tissue Asymmetry to Cell Polarity on the Plane**

Uemura T[1,2], Harumoto T[1,2], Shimada Y[1], Ueda, HR[3], Kobayashi TJ[3,4]
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Cells sense global axes of the tissue to which they belong and manifest polarity for specialized functions. One such example is planar cell polarity (PCP), which is seen in many animals and tissues, such as epidermal cuticular structures in insects. Mechanisms of PCP have been studied well in the Drosophila wing, where epidermal cells somehow sense the organ cue along the proximal-distal (P-D) axis, localize an assembly of actin filaments at the distal cell vertex, and generate wing hairs (prehairs) that point distally during pupal development.

The pertinent molecular players have been classified into at least the 2 following categories: Te first group includes atypical cadherins Dachsous (Ds) and Fat (Ft) that are thought to contribute to the tissue patterning information across the axis. Second, members of the "core group," including Frizzled (Fz) and the seven-pass transmembrane cadherin Flamingo (Fmi), assemble into asymmetric apicolateral complexes that straddle the proximodistal (P/D) junctions between adjacent

cells; and they specify the intracellular location of the wing hair formation by a feedback loop. In spite of a growing body of information regarding PCP, we are still far from understanding the overall mechanisms underlying it. Important questions include how the above 2 categories of regulators are functionally related to each other and why Fz is relocalized at distal cell borders in the first place.

We have been pursuing the cellular mechanisms that underlie how such a redistribution is achieved. Based on ultrastructural, immunohistochemical, and in vivo time-lapse analysis, we previously showed a role of polarized transport of Fz-containing vesicles along P-D-oriented non-centrosomal microtubules (Shimada et al., 2006). Nevertheless 2 critical questions have remained to be elucidated: First, how do the MTs become aligned along the P-D axis? Second, why are the Fz vesicles transported distally, but not proximally? To address these questions, we have been quantitatively analyzing dynamics of microtubules and movements of Fz-containing vesicles.

Reference:

Y. Shimada, S. Yonemura, H. Okura, D. Strutt, and T. Uemura. Polarized transport of Frizzled along the planar microtubule arrays in Drosophila wing epithelium. *Developmental Cell*, 10: 209-222 (2006).

P-5 **Control of Dendritic Self-Avoidance by the Seven-Pass Transmembrane Cadherin Flamingo and PET-LIM domain protein Espinas in Drosophila Sensory Neurons**

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The correct wiring of neuronal circuitry relies not only on axon targeting, but also precise patterning of the dendritic fields. Once the territory field is covered by dendrites, the growth and branching of dendrites would cease normally, so as to prevent overlap between branches of the same neuron and between the receptive fields of neighboring functionally homologous neurons. This complete coverage of a each receptive field is achieved by inhibitory interaction at dendro-dendritic interfaces. Although our knowledge of molecular mechanisms underlying dendritic elaboration has been accumulated, the cellular and molecular mechanisms governing the dendritic avoidance are still poorly understood. Drosophila dendritic arborization (da) neurons extend and elaborate dendritic branches within two dimensions on the basal surface of epidermis, and one particular class of da neurons provides a suitable system to study the mechanisms of the dendritic avoidance.

Drosophila Flamingo (Fmi; also designated as Starry night) is an evolutionally conserved seven-pass transmembrane cadherin and plays multiple roles in controlling epithelial planar cell polarity and patterning axons and dendrites. In contrast to its role in the epithelial polarity, little has been understood about molecular functions in neuronal cell morphogenesis. Although previous studies implicated a role of Fmi in dendritic avoidance, interpretations of an overlap phenotype in fmi strong loss-of-function mutants have been controversial. This is primarily because the avoidance defect could be a secondary consequence of initial robust overgrowth of branches in the mutant embryo (Grueber et al., 2002 and Kimura et al., 2006).

To solve this issue, we observed dendritic morphology of da neurons in fmi hypomorphic mutants that developed to mature larvae. Those mutant larvae displayed a highly-penetrant overlap of terminal sister branches, whereas overall shape of major branches looked hardly affected. We demonstrated that the expression of one of the two Fmi isoforms in the mutant neurons was sufficient to restore this overlap phenotype. To further understand how Fmi regulates the branch avoidance at the molecular level, we identified Espinas (Esn), a PET-LIM domain protein, as a downstream signaling component of Fmi. We found the physical interaction between cytoplasmic-tail domain of Fmi and the LIM domain of Esn was essential for the normal dendritic self-avoidance.

Grueber et al., Development 129: 2867-2878 (2002)
Kimura et al., Journal of Cell Science 119: 1116-1129 (2006)

P-6 **Sickie, a Neuron navigator homolog, is required for the axonal development of *Drosophila* mushroom body neurons**

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The Drosophila Mushroom Body (MB) is a center for the higher brain functions such as learning and memory. The MB consists of the 3rd-ordered interneurons called Kenyon cells, and forms a unique lobed structure in an olfactory circuit in the brain.

Using the *pBGay* vector based Gal4/UAS enhancer trap method, we have tried to identify key molecules expressing in MB and controlling its development. Among 1,500 *pBGay* insertion lines, 82 lines showed specific Gal4 expressions in MB. Next, we determined *gal4* insertion sites of those 82 lines and listed up candidate genes that are adjacent to the inserted *pBGay* vectors. By *in situ* hybridization assay, several genes expressed in the cell body region of MB neurons are identified. Among them, we found that *sickie* was expressed in both larval and pupal brains.

sickie encodes a putative cytoplasmic protein and is a human *Neuron navigator* and *C. elegans unc-53* homolog. This gene is thought to be required for the migration of cells and neuronal processes in *C. elegans* (*Stringham, et al, Development, 2009*), however, detailed molecular mechanisms and developmental roles of this gene in CNS remain elusive.

To tackle this issue, we generated FLP/FRT mediated deletion alleles of *sickie*. The mutants showed MB lobe formation defects. Short, thin, or truncated lobes were often observed. RNAi knock down of sickie induced by MB specific Gal4 driver resulted in similar lobe formation defects. MARCM technique revealed that *sickie* mutant single cell clones showed defects in axon growth, branching and guidance cell-autonomously. Sickie localized in axons of newborn MB neurons but could not be detected in mutant neurons. Furthermore, the lobe formation defect of the *sickie* mutant was rescued by the expression of *sickie* transgenes in new born MB neurons.

We thus conclude that Sickie is required for the axonal development of MB neurons in a cell-autonomous manner. We are now trying to identify the signaling pathway in which Sickie is involved. We will report some candidate genes that might play roles in the same signaling pathway.

P-7 **Dendritic and axonal targeting of synaptic partner neurons in the antennal lobe are regulated by Meigo, a putative UDP-sugar transporter**

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The wiring of functional neural network results from numerous synaptic connections among neurons. The proper "targeting" of both axons and dendrites actively contribute to the synaptic match making, however the molecular mechanism is largely unknown. During the development of *Drosophila* olfactory system, the axon of primary neuron (olfactory receptor neuron; ORN) target one of ~50 glomeruli in the antennal lobe (AL), and the dendrites of secondary neuron (projection

neuron; PN) also target single glomerulus independently of ORN axon, offering us a good model system to study and compare the targeting mechanisms of synaptic partners.

To obtain mutants that exhibit dendritic mistargeting in PN, we performed MARCM-based screen and isolated a mutant, *meigo*$^{B3-37}$. The dendritic targeting of PNs homozygous for *meigo*$^{B3-37}$ (*meigo*$^{B3-37}$ PN) shifted to the medial side of the AL, but the axonal trajectory and branching were largely normal, indicating that *meigo*$^{B3-37}$ mutations causes the targeting defect specifically in dendrite but not axon of PNs. Interestingly, however, *meigo*$^{B3-37}$ ORN exhibited the defects in axonal targeting. The axon of *meigo*$^{B3-37}$ ORNs mistargeted to the medial side of the AL, which was similar to those seen in *meigo*$^{B3-37}$ PN dendrites. The responsible gene (*meigo*) encodes a putative UDP-sugar transporter, which is conserved from yeast to human. Meigo was localized at ER and Golgi, and the null mutants for the other UDP-sugar transporters or HSPG synthase genes did not exhibit defects in PN dendritic targeting. Together with the mosaic rescue experiments, our result indicate that Meigo is cell-autonomously required for neuronal targeting along the medial to lateral (M-L) axis in the AL, probably by regulating the glycosylation and/or folding of cell surface proteins. Further analyses may offer new insights into the molecular mechanism for forming and/or recognizing the positional information along M-L axis in the neural wiring.

P-8 **RNA interference screening to identify genes required for the development of the *Drosophila* mushroom body**

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The mushroom body (MB) is a chemosensory neuropil in *Drosophila* brain that is essential for olfactory processing. One MB is derived from four neuroblasts, each of which sequentially generates three types of MB neurons that can be distinguished by their axon projection/fasciculation patterns. These neurons extend their axons from the cell bodies that are clustered on the posterior surface of the protocerebrum to the anterior direction. At the anterior side, MB axons are segregated into three sets of lobes. The γ lobe extends medially and consists of the axons derived from the MB neurons(γneurons) that are born before the mid-3rd instar stage. The α' and β' lobes, which project dorsally and medially, respectively, are derived from bifurcation of the axons of late larval-born MB neurons (α'/β' neurons). The α and β lobes, the other pair of perpendicular lobes, are composed of axonal branches of MB neurons (α/β neurons). To elucidate the mechanisms of the projection patterns of these MB neurons is important to understand MB functions, but they are largely unknown.

In order to elucidate the mechanisms of the morphogenesis of MB, we have performed RNAi screening using GAL4/UAS-IR system, which can disrupt gene function in specific tissues. We crossed *OK107-GAL4*, a MB specific GAL4 driver, with *UAS-IR* lines, each of which expresses dsRNA for a target gene under the control of UAS, and observed the phenotypes of adult MBs. We have screened over 1000 IR lines that down-regulate transcription factor and membrane-bound protein-related genes and found that about 34% of the genes showed phenotypes. We present the results of the screening and the molecular analysis of the genes that showed severe phenotypes.

P-9 **Hunting for target genes of transcription factors that control neuronal class-specific dendrite morphogenesis**

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Dendritic tree morphology is a hallmark of cellular diversity in the nervous system, and it has been suggested that this diversity supports the differential processing of information in each type of neurons. Drosophila dendritic arborization (da) neurons provide an excellent model system to study its molecular basis. da neurons are classified into classes I to IV in order of increasing branching complexity. We have shown that expression of the BTB-zinc finger protein Abrupt (Ab) is highly restricted to class I neurons and is necessary and sufficient to endow da neurons with the class-I-specific simplest dendritic pattern. In contrast to Ab, a transcriptional regulator of the Early B-Cell Factor (EBF)/Olfactory 1(Olf-1) family, Knot (Kn)/Collier (Col) is expressed selectively in class IV neurons, which generate the most expansive and complicated dendritic trees. We have also shown that Kn exerts its cell-autonomous function to control the formation, and possibly the function, of class IV-like elaborated dendritic arbors. To explore entire mechanisms of the class-specific dendritic morphogenesis, we have been searching for target genes of Ab or Kn by using an in vivo binding-site profiling technique, DNA adenine methyltransferase identification (DamID). We generated transgenic flies that expressed either Dam-Ab or Dam-Kn fusion protein to mark potential binding sites of each transcription factor with a unique methylation tag. Using genomic DNA from those transgenic flies, we mapped regions that were reproducibly tagged on the whole genome tiling array. To narrow down candidates of the target genes, we are now trying to perform pan-da or class-selective expression profiling. We will discuss our ongoing analysis to find the candidate genes that regulate type-selective dendrite morphogenesis.

P-10 **Roles of Wnt Signal in Development of *Drosophila* Mushroom Body**

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The *Drosophila* mushroom body (MB) is a paired neuropile structure composed of three distinct types of neurons; γ, α'/β', α/β Kenyon cells (KCs) and is required for olfactory memory. Cell bodies of KCs reside in the dorsoposterior part of the brain and project their axons anteriorly forming a massive fiber tract known as peduncle. At the frontal margin of the brain, while axons of γ KCs turn medially to form a γ lobe, axons of α'/β', α/β KCs bifurcate to form discrete dorsal and medial lobes. In this study, we demonstrate that Wnt/planar cell polarity (PCP) signal is required for proper formation of the structure of MB. Major components of Wnt/PCP signal are membrane protein Frizzled (Fz), Strabismus (Stbm), Flamingo (Fmi), cytoplasmic protein Dishevelled (Dsh), Prickle (Pk), and Diego (Dgo). We found lobe structures were lost or reduced in *fz*, *stbm*, *fmi* or *dsh* mutants. Rescue experiments of *dsh* and *fz* mutant and expression analysis of Fz, Stbm and Fmi strongly suggested autonomous function of Wnt/PCP components in development of MB. Wnt ligand is not identified in Wnt/PCP signal of *Drosophila*. Then we searched for ligand of PCP components in development of MB and found that *Wnt5* mutants showed the similar phenotype to *fz*, *dsh* or *stbm* mutant. Furthermore, *Wnt5* and some PCP genes genetically interacted. These data suggest that PCP genes and *Wnt5* function in the same or parallel pathway and control axon guidance of Kenyon cells. Single-cell analysis in *Wnt5* mutant revealed that *Wnt5* is not required for axon branching itself or extension but for correct targeting of bifurcated axons. Wnt5 protein was expressed broadly in the neuropile regions of pupal brain except lobe structures and peduncle of MB. Notably, Wnt5 was strongly localized at the branching point of lobes and in calyx of MB. *Wnt5* mutant phenotype was partially rescued by specific expression of Wnt5 in calyx. These data suggest that Wnt5 controls axon guidance of Kenyon cells apart from cites where *Wnt5* mutant phenotype become obvious. From above results, we would like to discuss several possibilities as for the roles of Wnt5 in development of MB.

P-11 ***wasted*, a gene essential for utilization of stored sperm in *D. melanogaster*, encodes a novel protein specific to the genus *Drosophila***

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The male sterile mutant named *wasted* (*wst*) of *D. melanogaster* was isolated from a natural population in Katsunuma, Japan. It shows defects in three independent processes of utilization of stored sperm. (1) Entry rate into eggs of the *wst* sperm decreases a half of the initial level in one day after copulation. (2) The *wst* sperm in seminal receptacles are lost within two days after copulation. (3) The inseminating *wst* sperm fail to form the male pronucleus. The *wst* locus was determined by complementation tests using deficiencies to the region between 49F to 50A. To identify the *wst* gene, we analyzed nucleotide sequences of putative exons of 17 genes located in a 170 kb region to which *wst* was mapped. We found a four-nucleotides deletion that introduced a premature stop codon in a gene. The wild-type allele of the gene rescued the male sterility due to *wst* mutation, indicating that it is the *wst* gene and three defects of the *wst* sperm are caused by a single gene mutation. Sequencing of cDNAs revealed a potential ORF of 1,503 amino acids that shows no homology to known conserved domains. The orthologs were found only in species of the genus *Drosophila*, suggesting that the *wst* gene had newly occurred after the divergence of the genus and had acquired novel *Drosophila*-specific functions in the reproductive system.

P-12 **The Hotspot Region for Male Recombination in *Drosophila ananassae***

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Since Kikkawa (1937) and Moriwaki (1937) reported spontaneous crossing over in *Drosophila ananassae* males, frequent male recombination has been observed in both the laboratory and natural populations (reviewed by Matsuda et al. 1993, Goni et al. unpublished). A cytogenetic analysis of male recombination revealed that cytological exchanges resulted in genetic crossing over (Matsuda et al. 1984, Goni et al., 2006). Furthermore, male recombination in *D. ananassae* is controlled by a complex system of genetic factors including Enhancers, Suppressors and Modifiers (for review, see Matsuda et al., 1993). In F_1 males from crosses between the *e pi; bri ru* (EP) strain, which carries Enhancer for male recombination, and the *Om(2C) Arc* strains, we demonstrated remarkably increased recombination in the *Om(2C)* $\sim$ *Arc* section of the proximal region of the 2nd chromosome. This rate of enhancement is 40 $\sim$ 50 times as higher than that at the standard map units (0.4 cM). Using SNP markers and *in situ* hybridization, we found that the hotspot of recombination between *Om(2C)* and *Arc* is located within about 6 kb on the 45C band of the proximal region of the right arm of the 2nd chromosome. An adjacent 1.6 kb deleted region of the hotspot was found in the EP strain, and an approximately 14kb insertion sequence was found in the *TyrR* (*Tyramine receptor*) gene, which is included in the hotspot region of the EP strain. The insertion sequence found in the EP strains was also present in other strains which carry Enhancer of male recombination, but not in those that don't. We discuss the enhancement of male recombination in the hotspot of *D. ananassae*.

P-13 **Long-term In Vivo Time-lapse Recordings of Dendritic Development and Organelle Dynamics**

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Dendrites allow neurons to integrate sensory or synaptic inputs, and the spatial disposition and local density of branches within the dendritic arbor limit the number and type of inputs. *Drosophila* dendritic arborization (da) neurons provide a model system to study the genetic programs underlying dendritic development *in vivo*. It has been recently demonstrated in larvae that several organelles and cargos of motor proteins play critical roles in shaping dendritic arbors; and those organelles examined include Golgi outposts (Ye et al., 2007), RNA particles (Brechbiel and Gavis, 2008), endosomes (Satoh et al., 2008; Zheng et al., 2008), and mitochondria (Tsubouchi et al., 2009). Unfortunately, however, a large volume of data is unavailable on the organelle behaviors that underlie the dynamics of branch elongation, sprouting, and retraction, from budding of primary branches to maturation of entire dendritic arbors. This is due to the difficulty in technique to keep da neurons in mounted larvae healthy for an hour or even for 10 min.

Compared to larval da neurons, one prominent advantage of observing pupal da neurons is accessibility to long-term time-lapse recordings. da neurons exhibit large-scale dendritic pruning and subsequent growth during metamorphosis of pupal stages. We recently identified da neurons in the adult Drosophila abdomen, visualized the dendritic arbors of the individual neurons, and traced the origins of those cells back to the larval stage (Shimono et al., 2009). We then started investigating dendritic patterns of one of the da neurons at various pupal stages and found that the neuron appeared to start dendritic growth around 40 hrs after pupal formation (APF). To understand dynamics of the growth of the dendrites, we acquired z-stacked images of dendrites at 30-seconds intervals for 4 hours or longer at the stages of 40hr APF or 60hr APF; and we observed not only branch elongation and elaboration, but also retraction and local pruning of filopodia-like structures. We are also performing simultaneous tracking of organelles or microtubule growth together with branches, which hopefully would allow much more quantitative analysis to determine cause-and-effect relationships between the intracellular dynamics and dendrite morphogenesis. We will discuss our ultimate goal, which is to collect and analyze all data on distinct neuronal types to understand neuronal type-specific dendrite morphogenesis.

P-14 **Functional analysis of genes from an anhydrobiotic chironomid, *Polypedilum vanderplanki*, using the transgenic fly system**

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Larvae of the sleeping chironomid, *Polypedilum vanderplanki*, live in temporary rock pools in semi-arid zone of Africa, and survive complete desiccation entering anhydrobiosis (i.e., life without water) during dry season. To reveal the mechanism underlying an extreme desiccation tolerance in *P. vanderplanki*, we researched expression profile of genes involved in anhydrobiosis, and analyzed function of the genes using transgenic fly. Using desiccating larvae we have constructed an anhydrobiosis-related EST database, consisting of 15,056 EST clones. Using the database, we isolated genes for trehalose (a sugar, one of the factors for successful induction of anhydrobiosis) synthetic enzymes (TPS and TPP) and a trehalose transporter (TRET1). Desiccation induced these trehalose-related genes expressed in the fat body, and the expression profiles could be the rationale for that trehalose synthesized in the fat body is transported to the hemolymph to be spread to the whole body of the desiccating larva. Desiccation tolerance-related genes (LEA protein genes, *Lea1* through *Lea4*), of which homologous proteins were found in anhydrobiotes, including prokaryotes, plants and nematodes, were also expressed in the desiccating larvae. Upon dehydration, LEA proteins reversibly transform random coils to α-helical coiled coil and can form a stable glass matrix with trehalose. Transcripts of the LEA proteins-coding genes accounted for about 11.9 % of total mRNAs during desiccation, according to estimation based on number of the corresponding EST clones.

Anhydrobiosis-related dynamic of LEA proteins mRNA indicate that they probably act as mechanical support for the intracellular matrix and as a thermal stabilizer of biological glasses in the desiccated larvae.

Further, in order to confirm biological functions of TRET1 and LEA1-4, we introduced these coding sequences to *D. melanogaster*, w^{1118} strain using pPUAST-9050. Transgenic flies carrying UAS-the chironomid sequence were crossed with the flies carrying GAL4 coding sequence under control of the Act5C promoter. Adult transgenic flies were counted and their mRNA was extracted to corroborate expression of the desiccation tolerance-related genes originated from *P. vanderplanki*. The flies expressing *Lea1-4* were reproductive normally comparing with the control crosses, whereas *Tret1* expression sterilized both male and female flies. This reproductive disruption might be due to derange trehalose distribution caused by constitutive expression of *Tret1*. We go on crossing flies to establish lines simultaneously expressing two or more genes participated in desiccation tolerance, suggesting that desiccation tolerant flies will emerge through continuous crossing and introducing.

P-15 **Identification of a mushroom body extrinsic neuron required during the consolidation phase for odor memory**

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Previous studies have suggested that *Drosophila* mushroom bodies (MB) and a few input neurons to the MB are required for olfactory memory. However it remains to be elucidated how memory traces involving MB are formed in memory processing. To determine a new neural circuit critical for memory formation, here we studied the function of newly identified MB output neurons. These neurons, we call them dorsal paired MB output neurons (PMO), receive inputs from the Mβ' lobe and convey output to the outside of the MB. By temporally inactivating these neurons with Gal4 enhancer trap lines expressing in PMO neurons and UAS-*shibire*^{ts}, we found that PMO neurons were dispensable for one-hour memory but necessary during the consolidation phase for three-hour memory.

These results suggest that PMO neurons are only required during memory storage but not during acquisition or retrieval, and that a loop, which consists of MBβ' neurons, PMO neurons and MB α neurons, is critical for the consolidation of mid-term odor memory, considering the fact that MBαlobe is necessary for memory retrieval. We revealed that memory trace formed in MB output neurons is critical for odor memory.

P-16 **Courtship decision making is regulated by signal comparison between two olfactory pheromone pathways in *Drosophila melanogaster***

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How does an animal evaluate its complex environment and generate a suitable behavioral response? In the fruit fly, *Drosophila melanogaster*, aphrodisiac pheromones produced by females are a strong attractive cue stimulating male courtship. On the other hand, *cis*-vaccenyl acetate (cVA), a product of the male ejaculatory bulb, works as a courtship inhibitory pheromone to suppress homosexual male-male courtship. In a previous study, we showed that cVA is transferred to the female during copulation and reduces the sexual attractiveness of the mated female. Since the mated female becomes unreceptive to a courting male, it is beneficial for a male to reduce his level of courtship towards mated females. Recently, two groups of pheromone odorant receptors (ORs) were identified by electrophysiological analysis: OR47b and OR88a which respond to fly odors, and OR65a and OR67d which respond to cVA. It was also found that gene expression of Or67d and synaptic activity of the Or65a odorant receptor neurons (ORNs) were required for cVA-responsive courtship suppression.

In order to examine how pheromonal information is processed in these ORNs by antennal lobe circuitry and leads to an appropriate behavioral response, we performed physiological analysis using an activity-sensitive fluorescent marker, synaptopHluorin. The " naked brain " expressing synaptopHluorin showed that cVA-responsive Or65a ORNs and fly odor-responsive Or47b ORNs received cholinergic excitatory input to their presynaptic terminals. Blocking expression of the acetylcholine receptor in either Or65a or Or47b ORNs diminished cVA-responsive courtship suppression, suggesting that lateral feedback modification of ORNs is critical for pheromone coding. Intriguingly, co-suppression of the excitatory feedback in both Or65a and Or47b ORNs rescued the cVA-response defect, indicating that the behavioral output is determined by the signal equilibrium between the Or65a courtship inhibitory pathway and the Or47b courtship stimulatory pathway.

P-17 **Subtype Specification of the *Drosophila* Longitudinal Glial Cells**

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Glial cells comprise many subtypes that differ in gene expression patters and physiological functions. The longitudinal glial cells (LG) of the *Drosophila* CNS is a heterogeneous population that enwrap the longitudinal axon bundle and are required for the correct axonal path-finding. Subtype specification of LG and how such specification contributes to their terminal differentiation are poorly understood.

LG can be subdivided into two classes with respect to the expression of a homeodomain transcription factor Prospero (PROS). PROS is expressed in six out of nine LG. In *pros* mutant embryos, PROS-positive LG form but fail to differentiate and support axonal growth. PROS expression requires Notch activation, and conversely, artificial activation of activated-Notch promotes ectopic PROS-expression. In contrast, in other CNS glial cells such as subperineural glial cells, *pros* is insensitive to Notch signaling. We found that Notch-dependent PROS induction require three transcription factors: homeodomain protein REPO, AT-rich interaction domain protein DRI/RETN, and ets transcription factor Pointed. These factors likely provide "context" for Notch-dependent LG subtype specification. We further show that PROS, in coordination with Pointed, repress the expression of *axotactin*, which is expressed in PROS-negative LG and encodes a secreted protein that is important for establishing the electrical properties of axonal membranes.

P-18 **A high resolution and throughput screen for subcellular mRNA localization in the *Drosophila* early embryos reveals a wide variety of mRNA localization patterns**

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It is generally believed that the transport of most subcellular localized proteins occurs subsequent to translation. Recently, however, it has been shown that protein localization can also be controlled by mRNA localization in advance of translation.

Subcellular mRNA localization has been known as a widespread mechanism for controlling efficient and prompt protein synthesis in several types of cells, such as embryonic, neuronal, yeast and fibroblast cells and also is conserved from *E. coli* to human. While the list of known localized mRNAs has grown steadily in a few decades, the prevalence, variety and overall importance of mRNA localization events is unknown. In addition, although evidence from cell fractionation experiments showed a number of mRNAs are localized to subcellular organelles, because of the low resolution of these signals, the numbers and types of mRNA localization have been difficult to estimate.

To unravel this challenging question, we developed a high resolution and throughput in situ hybridization procedure, and carried out an mRNA localization screen in early *Drosophila* embryos. In this screen, of the 3370 genes analyzed, the expression of 2314 genes was detected. Of the genes expressed during the developmental window analyzed, 71% of the mRNAs expressed are subcellulary localized, and a variety of different mRNA localization patterns were observed and were grouped into 35 localization categories, including anterior/posterior patterns, apicobasal patterns, membrane-associated patterns and cell-division and nuclei-associated patterns. "Fly-FISH", a web resource documenting these mRNA localization patterns has been established and opened to public.

P-19 **Analysis of genetic association between circadian clock genes and photoperiodic diapause incidence in *Drosophila triauraria***

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Diapause is an adaptive response to overcome unfavorable seasons. It is triggered by daylength and temperature. However, its physiological process and genetic architecture are still largely unknown. It has been argued that the photoperiodic clock has a common genetic architecture/components with circadian clock that is another time measurement system controlling daily-rhythmic physiological phenomena including locomotor activity, eclosion and oviposition. As a first step to clarify the argument, we examined association of five circadian clock genes (*period*, *timeless*, *Clock*, *cycle*, *cryptochrome*) with diapause incidence in *Drosophila triauraria*. *D. triauraria* shows robust reproductive diapause with clear photoperiodicity. Females of *D. triauraria* do not develop their ovaries under short-day condition at 15°C, although they do under long-day condition even at the same temperature. On the other hand, flies collected from low-latitude locations do not enter diapause-state even under the short-day condition (non-diapause strain). Using the diapause and non-diapause strains, we examined diapause incidence in females of F_1 hybrids and backcross progenies between the strains. Then, in the backcross progenies, we genotyped their alleles of the five circadian clock genes in 329 females by sequencing regions including the strain-specific SNPs or deletions. Our phenotyping and genotyping analysis revealed that the molecular markers within the two genes *timeless* and *cryptochrome* associated with the diapause incidence. The finding suggests a molecular link between the two circadian clock genes *timeless*, *cryptochrome* and the photoperiodic diapause incidence.

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E-1 **Summary of National Bioresource Project (NBRP) in Japan**

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The Ministry of Education, Culture, Sports, Science and Technology (MEXT) has decided to continue the operation of the National Bioresource Project from 2007 to 2011. Bioresources as experimental materials for research and development include strains, populations, tissues, cells, genetic materials, and associated information of animals, plants, and microorganisms. The goals of this project are to build up strategically bioresources of the highest level in the world by 2010, and also to facilitate the use of them.

The major purpose of this projects is to collect, preserve, and provide bioresources (such as experimental animals and plants) that are essential experimental materials for life science research. The project also aims to improve the bioresources by increasing the value of bioresources through developing technologies for preservation and other necessary procedures and enriching genome information, in order to meet to the current scientific demands. In addition, reinforcement of functions of the information center is included in the project to enhance dissemination of the information regarding the whereabouts and biological characteristics of bioresources.

In our poster, we would like to report the summary of the National Bioresource Project in Japan.

E-2 **National BioResource Project "*Drosophila*"**

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The National BioResource Project (NBRP) is a Japan's national project that aims to provide systematic accumulation, storage and provision structure for nationally recognized bioresources. The first stage of NBRP started in 2002 and Drosophila was selected as a subject in NBRP among 24 species. It ended in March 2007 and the second stage of NBRP Drosophila started since April 2007. Drosophila Genetic Resource Center, Kyoto Institute of Technology plays a role as the central resource center of NBRP Drosophila. National Institute of Genetics (RNAi strains), Ehime University (wildtype strains of other species) and Kyorin University (mutants of other species and "12 genomes" species) participate in NBRP Drosophila. We are keeping totally more than 42,000 strains and provide an essential resource for the fly community. Following the closure of Szeged Stock Centre, Hungary in June 2009, the majority of the stocks have now been transferred to DGRC Kyoto and are now available for distribution.

E-3 **GRED: Program for Genetic Resource Education & Development**

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Humans have exerted strong negative effects on biodiversity. Global economy and global warming appear to cause the loss of species. One of the important issues over the globe is to preserve biodiversity. On the other hand it should be continued to study and develop genetic resources, because they are useful for progression of fundamental biology, medical science, agricultural biology, and industrial benefits. Here we introduce the program to train genetic resource curators, which learn how to analyze genetic resources, how to maintain genetic resources, and what are the rules when handle genetic resources. This educational program is for graduate students and industrial workers etc. The core curriculum of the program consists of two classes and two training courses as follow. 1) Genetic resources and Society —rules and laws—; 2) Genetic diversity; 3) Training course I for studying model genetic resources; 4) Training course II for studying model genetic resources.


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