Platform session I

July 29 (Tue) 13:30-15:15

(Poster: July 29 (Tue) 17:30-19:30)

O-I-1
The target recognition molecule Capricious concentrates at the tips of protrusion of muscle cells
p=Hiroshi Kohsaka, Akinao Nose
Dept. Physics., Grad. of Sci., Univ. Tokyo, Tokyo

O-I-2
Homeotic gene Antp controls axonal pattern by enhancing fasII expression in Drosophila CNS
p=Kazuma Fushima, Youichi Hatatani, Hidenobu Tsujimura.
Developmental Biology, Tokyo Univ. Agri. Tech.

O-I-3
Structure-functional analysis of Flamingo in dendritic morphogenesis
p=Hiroshi Kimura-1, Tadao Usui-1 and Tadashi Uemura-1, 2
1) Inst. Virus Research, Kyoto University, Kyoto: 2) CREST, JST

O-I-4
Flamingo Regulates R8 Axon-Axon and Axon-Target Interactions in the Drosophila Visual System
p=Tadao Usui-1, Kirsten-Andre Senti-2, Barry J. Dickson-2, Tadashi Uemura-1, -3.
1) Inst. Virus Research, Kyoto Unuv., Kyoto: 2) Research Inst. Molecular Pathology, Vienna: 3) CREST, JST

O-I-5
Molecular anatomy of auditory system in Drosophila melanogaster.
p=Azusa Kamikouchi-1, Kei Ito-1,2
1) BIRD, JST: 2) IMCB, Univ. of Tokyo.

O-I-6
Clustering analysis of projection patterns of the antennal lobe relay interneurons
p=Takashi Shimada-1,2, Nobuaki Tanaka-1,4 Takeshi Awasaki-1,3, Kei Ito-1,2
1) IMCB, Univ. of Tokyo: 2) BIRD, JST: 3) PRESTO, JST: 4) Graduate Univ. for Advanced Studies

O-I-7
Behavioral Analysis of Olfactory Reward Learning in Drosophila Larvae
p=K. Honjo, K. Furukubo-Tokunaga.
Institute of Biological Sciences, University of Tsukuba

Platform session II

July 29 (Tue) 15:35-17:20

(Poster: July 29 (Tue) 17:30-19:30)

O-II-1
p170 a novel Drosophila transcriptional regulator of the FTZ-F1 gene.
p=Yasuo Agawa-1, Susumu Hirose-1,-2, Hitoshi Ueda-1,-2.
1) Dept. Devt. Genet., Natl. Inst. Genet. Mishima, Shizuoka: 2) Dept. Genet, Grad. Univ. Adv. Stud. Mishima, Shizuoka

O-II-2
Direct evidence for alternative trans-splicing in the Drosophila lola locus.
p=Takayuki Horiuchi-1, Edward Giniger-2, Toshiro Aigaki-1
1) Dept. Biol., Tokyo Metropolitan Univ., Tokyo: 2) Fred Hutchinson Cancer Research Center, WA.

O-II-3
Functional Analysis of Apoptosis-Related Genes, POSH, ALG-2 and ALIX in Drosophila
p=Manabu Tsuda, Ki-Hyeon Seong, Takashi Matsuo, Toshiro Aigaki
Dept. Biol. Sci., Tokyo Metropol. Univ

O-II-4
Diversity of the Golgi apparatus as functional units
p=Hiroyuki Yano, Reiko Kuwahara, Shuka Haraguchi, Satoshi Goto
Cell Function Unit, Mitsubishi Kagaku Institute of Life Science

O-II-5
Drosophila Deltex regulates endocytic trafficking of the Notch protein and promotes its intramembrane cleavage.
p=Kazuya Hori-1, Mikiko Ito-2, Takashi J. Fuwa-1, Masahiro J. Go-3, Hideyuki Okano-4 and Kenji Matsuno-1,5,6
1) Dept. Biol. Sci./Tech., Tokyo Univ. of Sci., Chiba: 2) Dept. Nutrition, Sch. Med., Univ. of Tokushima, Tokushima: 3) Dept. Neuroscience and Immunology, Kumamoto Univ., Kumamoto: 4) Dept. Physiology, Keio Univ., Tokyo: 5) Genome and Drug Research Center, Tokyo Univ. of Sci., Chiba: 6) PRESTO, JST

O-II-6
Analysis of Notch signaling in Drosophila postembryonic neuroblast
p=Masako Toriya-1,-2, Keiko Nakao-1, Hideyuki Okano-1,-3.
1) Dept.Physiol., Med., Keio Univ.: 2)Dept.Cell Biol. Neurosci., Grad. Sch. Med., Osaka Univ.: 3)CREST-JST

O-II-7
Genetic and developmental analyses of left-right asymmetry in the Drosophila embryonic guts.
p=Shunya Hozumi-1, Reo Maeda-1, Asako Minami-1, Kiichiro Taniguchi-1, Masasi Ooike-1, Takeshi Sasamura-1-2, Toshiro Aigaki-3, Ryutaro Murakami-4, and Kenji Matsuno-1-2
1)Dept. Biol. Sci./Tec., Tokyo Univ. of Sci., Chiba: 2) PREST, JST: 3) Dept. Bio. Sci., Tokyo Met. Univ., Tokyo: 4)Dept. Phys. Bio. Inf., Yamaguchi Univ., Ymguchi

Platform session III

July 30 (Wed) 9:00-10:30

(Poster: July 30 (Wed) 15:00-17:30)

O-III-1
A novel formin homology protein implicated in tracheal formation in Drosophila.
p=H. Tanaka-1, E. Takasu-1, T. Umemiya-1, T. Aigaki-2, K. Kato-3, S. Hayashi-3, A. Nose-1
1) Dept. Phys., Univ. Tokyo, Tokyo 2) Dept. Biol., Tokyo Metropolitan Univ., Tokyo 3) Lab Morphogenetic Signaling, Riken CDB, Kobe

O-III-2
RAS activation downstream of Drosophila EGF and FGF receptors is essential for survival and motility of tracheal cells
p=Ryo Matsuda, Chie Hosono and Kaoru Saigo
Dept. of Biophys. & Biochem., Univ. of Tokyo

O-III-3
Drosophila PVR receptor tyrosine kinase is required for thorax closure during metamorphosis
p=Satoshi Ishimaru-1, Ryu Ueda-2,4 Yoshimi Hinohara-3, Shynzo Kondo-3, Mayumi Ohtani-1, Akiko Fujita-1, Hidesaburo Hanafusa-1
1) Lab. Mol, Oncl., Osaka Biosci. Inst., Suita: 2) Genetic Networks, MITILS, Machida: 3) Fine Struc. Anal., MITILS, Machida: 4) Invertebrate Genetics, NIG, Mishima

O-III-4
Discovery of genes with segment-specific expression in the Drosophila leg disc using cDNA microarray
p=Reiko Tajiri, Tetsuya Kojima, Kaoru Saigo
Dept. Biophys. & Biochem., Univ. of Tokyo

O-III-5
Species-specific activation of EGF receptor signaling underlies evolutionary diversity in the dorsal appendage number of the genus Drosophila eggshells
p=Yukio Nakamura-1, Kenji Matsuno-1
1) Dept. Biol. Sci./Tech., Tokyo Univ. Sci., Chiba

O-III-6
Towards the establishment of gene function analysis systems in a nonmodel insect, ladybird beetle
p= Teruyuki Niimi-1, -2, Hisashi Kuwayama-1 and Toshinobu Yaginuma-1
1) Grad. Sch. Bioagr. Sci., Nagoya University: 2) PRESTO, JST

Platform session IV

July 30 (Wed) 10:45-12:00

(Poster: July 30 (Wed) 15:00-17:30)

O-IV-1
Spindle orientation ensures the asymmetric outcome of the male germ line stem cell division
p=Yukiko Yamashita, Margaret Fuller
Department of Developmental Biology, Stanford University

O-IV-2
Analysis of innate immune response defective mutant
p=Yoshimasa Yagi-1,-2, Y. Tony Ip-1.
1) Program in Molecular Medicine, UMass Medical School, USA: 2) Div. Biol. Sci., Sch. Science, Nagoya Univ., Nagoya

O-IV-3
THE DROSOPHILA INHIBITOR OF APOPTOSIS PROTEIN 1 (DIAP1) DEGRADATION IS REGULATED BY A DEATH KINASE
p=Erina Kuranaga-1, Hirotaka Kanuka-2, Masayuki Miura-3.
1)Dept. Genetics, Grad. Sch. Pharma. Sci, Univ. Tokyo, Tokyo: 2)Lab. Cell Recov. Mech., RIKEN BSI, Saitama: 3)Dept. Cell Biol. Neurosci., Osaka Univ., Osaka:

O-IV-4
A Genetic Screen for Downstream Molecules of a Drosophia TNF Superfamily Protein, Eiger
p=Hiroshi Kanda-1,-2, Tatsushi Igaki-1, Jun Takahashi-1,-3, and Masayuki Miura-1
1)Dept. Genetics, University of Tokyo, 2)Lab. for Integrated Biol., Osaka Univ., 3)Dept. of Biol. Sci., Shimane Univ.

O-IV-5
The nucleotide changes governing cuticular hydrocarbon variation in Drosophila melanogaster
p=Aya Takahashi-1, Shun-Chern Tsaur-2, Jerry A. Coyne-3, and Chung-I Wu-4
Dept. of Ecology and Evolution, Univ. of Chicago

Platform session V

July 30 (Wed) 13:30-14:45

(Poster: July 30 (Wed) 15:00-17:30)

O-V-1
Taco is a direct target of the Ebi/Smrter/Su(H) corepressor complex
p=Leo Tsuda-1, Masako Kaido-1, Toshiro Aigaki-2, Shigeo Hayashi-1
1) Riken Center for Developmental Biology, Kobe: 2) Tokyo Metropolitan University, Tokyo

O-V-2
A novel Cystein Rich Domain protein, Drosophila Corin, is involved in the determination of the proximo-distal axis.
p=Atsushi Sato, Andrew Tomlinson
Department of Genetics and Development, College of Physicians and Surgeons, Columbia University

O-V-3
Three Drosophila EXT genes regulate morphogen gradients through synthesis of Heparan Sulfate Proteoglycans.
p=Yuki Takei, Yutakahiko Ozawa, Makoto Sato, Akira Watanabe, and Tetsuya Tabata
Inst. Mol. Cell. Biol., Univ. Tokyo, Tokyo

O-V-4
Drosophila GATA factor genes, serpent and dGATAe, act sequentially to induce the endoderm development
p=Takashi Okumura,Ryutaro Murakami
Department of Physics, Biology, and informatics, Yamaguchi University

O-V-5
Application of the Gene Search system to screen for genes affecting nervous system development or function.
p=Peyre Jean-Baptiste, Matsuo Takashi, Aigaki Toshiro
Dept. Biological Sciences, Tokyo Metropolitan University

O-I-1

The target recognition molecule Capricious concentrates at the tips of protrusion of muscle cells

p=Hiroshi Kohsaka, Akinao Nose
Dept. Physics., Grad. of Sci., Univ. Tokyo, Tokyo

During synaptogenesis, both presynaptic cells and postsynaptic cells extend dynamic protrusions. The roles of microprocesses on postsynaptic cells have been recently examined in various model systems. In Drosophila, microprocesses on muscles, myopodia, are suggested to play roles in guiding specific motoneurons during the formation of neuromuscular network. In this study, we investigated the relationship between myopodia and the target recognition molecule Capricious (CAPS). CAPS, a transmembrane protein, is expressed on both small subsets of muscles and the innervating motoneurons and proposed to function as a target recognition molecule on muscle 12. We expressed a CAPS-GFP fusion protein on muscle 12 by using GAL4-UAS system and examined its distribution in live dissected embryos during target recognition and synapse formation. We found that CAPS-GFP concentrates at the tips of myopodia during the period when motoneurons initiate contact with muscles. The concentration of CAPS-GFP at the tips of myopodia was observed in prospero mutant which have severe delays in axon outgrowth, indicating that the concentration of CAPS doesn't require interaction between myopodia and motoneurons. Presentation of a target recognition molecule at the tips of cellular protrusions such as myopodia may be an efficient strategy to ensure precise recognition between pre- and postsynaptic cells.

O-I-2

Homeotic gene Antp controls axonal pattern by enhancing fasII expression in Drosophila CNS

p=Kazuma Fushima, Youichi Hatatani, Hidenobu Tsujimura.
Developmental Biology, Tokyo Univ. Agri. Tech.

Homeotic genes are expressed in a region specific pattern in the developing CNS and likely to control regional identity of the CNS. Only a few studies has been reported on their role in the CNS development. They work for the differentiation of neurons. They control the region specific period of NB division during the larva. However, their role is unknown in the development of neural wiring such as axonal or dendritic patterning. We studied it by ectopically expressing Antp in the developing adult mushroom body, a brain substructure known in the most detail in Drosophila, and analyzing its phenotype. Antp mis-expression changed axonal patterns of adult mushroom bodies by enhancing fasII expression. The mis-expression resulted in two phenotypes: lobe misdirection and horizontal lobe overextension. The phenotypes were first observed in pupal stages. We found that α'-lobe neurons, which normally did not express FASII, often expressed it on the pupal stage. When fasII was over-expressed in the mushroom body, lobes misdirected as in Antp mis-expression. When Antp was mis-expressed in fasII mutants, misdirected lobe phenotype was suppressed. Finally, our recent study suggests that Antp control the embryonic axonal pattern in the same way.

O-I-3
Structure-functional analysis of Flamingo in dendritic morphogenesis

p=Hiroshi Kimura-1, Tadao Usui-1 and Tadashi Uemura-1, 2
1) Inst. Virus Research, Kyoto University, Kyoto: 2) CREST, JST

Flamingo (Fmi) is an evolutionally conserved seven-pass transmembrane cadherin and plays multiple roles in controlling epithelial planar cell polarity (PCP) and patterning neuronal processes. In controlling PCP, it has been strongly suggested that Fmi-Fmi homophilic interaction is required for formation of a signaling complex including Frizzled; in contrast, little has been understood about molecular functions of Fmi in neurons. We conducted structure-function analysis and investigated which domain is responsible for dendritic morphogenesis of dendritic arborization (da) neurons. Dendrites of da neurons grow and branch within two dimensions on the basal surface of epidermis; and in fmi mutant embryos, they overextend dorsally. This overextention phenotype was rescued by expression of Fmi::EYFP only in da neurons. Interestingly, the mutant phenotype was also rescued partially by expression of a truncated form of Fmi::EYFP that totally lacks extracellular cadherin repeats, presumptive homophilic binding modules. These and other results suggest that the homophilic interaction doesn't play a role in regulating dendritic outgrowth of da neurons and that Fmi functions as a receptor for an as yet unidentified ligand. To identify such a hypothetical ligand, we prepared a "receptor probe" that contains a small portion of the extracellular domain, and initiated expression screening.

O-I-4
Flamingo Regulates R8 Axon-Axon and Axon-Target Interactions in the Drosophila Visual System

p=Tadao Usui-1, Kirsten-Andre Senti-2, Barry J. Dickson-2, Tadashi Uemura-1, -3.
1) Inst. Virus Research, Kyoto Unuv., Kyoto: 2) Research Inst. Molecular Pathology, Vienna: 3) CREST, JST

Photoreceptors (R cells) in the Drosophila retina connect to targets in three distinct layers of the optic lobe of the brain: R1-R6 connect to the lamina, and R7 and R8 connect to two distinct layers in the medulla. In each of these layers, R cell axon termini are arranged in evenly spaced topographic array. Genetic mosaic analyses revealed that flamingo (fmi) was required to form precise retinotopic projections of R8 growth cones in the medulla. fmi encodes a seven-transmembrane cadherin, previously shown to function in planar cell polarity (PCP) and in dendritic patterning. Here, we show that fmi plays two specific roles in R8 axons: it facilitates competitive or inhibitory interactions between adjacent R8 axons to ensure their correct spacing, and it stabilize the connections between R8 axons and their target cells in the medulla. The former suggests a general role for Fmi in establishing nonoverlapping dendritic and axonal target fields. Importantly, PCP genes other than Fmi do not appear to be required for projection of R8 axons, indicating that Fmi must act with a different set of proteins in navigating R8 growth cones. To identify such down-stream factors, we are now searching for cytoplasmic binding partners of Fmi.

O-I-5
Molecular anatomy of auditory system in Drosophila melanogaster.

p=Azusa Kamikouchi-1, Kei Ito-1,2
1) BIRD, JST: 2) IMCB, Univ. of Tokyo.

To clarify the mechanisms how acoustic information is processed in the brain, we started the identification of the auditory neural network using GAL4 enhancer-trap lines. We screened the strains that label the neurons of the Johnston ’s organ (JONs), the mechanoreceptive auditory organ located in the 2nd antennal segment, and found 34 positive lines. Comparison of the labeling patterns revealed that the distribution of GAL4-expressing cells can be classified into several categories, indicating that JONs consist of several subgroups that exhibit discrete patterns of enhancer activity. To precisely identify the primary processing sites in the brain that receive the output from JONs, we further searched for the lines that exclusively label JONs without labeling other mechanoreceptive sensilla. Analysis of four such lines revealed that the projection area of JONs can be divided into at least seven subareas that collectively cover essentially all the antennal mechanosensory regions. We further examined the projections of individual JONs using the heat-shock flip-out system, and identified at least nine types of JONs, each of which has different projection pattern. These results demonstrate that JONs are not an array of homogeneous neurons but an assembly of heterogeneous subpopulations.

O-I-6
Clustering analysis of projection patterns of the antennal lobe relay interneurons

p=Takashi Shimada-1,2, Nobuaki Tanaka-1,4 Takeshi Awasaki-1,3, Kei Ito-1,2
1) IMCB, Univ. of Tokyo: 2) BIRD, JST: 3) PRESTO, JST: 4) Graduate Univ. for Advanced Studies

The antennal lobe (AL) of Drosophila has about 50 glomeruli, each of which receives information from a distinct type of olfactory sensory neurons. The relay interneurons connect the AL with the secondary olfactory centers, the mushroom body (MB) and lateral horn (LH). Analyses of GAL4 enhancer-trap strains suggest that the projection patterns of specific subsets of these neurons form characteristic substructures within the MB and LH. The detailed examination of these structures, however, has been difficult because of the lack of consistent procedure for the assessment of their spacial distribution. To overcome this problem, we developed an algorithm for the clustering analysis. The cross-section pictures of the MB and LH labeled with various GAL4 enhancer-trap strains are coarse-grained and collectively regarded as a single "initial cluster". The cluster is then divided into two subclusters by the k-means algorithm using the calculated "distance" between different labeling patterns. The procedure is repeated recursively until all the clusters are decomposed to each original picture. By choosing the proper coarse-graining size and metric measurement, we found that projection patterns in the MB and LH can surely be separated into distinct clusters. The clustering patterns showed clear correlation between MB and LH, as well as between relay interneurons and MB neurons, and between relay interneurons and LH neurons.

O-I-7
Behavioral Analysis of Olfactory Reward Learning in Drosophila Larvae

p=K. Honjo, K. Furukubo-Tokunaga.
Institute of Biological Sciences, University of Tsukuba

The fruit fly, Drosophila melanogaster has been used successfully as a model animal for the study of learning and memory because of its powerful genetics. Though most of the previous studies were performed using adult flies, the relative complexity of the adult fly brain hiders functional studies at cellular and molecular resolution. Here, we show that Drosophila larvae can be useful to investigate neuronal connectivity in olfactory learning and memory formation. We have established a new olfactory behavior assay system with positive reinforcement such as sucrose. With this new paradigm, wild type larvae are able to associate a given odor with sucrose. The fact that adult learning mutants such as dunce and rutabaga are also defective in larval learning support that this paradigm represents genuine associative learning, and indicate that similar biochemical pathways are involved as compared to the adult learning behavior. We are currently dissecting anatomical properties of the larval learning using dominant paralytic mutant flies.

O-II-1
p170 a novel Drosophila transcriptional regulator of the FTZ-F1 gene.

p=Yasuo Agawa-1, Susumu Hirose-1,-2, Hitoshi Ueda-1,-2.
1) Dept. Devt. Genet., Natl. Inst. Genet. Mishima, Shizuoka: 2) Dept. Genet, Grad. Univ. Adv. Stud. Mishima, Shizuoka

Transcription factor beta-FTZ-F1 is induced after a pulse exposure of ecdysteroids and is expressed just before hatching, ecdysis and pupation in a stage specific manner. To understand temporary precise regulation mechanism of the beta-FTZ-F1 gene, we have been analyzing cis-regulatory elements and transacting factors that bind to these elements. Factor I-4 is one of the identified factors from Drosophila nuclear extracts by these analyses. The factor is present during high ecdysteroid periods, so that it was thought as a repressor of the beta-FTZ-F1 gene. However, reporter assays showed that the recognition sequence of Factor I-4 is required for high-level expression of beta-FTZ-F1. To clarify the discrepancy and elucidate the regulatory mechanisms of the beta-FTZ-F1 gene, I tried purification, identification and characterization of Factor I-4. A 170-kD protein p170 was identified as a candidate for Factor I-4 by purification. p170 polypeptide revealed it to be a zinc finger protein. Several biochemical features showed that p170 is Factor I-4. RNAi experiments were performed to see loss of function phenotypes of p170. Flies suffered RNAi of p170 were prepupal or pharate adult lethal with malformed legs in several independent lines, which was similar to phenotypes of hypomolphic FTZ-F1 mutants. The result suggested that p170 is a regulator of the beta-FTZ-F1 gene.

O-II-2
Direct evidence for alternative trans-splicing in the Drosophila lola locus.

p=Takayuki Horiuchi-1, Edward Giniger-2, Toshiro Aigaki-1
1) Dept. Biol., Tokyo Metropolitan Univ., Tokyo: 2) Fred Hutchinson Cancer Research Center, WA.

The longitudinals lacking (lola) gene produces at least 80 mRNA variants, each consisting of a constant region with a highly variable 3' region. Here we show that lola mRNA variants are generated through alternative cis- and trans-splicing. Interallelic complementation between mutations in the constant exons and those in the variable exons was explained by trans-splicing at the constant/variable junction of lola mRNA. Quantitative analyses of lola mRNA variants using single nucleotide polymorphism revealed a frequent interallelic trans-splicing. The frequency of interallelic trans-splicing was dramatically reduced when the two loci were improperly located by chromosome rearrangements. Furthermore, a variable exon-specific promoter was identified within the intronic sequence, implying that the ‘gene expression factory’ coordinates the promoter activities for both constant and variable exons of both loci on the homologous chromosomes. The mechanism may serve to simplify otherwise complex tissue-specific regulation of alternative splicing.

O-II-3
Functional Analysis of Apoptosis-Related Genes, POSH, ALG-2 and ALIX in Drosophila

p=Manabu Tsuda, Ki-Hyeon Seong, Takashi Matsuo, Toshiro Aigaki
Dept. Biol. Sci., Tokyo Metropol. Univ

POSH, a scaffold protein containing a RING finger domain and four SH3 domains has been identified as Rac1-interacting protein. It has been thought to be involved in activation of JNK signaling pathway as a scaffold for signaling molecules, such as MLKs, MKKs, and JNK. Here we identified Drosophila homolog of ALG-2 (Apoptosis-linked gene-2) and ALIX (ALG-2 interacting protein X) as additional components for POSH-involved complex. POSH is capable of forming a complex with ALG-2 and ALIX both in vitro and in vivo. Overexpression of either ALG-2 or ALIX activates JNK signaling pathway, which in turn produces various morphological defects, such as rough eye and disordered wing hair polarity. Furthermore, POSH, ALG-2, and ALIX interact with components involved in the Drosophila TNF (Eiger) signaling pathway, suggesting that POSH, in concert with ALG-2 and ALIX, modulates Eiger-induced JNK signaling pathway.

O-II-4
Diversity of the Golgi apparatus as functional units

p=Hiroyuki Yano, Reiko Kuwahara, Shuka Haraguchi, Satoshi Goto
Cell Function Unit, Mitsubishi Kagaku Institute of Life Science

Various kinds of modification and processing of proteins are simultaneously occurred in the Golgi apparatus that are scattered throughout a cell in Drosophila. It has not been revealed how such various Golgi functions are controlled. To approach this problem, we focused on glycosylations of proteins. FRINGE CONNECTION (FRC) is a UDP-sugar transporter that is localized on the Golgi membrane and transports UDP-sugars from the cytoplasm into the Golgi apparatus for glycosylation. We found that FRC was localized in a subset of the Golgi apparatus in a cell and that Notch glycosylation was selectively defected in frc mutant. These results suggest that the FRC-localized Golgi apparatus would be functionally different from the other Golgi apparatus. We call these distinct subgroups ｢ Golgi Units ｣ and this organization of Golgi units may be responsible for the many functions of the Golgi apparatus.

O-II-5
Drosophila Deltex regulates endocytic trafficking of the Notch protein and promotes its intramembrane cleavage.

p=Kazuya Hori-1, Mikiko Ito-2, Takashi J. Fuwa-1, Masahiro J. Go-3, Hideyuki Okano-4 and Kenji Matsuno-1,5,6
1) Dept. Biol. Sci./Tech., Tokyo Univ. of Sci., Chiba: 2) Dept. Nutrition, Sch. Med., Univ. of Tokushima, Tokushima: 3) Dept. Neuroscience and Immunology, Kumamoto Univ., Kumamoto: 4) Dept. Physiology, Keio Univ., Tokyo: 5) Genome and Drug Research Center, Tokyo Univ. of Sci., Chiba: 6) PRESTO, JST

The Notch signaling pathway is an evolutionarily conserved mechanism that regulates various cell-fate decisions. Deltex acts as a positive regulator of Notch signaling through interaction with the intracellular domain of Notch. However, the precise mechanism of Deltex action is unknown. In this study, first, we found that overexpression of Deltex was sufficient to activate the dorsoventral compartment boundary enhancer of vestigial (vgBE), which is a target of Notch signaling during the development of the wing disc. The activation of vgBE by Deltex depended on Notch. Deltex has a RING-H2 finger motif that commonly exists in proteins that belong to the E3 ubiquitin ligase family. Therefore, next, we examined the possibility that Deltex regulates the endocytic trafficking and stability of Notch protein. We found that Deltex caused Notch to be removed from the cell surface into intracellular endocytic vesicles, where Notch became more stable. Furthermore, Notch protein stabilized by Deltex leaded to proteolytic release of the intracellular domain of Notch, which translocated to the nucleus where it activated the reporter gene. Our results suggest that Deltex regulates Notch signaling through the stabilization of the Notch protein in a specific class of endosomal vesicles.

O-II-6
Analysis of Notch signaling in Drosophila postembryonic neuroblast

p=Masako Toriya-1,-2, Keiko Nakao-1, Hideyuki Okano-1,-3.
1) Dept.Physiol., Med., Keio Univ.: 2)Dept.Cell Biol. Neurosci., Grad. Sch. Med., Osaka Univ.: 3)CREST-JST

Stem cells either maintain self-renewing activity or differentiate into neuron and glia, based on their intrinsic mechanism or by responding to extracelllular signaling including Notch. The activity of Notch signaling could be further modulated by factors such as Numb that is an antagonist of Notch and collaborates with a-Adaptin to recruit Notch to the endocytosis complex. Drosophila embryonic neuroblasts cease to divide and become dormant at the late embryonic stage, and are reactivated to proliferate during the larval period. These reactivated Nbs are called postembryonic neuroblasts (pNbs). pNbs are distinct from embryonic Nbs in that pNbs initially increase their number by symmetrically dividing before they start to differentiate into postembryonic neurons and glia by asymmetric division. We found that, from the early larval stage, Notch is highly expressed in pNbs, and Numb is also expressed in pNbs but asymmetrically distributed into their siblings. On the other hand, a-Adaptin expression starts at later larval stage. Based on genetic and biochemical analyses, we have demonstrated that Notch is required for pNbs to continue proliferating and that co-expression of Numb and α-Adaptin at late larval stage decrease nuclear Notch level presumably through degradation during endocytosis of Notch. This decrease of nuclear Notch level turned out to be crucial for initiating differentiation of pNbs.

O-II-7
Genetic and developmental analyses of left-right asymmetry in the Drosophila embryonic guts.

p=Shunya Hozumi-1, Reo Maeda-1, Asako Minami-1, Kiichiro Taniguchi-1, Masasi Ooike-1, Takeshi Sasamura-1-2, Toshiro Aigaki-3, Ryutaro Murakami-4, and Kenji Matsuno-1-2
1)Dept. Biol. Sci./Tec., Tokyo Univ. of Sci., Chiba: 2) PREST, JST: 3) Dept. Bio. Sci., Tokyo Met. Univ., Tokyo: 4)Dept. Phys. Bio. Inf., Yamaguchi Univ., Ymguchi

The viseral organs of most animals are placed precisely as three-dimensional structure determined genetically. While in Drosophila melanogaster genetic bases of dorsal-ventral and anterior-posterior axis formations were understood very well, left-right axis has not been studied until very recently. We are interested in the genetic mechanisms responsible for left-right asymmetry of the visceral organs. Taking advantages of advanced genetic approach, we decided to study left-right asymmetry in the digestive organs of Drosophila embryos. First, to illustrate left-right asymmetry in the three-dimensional structure of the embryonic guts, we carried out time-laps analyses. We found that the left-right asymmetrical structure of each part of guts was arisen according to the genetically determined spatial and temporal patterns. Second, we performed genetic screens of the mutations that showed situs inversus or randomization of the gut left-right asymmetry to identify genes that are responsible for left-right asymmetry. We have identified several mutations that showed mirror image or randomization of left-right the asymmetrical structure of the guts. For example, in souther mutant embryos, left-right inversion in the shape of the midgut and hindgut was observed at approximately 80%, suggesting that left-right axis is reversed in this mutant. We will report a phenotypic analysis of souther mutation.

O-III-1

A novel formin homology protein implicated in tracheal formation in Drosophila.

p=H. Tanaka-1, E. Takasu-1, T. Umemiya-1, T. Aigaki-2, K. Kato-3, S. Hayashi-3, A. Nose-1
1) Dept. Phys., Univ. Tokyo, Tokyo 2) Dept. Biol., Tokyo Metropolitan Univ., Tokyo 3) Lab Morphogenetic Signaling, Riken CDB, Kobe

During embryogenesis of Drosophila, tracheal cells change their shape, migrate, branch and some of them fuse to form luminal network. These morphogenetic processes accompany highly regulated cytoskeletal reorganizations of each tracheal cell. Formin3 is a novel formin homology protein (FHP) isolated by misexpression screening. FHPs are members of a widely conserved protein family implicated in cytoskeletal reorganization. formin3 mRNA are mainly expressed in tracheal cells. We obtained an EMS-induced mutant of formin3, Em31, which has a point mutation that substitutes stop codon for the 428th R in the FH2 domain. In Em31, the dorsal trunk (DT) lumen is discontinuous at some of anastomosis sites, and forced expression of formin3 in the dorsal trunk could rescue this phenotype. We performed live analysis of tracheal cells of Em31 using GFP and GFP-moesin. At stage14, although the tip cells of DTp migrate normally, following cells migrate only slowly or not at all. F-actins do not accumulate in the future axis of the lumen in the fusion cells. The dynamics of fusion cells are abnormal. At later stages, some cells drop out from the tracheal network. These observations suggest that Formin3 participates in the connection of tracheal lumens by regulating actin cytoskeleton.

O-III-2

RAS activation downstream of Drosophila EGF and FGF receptors is essential for survival and motility of tracheal cells

p=Ryo Matsuda, Chie Hosono and Kaoru Saigo
Dept. of Biophys. & Biochem., Univ. of Tokyo

Robust activation of MAPK is reported to occur twice in Drosophila tracheal cells. SPITZ/dTGF-alpha, cleaved by RHOMBOID expressed in the tracheal placode activates MAPK through DER/dEGFR and promotes tracheal cell invagination. Then at the tracheal pit, BRANCHLESS/dFGF expressed in the surrounding tissues activates MAPK through BREATHLESS/dFGFR at the tip of each branch, which is responsible for both the guidance of and the global cell fate organization of each branch. Several questions were raised against morphogenesis of the trachea, the answers of which will be presented in the meeting. 1, What happens to the trachea if activities of both DER and breathless (btl) signaling were simultaneously reduced or eliminated? 2, Is ras involved in tracheal development downstream of DER and btl? 3, Why is the tracheal phenotype of DER null mutant severer than that of rho null mutant? 4, pointed, encoding an ETS domain containing transcription factor known to function downstream of btl, has any role as an effector of DER in tracheal development? 5, How do tracheal cells invaginate? 6, What is the regional relationship between tracheal placode and tracheal pit? 7, How are branch identity genes of spalt and knirps turned on?

O-III-3

Drosophila PVR receptor tyrosine kinase is required for thorax closure during metamorphosis

p=Satoshi Ishimaru-1, Ryu Ueda-2,4 Yoshimi Hinohara-3, Shynzo Kondo-3, Mayumi Ohtani-1, Akiko Fujita-1, Hidesaburo Hanafusa-1
1) Lab. Mol, Oncl., Osaka Biosci. Inst., Suita: 2) Genetic Networks, MITILS, Machida: 3) Fine Struc. Anal., MITILS, Machida: 4) Invertebrate Genetics, NIG, Mishima

The primary function of the skin is to serve as a protective barrier against the environment. Efficient wound healing is essential for animals ranging from insects to mammals to recover from epithelial injury. It is likely that genes involved in wound healing are conserved through the phylogeny. Furthermore, epithelial cell movement during specific developmental processes, such as dorsal closure (DC) during embryogenesis and thorax closure (TC) during metamorphosis, is considered to resemble those seen in mammalian wound healing. Genetic analyses revealed that JNK activation is required for both TC and DC. However, little is currently known about the signaling pathway(s) regulating the JNK activity for these epidermal cell movements. We demonstrate that Drosophila PDGF/VEGF receptor homolog, PVR, is required for TC during metamorphosis. PVR RNAi represses JNK activation in a developing wing imaginal disc. Genetic and biochemical experiments indicate that PVR activates JNK pathway through Rac. An adaptor molecule, Crk and an atypical Rac-GEF complex, Mbc/DELMO have pivotal roles for the Rac activation downstream of PVR. PVR also regulates border cell migration during oogenesis and hemocyte migration during embryogenesis. We speculate that PVR-Crk-Mbc/DELMO pathway is one of the key regulators for those cell migrations during Drosophila development.

O-III-4

Discovery of genes with segment-specific expression in the Drosophila leg disc using cDNA microarray

p=Reiko Tajiri, Tetsuya Kojima, Kaoru Saigo
Dept. Biophys. & Biochem., Univ. of Tokyo

It has been proposed that in the development of higher eucaryotes, graded morphogen activity subdivides the corresponding developing field through instruction of region-specific expression of genes encoding transcription factors. However, what kinds of and how many genes are under the control of each transcription factor is largely unknown. The adult leg of Drosophila is segmented along the proximodistal axis and several transcription factors have been implicated in the specification of these segments. As the first step towards identifying downstream genes of these transcription factors in a comprehensive manner, we applied cDNA microarray analysis to discover genes that are expressed in specific segment(s) in the pupal leg disc. Transcripts that are known to be expressed segment-specifically, such as BarH1 (tarsal segments 4-5), apterous(tarsal segment 4), aristaless and Lim1 (pretarsus), showed consistent transcript enrichment on the arrays. We are carrying out in situ hybridization for a subset of the genes that showed strong differential expression on the arrays and have so far confirmed the segment-specific expression patterns of 14 genes.

O-III-5

Species-specific activation of EGF receptor signaling underlies evolutionary diversity in the dorsal appendage number of the genus Drosophila eggshells

p=Yukio Nakamura-1, Kenji Matsuno-1
1) Dept. Biol. Sci./Tech., Tokyo Univ. Sci., Chiba

In Drosophila melanogaster, the patterning of dorsal appendages on the eggshell is strictly controlled by EGFR signaling. However, the number of dorsal appendages is remarkably diverse among Drosophila species. For example, D. melanogaster and D. virilis have two and four dorsal appendages, respectively. Here we show that during oogenesis the expression patterns of rhomboid (rho) and argos (aos), positive and negative regulators of EGFR signaling, respectively, were substantially different between D. melanogaster and D. virilis. Importantly, the number and position of both the rho expression and MAPK activation coincide with those of the dorsal appendages in each species. Despite these differences in the spatial patterns, these results also suggest that the function of EGFR signaling in dorsal appendage formation is largely conserved between these two species. Thus, our results link the species-specific activation of EGFR signaling and the evolution of eggshell morphology in Drosophila.

O-III-6

Towards the establishment of gene function analysis systems in a nonmodel insect, ladybird beetle

p= Teruyuki Niimi-1, -2, Hisashi Kuwayama-1 and Toshinobu Yaginuma-1
1) Grad. Sch. Bioagr. Sci., Nagoya University: 2) PRESTO, JST

The ladybird beetle Harmonia axyridis (Coleoptera) has conspicuous wing color patterns consisting of black and red pigments. Wing color patterns in H. axyridis are extraordinarily variable. Classical genetic studies revealed that the elytral color patterns are formed from the superimposition of any combinations of four major alleles. In addition, in any heterozygote, the black-pigmented area invariably appears as dominant character at the overlapping two different pigmentations where the color patterns of two alleles are superimposed. The mechanisms involved in wing color pattern formation are yet unknown. Therefore, our ultimate goal is to understand the molecular mechanisms underlying the variations of wing color patterns. As shown in Drosophila, methods for germline transformation are essential for solving many problems in developmental genetics. We have established a technique for germline transformation using a piggyBac vector and RNA interference in the ladybirds. Additionally, we have constructed piggyBac vectors for tetracycline-OFF system, reporter assay and enhancer trapping. First, these vectors have been confirmed to be effective in Drosophila. Subsequently, we are currently investigating the utility of these vectors in transgenic ladybird beetles.

O-IV-1
Spindle orientation ensures the asymmetric outcome of the male germ line stem cell division

p=Yukiko Yamashita, Margaret Fuller
Department of Developmental Biology, Stanford University

The Drosophila male germ line stem cells divide asymmetrically, giving rise to one stem cell and one goniablast which initiates differentiation, thus keeping balance between stem cell and differentiating cell populations. In adult males, 8-10 germ line stem cells surround the somatic apical hub. The hub functions as niche by secreting a signalling ligand, unpaired (Upd), which activates the JAK-STAT pathway in germ line stem cells to maintain stem cell identity. Here we show that spindle orientation of male germ line stem cell division is always perpendicular to the hub to ensure asymmetric outcome of the division. Spindle orientation is established in interphase by the stereotyped positioning of centrosomes. Both interphase centrosome positioning and spindle orientation are disrupted in flies mutant for centrosomin (cnn), apc1 and apc2, resulting in increased number of stem cells, possibly because of symmetric stem cell division. We propose that polarization of the germ line stem cell toward the apical hub both in interphase and mitosis provides a mechanism to ensure the normally asymmetric outcome of stem cell divisions by placing one daughter cell close to and the other apart from the hub.

O-IV-2
Analysis of innate immune response defective mutant

p=Yoshimasa Yagi-1,-2, Y. Tony Ip-1.
1) Program in Molecular Medicine, UMass Medical School, USA: 2) Div. Biol. Sci., Sch. Science, Nagoya Univ., Nagoya

Innate immunity is the first line defense against microbial invasion. In Drosophila, Toll and imd pathway are activated by microbial infection and induce expression of anti-microbial peptides. Mammalian orthologs of these signaling pathway genes are also involved in innate immune response. It suggests that origin of innate immunity is old and there is conserved mechanism between insect and mammal. To identify new components of innate immune system, we screened P-element insertion lines. We injected bacteria to third instar larvae and the changes of transcription level of anti-microbial peptides were examined by Northern blot. We analyzed one of the P element lines which showed anti-microbial peptide transcription defect. Two transcription units were found around the P element insertion point. Northern blot analysis showed that the expression of one of the transcript is lost in mutant larvae. Expression of this transcript in mutant larvae using UAS-Gal4 system could rescue the immune response defect. Thus we concluded loss of the expression of the transcript is affecting innate immune response of Drosophila.

O-IV-3

THE DROSOPHILA INHIBITOR OF APOPTOSIS PROTEIN 1 (DIAP1) DEGRADATION IS REGULATED BY A DEATH KINASE

p=Erina Kuranaga-1, Hirotaka Kanuka-2, Masayuki Miura-3.
1)Dept. Genetics, Grad. Sch. Pharma. Sci, Univ. Tokyo, Tokyo: 2)Lab. Cell Recov. Mech., RIKEN BSI, Saitama: 3)Dept. Cell Biol. Neurosci., Osaka Univ., Osaka:

The moleculer mechanisms of apoptosis are highly conserved throughout evolution. Reaper(Rpr) has been identified as one of key regulators of apoptosis during Drosophila embryogenesis and can lead to the activation of caspase-dependent cell death. Recently, other groups and we showed that Rpr induces cell death through the degradation of DIAP1, which degrades the DTRAF1 or the DRONC, to protect cells in normal conditions. These data suggest that DIAP1 degradation is a key event for cell death in Drosophila. To identify genes involved in DIAP1 degradation, we performed genetic screen using a collection of chromosomal deletions that cover more than 80% of the Drosophila genome. Some regions were identified as dominant modifiers of the GMR-Rpr eye phenotype, and then available mutants in suppressor regions were crossed with GMR-Rpr flies. As a result, we newly identified three mutant alleles named APTX7 as modifiers of Rpr-induced cell death. These alleles encode the same protein, which has a kinase domain. APTX7 induced cell death in both S2 cells and fly compound eyes. Like Rpr, overexpression of APTX7 induced DIAP1 degradation in a dose dependent manner. We will present our results to elucidate the mechanisms of APTX7-induced DIAP1 degradation and cell death.

O-IV-4

A Genetic Screen for Downstream Molecules of a Drosophia TNF Superfamily Protein, Eiger

p=Hiroshi Kanda-1,-2, Tatsushi Igaki-1, Jun Takahashi-1,-3, and Masayuki Miura-1
1)Dept. Genetics, University of Tokyo, 2)Lab. for Integrated Biol., Osaka Univ., 3)Dept. of Biol. Sci., Shimane Univ.

Eiger is a Drosophila tumor necrosis factor (TNF) superfamily ligand obtained by the gain of function screen for cell death genes(1). Eiger induced the activation of Drosophila JNK (Bsk) and caspase-independent cell death. To identify the downsteam signal molecules of Eiger, we conducted a genetic screen for dominant modifiers of Eiger. We performed a series of chromosomal deficiency screening and found 20 candidate genes which would act downstream of Eiger. Wengen, which was a novel Drosophila TNF receptor superfamily protein, was identified in this screen(2). Eiger-induced small eye phenotype was dramatically suppressed when Wengen was downregulated using RNAi. Moreover, Eiger and Wengen interacted each other through the TNF homology domain of Eiger and TNF receptor homology domain of Wengen. These results suggest that Wengen can act as a component of a receptor for Eiger. Analysis of other downstream components of Eiger signaling will be presented. 1) Igaki, T., Kanda, H, et al. (2002) EMBO J. 21(12):3009-18. 2) Kanda, H., Igaki, T, et al. (2002) J Biol. Chem. 277(32):28372-5.

O-IV-5
The nucleotide changes governing cuticular hydrocarbon variation in Drosophila melanogaster

p=Aya Takahashi-1, Shun-Chern Tsaur-2, Jerry A. Coyne-3, and Chung-I Wu-4
Dept. of Ecology and Evolution, Univ. of Chicago

The cuticular hydrocarbon (CH) pheromones in Drosophila melanogaster exhibit strong geographic variation. African and Caribbean populations have a high ratio of 5,9 heptacosadiene / 7,11 heptacosadiene (the High CH type) whereas populations from all other areas have a low ratio (Low type). Based on the genetic mapping, DNA markers were developed that localized the genetic basis of this CH polymorphism to within a 13kb region. We then carried out a hierarchical search for diagnostic nucleotide sites starting with 4 lines, and increasing to 24 and 43 lines from a worldwide collection. Within the 13 kb region, only one variable site shows a complete concordance with the CH phenotype. This is a 16 bp deletion in the 5' region of a fatty acid desaturase gene (desat2) that was recently suggested to be responsible for the CH polymorphism on the basis of its expression (Dallerac R, Labeur C, Jallon J-M, Knipple DC, Roelofs WL &Wicker-Thomas C, 2000. Proc. Natl. Acad. Sci. 97: 9449-9454). The cosmopolitan Low type is derived from the ancestral High type and DNA sequence variations suggest that the former spread worldwide with the aid of positive selection.

O-V-1
Taco is a direct target of the Ebi/Smrter/Su(H) corepressor complex

p=Leo Tsuda-1, Masako Kaido-1, Toshiro Aigaki-2, Shigeo Hayashi-1
1) Riken Center for Developmental Biology, Kobe: 2) Tokyo Metropolitan University, Tokyo

During Drosophila eye development, photoreceptor cells start expressing Delta (Dl) under Egfr signaling control. The Dl expression is required for cone cell development, so the transcriptional regulation of Dl in the photoreceptor cell is one of the key event for the communication between photoreceptor and cone cells. It has been shown that Ebi/SMRTER/Su(H)/Sno, a co-repressor complex, is acting under Egfr signaling for regulating Dl expression during eye development. We identified the molecular nature of the transcriptional regulation for Dl expression by the corepressor complex. From genetic screening we isolated taco (a target of the corepressor complex) as a direct target of the corepressor complex. Taco is a C2H2-type zinc finger protein and shows specific binding to taco response element (TRE), a 21bp cis-regulatory sequence in vitro. We recognized TRE-like sequences in the promoter region of Delta genomic sequence. The Delta expression is eliminated in the photoreceptor cells by over expression of taco. These results suggest that a double negative regulation, which is mediated by the corepressor complex and taco, has an important role for regulating Dl expression under Egfr signaling.

O-V-2

A novel Cystein Rich Domain protein, Drosophila Corin, is involved in the determination of the proximo-distal axis.

p=Atsushi Sato, Andrew Tomlinson
Department of Genetics and Development, College of Physicians and Surgeons, Columbia University

Wnt / Wg signaling pathway is essential for many developmental processes in Drosophila. Members of the Frizzled family function as a Wnt / Wg receptors. The Cystein Rich Domain (CRD) of Frizzleds binds Wnt / Wg. In the Drosophila genome, several other genes encode CRD proteins, but their functions are still unknown. We are interested in one such CRD protein, called Drosophila Corin (DCorin). DCorin is a homologue of human Corin and mouse LRP4. Originally, Corin was identified from a human heart cDNA library, and is required for the maturation of atrial natriuretic peptide (ANP) hormone, which controls blood pressure. DCorin is a type II transmembrane protein and has one CRD, two LDL Receptor related Protein (LRP) domains, one Scavenger Receptor Cysteine Rich (SRCR) domain and one serine protease catalytic domain; in contrast, human Corin has two CRDs, seven LRPs, one SRCR and one protease domain, respectively. RNAi experiments with dcorin and the over-expression of dominant negative forms show that Corin is involved in the determination of the proximo-distal axis.

O-V-3

Three Drosophila EXT genes regulate morphogen gradients through synthesis of Heparan Sulfate Proteoglycans.

p=Yuki Takei, Yutakahiko Ozawa, Makoto Sato, Akira Watanabe, and Tetsuya Tabata
Inst. Mol. Cell. Biol., Univ. Tokyo, Tokyo

The signaling molecules Hedgehog (Hh), Decapentaplegic (Dpp) and Wingless (Wg) function as morphogens and organize wing patterning in Drosophila. The morphogens can be transported over a long distance and make a long range gradient by the mechanisms that are not fully understood. In this study we performed mutant screens for the loci that regulate Dpp activity in the wing development and identified three independent loci that showed almost identical phenotypes. Genetic mapping has identified them as tout-velu (ttv), and its related genes sister of ttv (sotv) and brother of ttv (botv). The predicted products of these genes belong to an EXT family of proteins that have or are closely related to glycosyltransferase activities required for biosynthesis of heparan sulfate proteoglycans (HSPGs), a family of the cell surface glycoprotein. Mutations in these genes reduced protein and signaling levels of Hh, Dpp and Wg. Moreover, Hh accumulated in front of EXT mutant cells, suggesting that Hh requires HSPGs to move efficiently. We propose that HSPGs facilitate the spreading of morphogens that generates concentration gradients.

O-V-4

Drosophila GATA factor genes, serpent and dGATAe, act sequentially to induce the endoderm development

p=Takashi Okumura,Ryutaro Murakami
Department of Physics, Biology, and informatics, Yamaguchi University

GATA factor genes are essential for development of various tissues, and conserved across protostomes and deuterostomes. We identified a novel endoderm-specific Drosophila GATA factor gene, dGATAe. Expression of dGATAe is first detected at stage 8 in the endoderm, and continues throughout life. serpent (srp), another GATA factor gene required for the endoderm development, is expressed in the prospective endodermal domain in early stages and disappears by stage 10-11. srp mutant failed to express dGATAe, and forced-expression of srp caused ectopic expression of dGATAe. In embryos either deficient for dGATAe or treated with dGATAe RNAi failed to express endodermal markers. Forced-expression of dGATAe, conversely, induced ectopic expression of endodemal markers. While both the dGATAe and srp are required for normal development of the endoderm, forced-expression of dGATAe in srp mutant, and forced-expression of srp in an embryo deficient for dGATAe restored expression of endodermal markers. dGATAe, as well as srp, has an activity to repress brachyenteron that determines developmental fate of the hindgut, thus contributes to maintain the boundary between the endodermal midgut and ectodermal hindgut. We present a gene regulatory pathway that leads to the specification of Drosophila endoderm.

O-V-5

Application of the Gene Search system to screen for genes affecting nervous system development or function.

p=Peyre Jean-Baptiste, Matsuo Takashi, Aigaki Toshiro
Dept. Biological Sciences, Tokyo Metropolitan University

The ongoing Gene Search Project initiated in our laboratory has led to the establishment of more than 12.000 independent transgenic lines, each one bearing an insertion of an UAS containing P element, termed Gene-Search (GS) Vector. This vector, in combination with various Gal4 lines, allows for controlled expression of the genome sequence flanking its insertion point. Inverse PCR of the sequences flanking the GS vectors allow us to rapidly identify their insertion point, and to localize them directly on the Drosophila Genome. We have undertaken to screen this collection using Appl-Gal as a driver of GS expression, to identify genes that would have effects on fly viability, development,, or behaviour when ectopically expressed in Appl-Gal4 expressing organs, namely the ring gland and the nervous system. We have screened over 8.000 GS lines. Overall, 15% of the crosses resulted in F1 lethality, whereas 5% induced abnormalities in offspring. Details and current results of the screen will be presented.

Poster session

Odd number: July 29 (Tue) 17:30-19:30

Even number: July 30 (Wed) 15:00-17:30

P-001
The comparison of intrinsic rates of increase among chromosome-substituted lines resistant and susceptible to organophosphate insecticides in Drosophila melanogaster
p=Takahiro Miyo-1, Yuzuru Oguma-2, Brian Charlesworth-1
1) ICAPB, Univ. Edinburgh, Scotland: 2) Inst. Biol. Sci., Univ. Tsukuba, Japan

P-002
Functional identification of water gustatory receptor neurons and their projection pattern in Drosophila.
p=Tsuyoshi Inoshita-1, Nicolas Meunier-1, 2 and Teiichi Tanimura-1
1)Department of Biology, Graduate School of Sciences, Kyushu University, Ropponmatsu, Fukuoka: 2)INRA Station de Phytopharmacie et Mediateurs Chimiques, France.

P-003
A Screen for cell adhesion molecules involved in proximodistal axis formation in the Drosophila leg
p=Kayoko Sakurai-1,2, Toshiro Aigaki-1, Shigeo Hayashi-2.
1)Dept. Biol. Sci.,Tokyo Met. Univ.: 2) Morphogenetic Signaling Group, CDB, RIKEN:

P-004
Divergent sensitivity for sugars, salts and bitter compounds in labellar chemosensilla of Drosophila
p=Makoto Hiroi-1, Frederic Marion-Poll-2, Teiichi Tanimura-1
1) Dept. Biol., Grad. School Sci., Kyushu Univ. Fukuoka: 2) Station de Phytopharmacie et Mediateurs Chimiques, INRA de Versailles, France

P-005
How to localize regulators of planar cell polarity: what drives the biased sorting of Fz::GFP particles?
p=Yuko Shimada-1,2 David I. Strutt-3, Tadao Usui-2, Tadashi Uemura-1,2,4
1) Graduate School of Biostudies, Kyoto Univ.: 2) The Institute for Virus Research, Kyoto Univ.: 3) Biomedical Science, University of Sheffield: 4) CREST, JST

P-006
Distinct functions of Rac1 and Cdc42 during axon guidance and growth cone morphogenesis in Drosophila
p=Ryouta Matsuura, Hideaki Tanaka and Masahiro J. Go
Div Dev Neurobiol, Kumamoto Univ Grad Sch Med Sci

P-007
Ggmma1 subunit of heterotrimeric G proteins is required for neuroblast asymmetric division
p=Yasushi Izumi, Nao Ohta, Asako Furuya-Ito, Mai Saito and Fumio Matsuzaki
RIKEN, CDB: CREST JST:

P-008
Molecular mechanism for the dynamic switching of axonal transport in the developing Drosophila brain
p=Masaki Sone-1,2, Megumi Utsugi-Asada-2, Mikio Hoshino-1,2, Yo-ichi Nabeshima-2
1) PRESTO, JST, Kawaguchi: 2) Kyoto Univ. Sch. Med., Kyoto:

P-009
Expression of Wnt and Frizzled family genes in the developing Drosophila optic lobe
p=Makoto Sato, Tetsuya Tabata
Institute of Molecular and Cellular Biosciences, Univ. Tokyo, Tokyo

P-010

B type lamin plays crucial roles during late development in Drosophila.

p=Shinichi Osouda-1, Suguru Toda-1, Shin Sugiyama-2, Tsuneyoshi Horigome-3, Paul A. Fisher-4 and Kazuhiro Furukawa-1

1)Cell Regulation Lab. in Biochemistry and 3)Biochemistry, Department of Chemistry, Faculty of Science, Niigata University; 2)Division of Biological Science, Graduate School of Science, Nagoya University; 4)Department of Pharmacological Sciences, School of Medicine, University Medical Center, State University of New York at Stony Brook.

P-011

Antagonistic interaction between Notch and EGFR signaling establishes the joint of the Drosophila leg

p=Tetsuya Shirai, Hideki Nakagoshi

Okayama Univ., Grad. Sch. of Nat. Sci. and Tech

P-012

Brain drain (Bdr) is a novel regulatory factor of EGFR signaling pathway

p=Ki-Hyeon Seong-1, Takashi Matsuo-1, Toshiro Aigaki-1

Dept. Biol. Sci., Tokyo Metropol. Univ.

P-013

Gain-of-function screening for circadian rhythm mutants using a gene search system

p=Masami Shimoda-1, Toshiro Aigaki-2

1) Nat. Inst. of Agrobiol. Sci., Tsukuba, Ibaraki: 2) Tokyo Metropolitan Univ., Hachioji, Tokyo

P-014

Comprehensive identification of neural circuits for olfactory processing of the Drosophila brain

p=Nobuaki Tanaka-1, Takeshi Awasaki-2, Kei Ito-3

1) Grad. Univ. Advanced Studies, IMCB, Univ. of Tokyo, Tokyo: 2) IMCB, Univ. of Tokyo, PRESTO, JST, Tokyo: 3) IMCB, Univ. of Tokyo, BIRD, JST, Tokyo

P-015

Functional analysis of eyeless and homothorax genes in antennal lobe development in Drosophila melanogaster

p=Totani, Y., Kurusu, M., and Furukubo-Tokunaga, K.

Inst. Biol. Sci., Univ. Tsukuba

P-016

Ubiquitin ligase Dnedd4 regulates Notch signal transduction pathway

p=Tadashi Sakata-1-2, Hiromi Sakaguchi-1, Leo Tsuda-1, Shigeo Hayashi-1

1)CDB,RIKEN: 2)Grad. school of Life Science Tohoku univ.

P-017

Developmental Stage-dependent Effects of Postsynaptic Activation of CaMKII on Synapse Formation at Drosophila Neuromuscular Junctions

p=Takako Morimoto-Tanifuji, Hokto Kazama, and Akinao Nose

Dept. Phys., Grad. Sch. of Sci., Univ. of Tokyo

P-018

Interaction between male-killing spiroplasma and Drosophila immune system

p=Hisashi Anbutsu-1, Greg Hurst-2, Mayako Kutsukake-1, Takema Fukatsu-1

1) Nat. Inst. of Advanced Industrial Sciences & Technology, Tsukuba:2) Dept. Biol., UCL, London

P-019

Drosophila heterotrimeric G proteins regulate daughter cell size in neuroblast asymmetric divisions

p=Naoyuki Fuse-1, Kanako Hisata-1, Alisa L. Katzen-3, Fumio Matsuzaki-12

1) CDB, RIKEN, Kobe: 2) CREST, JST: 3) Univ. of Illinois College of Med., Chicago:

P-020

How did the number of the MOL diverge in evolution?

p=Minoru Tateno, Daisuke Yamamoto

School of Science & Engineering, Waseda Univ.

P-021
Specificity of olfactory receptor neurons responding to sex pheromones in a moth, Ostrinia furnacalis
p=Takuma Takanashi-1, Peter Anderson-2, Christer Lofstedt-3, Bill S. Hansson-2
1) Insect Neurobiology Laboratory, National Institute of Agrobiological Sciences, Tsukuba, Ibaraki 305-8634, Japan: 2) Department of Crop Science, Swedish University of Agricultural Sciences, SE-230 53 Alnarp, Sweden: 3) Department of Ecology, Lund University, SE-223 62 Lund, Sweden

P-022
Axon branch pruning of Drosophila mushroom bodies during metamorphosis
p=Takeshi Awasaki-1,2, Kei Ito -1,3
1)IMCB, Univ. of Tokyo: 2)PRESTO, JST: 3)BIRD, JST

P-023
Dual Function of Src Proto-oncogene in Epitherial Morphogenesis in Drosophila.
p=Masayo SHINDO-1,2, Leo TSUDA-1, Toshiro AIGAKI-3, Shigeo HAYASHI-1
1) RIKEN. CDB, 2) Graduate Univ. for Advanced Studies, 3) Tokyo Meteopolitan Univ.

P-024
Btk29A is required for normal development of male and female reproductive organs in Drosophila
p=Noriko Hamada-1, C. M. Backesjo-2, C. I. Edvard Smith-2 and Daisuke Yamamoto-1
1) School of Science and Engineering, Waseda Univ: 2) Clinical Research Center, Karolinska Institutet:

P-025
Homology of polytene chromosomal banding patterns of X chromosome among three species, D. ananassae, D. vallismai, and D. melanogaster
p=Eiko Kataoka-1, Ryoko Ogawa-1, Hajime Sato-1, Yoshihiko Tomimura-2, Muneo Matsuda-1
1) Sch. Med., Kyorin Univ., Tokyo: 2) Shiba Gakuen, Tokyo

P-026
Drob-1, a Drosophila Bcl-2 family member, regulates pro- or anti-apoptotic signaling depending on specific death stimulus: Genetic analyses using drob-1 RNAi-mediated 'knock-down' flies
p= Nanami Senoo-Matsuda-1, -2, Tatsushi Igaki-1, -2, Masayuki Miura-1, -2
1) Cell Recov. Mech., RIKEN BSI, Wako, 2) Dept. Genet., Grad. Sch. Pharmac. Sci., Univ. Tokyo, Tokyo

P-027
G-protein gamma subunit 1 is required for the gustatory response to sugars.
p=Hiroshi Ishimoto, Teiichi Tanimura
Department of Biology, Graduate School of Sciences, Kyushu University

P-028
Development of Drosophila central complex during metamorphosis.
p=Mariko Kamiya-1、2, Kei Ito-1、2, Takeshi Awasaki-1, 3
1) IMCB, Univ. of Tokyo: 2) BIRD, JST: 3) PRESTO, JST

P-029
Possible involvement of the synaptic molecules for the formation of arousal consciousness in Drosophila.
p=Ikue Ibuki-1,2, Masaki Sone-1,3, Mikio Hoshino-1,3, Hideki Nakagoshi-2, Yo-ichi Nabeshima-1
1) Kyoto Univ. Grad. Sch. Med., Kyoto: 2) Okayama Univ., Okayama: 3) PRESTO, JST, Kawaguchi:

P-030
Age-related accumulation of deletions in the mitochondrial DNA of Drosophila
p=Ryoko Yui-1, Etsuko T. Matsuura-2
1) Dept. of Advanced Biosci., Ochanomizu Univ., Tokyo: 2) Dept. of Biol., Ochanomizu Univ., Tokyo:

P-031
Identification of Drosophila genes involved in the determination of organ identity using a gene search system
p=Tomonori Katsuyama-1, Tomo Sugawara-1, Yoshiteru Oshima-1, Toshiro Aigaki-2, Shoichiro Kurata-1
1) Tohoku Univ. Grad. Sch. Pharm. Sci. 2) Tokyo Met. Univ. Dept. Biol .Sci.

P-032
Visualization of unconstrained negative supercoils of DNA on polytene chromosomes of Drosophila melanogaster
p=Kuniharu Matsumoto-1, Susumu Hirose-2
Dept of Developmental Genetics, National Inst. of Genetics, Mishima, Shizuoka

P-033
Comprehensive identification of the neuronal target recognition molecules in Drosophila neuromuscular system using GeneChip
p=Mikiko Inaki, Akinao Nose
Dept. Phys., Univ. Tokyo, Tokyo

P-034
Isolation and Analysis of novel deltex alleles
p=Takashi J. Fuwa, Kazuya Hori, Takeshi Sasamura, Kenji Matsuno
Biol Sci/Tec, Tokyo Univ. of Sci. Noda, Chiba, Japan

P-035
Glomerular organisation of larval mushroom body calyx of Drosophila
p=Liria M. Masuda-Nakagawa-1, Cahir J. O'Kane-2, Mitsuhiko Kurusu-3, Makoto Hayashi-3 and Katsuo Furukubo-Tokunaga-3
1) Lab.Cell Regulation, Dept.Animal Sciences, Graduate School of Agriculture and Life Sciences, Univ.Tokyo, Tokyo: 2) Dept.Genetics, Univ.Cambridge, Cambridge, England: 3) Insititute Biol.Sciences, Univ.Tsukuba, Tsukuba

P-036
Drosophila larvae showed abnormal behavior when abd-A was mis-expressed in the nervous system
p=Nobuaki Saitoh,Youichi Hatatani,Hidenobu Tsujimura
Developmental Biology,Tokyo University of Agriculture and Technology

P-037
Functional analysis of ik2 kinase in Drosophila tracheal tubulogenesis
p=Kenji Oshima-1, Shigeo Hayashi-2
Riken Center for Developmental Biology, Kobe

P-038
Dm RECQ5/QE DNA helicase and retrotransposon mdg3
p=Katsumi Kawasaki-1-2, Minoru Nakayama-1,Takehiko Shibata-1-2
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P-039
Molecular evolution and population genetics of the Drosophila PGRP-LE genes
Naoko Ishida, p=Atsuko Date-Ito
Grad. Sch. of Hum. and Sci., Ochanomizu Univ., Tokyo

P-040
Control of photoreceptor targeting by layer-specific expression of the LRR protein Capricious
p=Makiko Kameda-Shinza-1,2,3 and Akinao Nose-1
1) Dept. Phys., Univ. of Tokyo, Tokyo: 2)IMCB, Univ. of Tokyo, Tokyo: 3) BIRD, JST:

P-041
Analyses of a male-female-sterile mutant, mfs(3)GS7354: behavioral sterility in male and physiological sterility in female?
p=Naoto JUNI, Ayako NIIKURA, Kazushi ISAWA, Takashi OHSAKO, and Masa-Toshi Yamamoto.
DGRC, Kyoto Inst. of Tech.

P-042
EARLY STAGE OF WING DISC DEVELOPMENT IN DROSOPHILA: TIME LAPSE ANALYSES OF CELL MOVEMENT
p=Yoshiko Inoue, Shigeo Hayashi
Morphogenetic Signaling Group, CDB, RIKEN

P-043
Possible roles of RecQ5 : Complementation study in yeast
p=Minoru Nakayama-1, 2. Katsumi Kawasaki-1, 3, 4. Kouji Matsumoto-2. Takehiko Shibata-1, 2, 3, 4.
1)RIKEN.Cel.Mol.Biol.: 2)Dept.Mol.Biol.,Univ.Saitama: 3)Bioarchitect: 4)CREST, JST

P-044
Evolution of olfactory receptor gene family in Drosophila and other insects.
p=Rumi Kondo
Dept. Biology, Ochanomizu University, Tokyo

P-045
A genetic screen for mutations that affect the formation of the muscle of Lawrence in concert with fruitless.
p=Yuki Watanabe-1, Kazue Usui-Aoki-2, Daisuke Yamamoto-2,3,
1)Grad. Sch. of Human Sci.: 2)Adv.Inst. Sci. Engin.: 3)Sch. of Sci. Engin., Waseda Univ.

P-046
Musashi is required for asymmetric cell division of Drosophila sensory organ precursor cells, and possibly modified asymmetrically in non-neural precursor cell.
p=Takao Imai-1, Masataka Okabe-2, Hironori Kawahara-1, Keiko Nakao-1, Hideyuki Okano-1
1)Dept. Pysiol. Keio Univ. Med. Shinjuku, Tokyo: 2)Dept. Dev. Genetics, National Institute of Genetics, Mishima, Shizuoka

P-047
Speculation of the relationship between fatty acids and DNA metabolism in Drosophila.
p=S. Murakami-1, K. Takata-1, S.Kamisuki-1, N. Kasai-1, Y. Mizushina-2, F. Sugawara-1, K. Sakaguchi-1.
1) Applied Biological Science, Tokyo University of Science, chiba-ken: 2) Kobegakuin University, Hyougo-ken

P-048
Identification of Drosophila Laval Common Motoneurons and Analysis of their Neuromuscular Junctions.
p=EIJI TAKIZAWA,HIDENOBU TSUJIMURA
Dev Biol.,Tokyo University of Agriculture and Technology.,Futyu,Tokyo

P-049
A role of nuclear matrix protein, Slender lobes, in accelerated proliferation of mushroom body neuroblasts
p=Minako Orihara-1, Mai Saito-2, Yuka yoda-3, Toshiro Aigaki-4, Chihiro Hama-5
1) RIKEN CDB, Kobe: 2) Dept. Biol., Tokyo Metro. Univ., Tokyo:

P-050
Molecular analysis of Drosophila myelodysplasia/myeloid leukemia factor (dMLF) and the interacting protein dCSN3
p=Wakana Sugano-1, Katsuhito Ohno-1, Noriko Kato-2, Jun-ya Kato-2, Yoshitaka Nagai-3, Masamitsu Yamaguchi-1
1) Div. Appl. Biol., Kyoto Inst. Tech., Kyoto: 2) Nara Inst. Sci. Tech., Nara: 3) Div.Functional Genomics, Grad. Sch. Med., Osaka Univ.,Osaka

P-051
Effects of over-expression of growth factors on the development of germline cells of Drosophila.
p=Ueda S.-1, Sato, T.-1, Niki, Y.-1 and Mahowald, A. P.-2
1)Dept. Biol. Univ. Ibaraki 2)Univ. Stanford Sch.Med.

P-052
Mutational Analysis of the Drosophila neural zinc finger gene, dNZF-1 that is expressed in neuronal subsets.
p=Emi Kinameri, Takafumi Yamada, and Shunji Ohsako
Tokyo Metropol. Inst. for Neurosci.,Tokyo

P-053
Molecular basis of distance-dependent variation of nonautonomous apoptosis mediated by Ras
p=Toshiyuki Harumoto-1,Takashi Adachi-Yamada-1,-2.
1) Fac. Human Dev., Univ. Kobe, Kobe: 2) JST, PRESTO:

P-054
GABA-mediated inhibitory neural connections in the Drosophila antennal lobe.
p=Ryuichi Okada-1,-2,-3, Takeshi Awasaki-1,-2,-4, Kei Ito-1,-2,-3
1) Natl. Inst. Basic Biol.: 2) IMCB, Univ. of Tokyo: 3) BIRD, JST: 4) PRESTO, JST

P-055
TRAP240, as a component of the mediator complex, represses transactivation function of androgen receptor
p=Ken-ichi Takeyama-1,2, Saya Ito-1, Syun Sawatsubashi-1,Yuko Shirode-1,2, Eriko Suzuki-1, Akio Maki-1, Etsu Cho-1,Kaoru Yamagata-1, Alexander Kouzmenko-1,2, Tetsuya Tabata-1 and Shigeaki Kato-1,2
1) IMCB. Univ. Tokyo: 2)SORST, JST

P-056
AN ANALYSIS OF THE MALE STERILE MUTANT ms(2)n55 IN DROSOPHILA MELANOGASTER
p=HARA, Masanori, OHSAKO, Takashi ,YAMAMOTO, Masa-Toshi .
Dros. Genet. Res. Ctr., Kyoto Inst. Tech.

P-057
The misfire gene functions in male pronuclear formation at fertilization of Drosophila
p=Takashi Ohsako-1,-2, Kazuyuki Hirai-1, Masa-Toshi Yamamoto-1
1) Dros. Genet. Res. Ctr., Kyoto Inst. Tech., 2) Soc. Edu. Fund.

P-058
A role of microtubule-associated protein, Orbit in chromosome segregation and cytokinesis in Drosophila.
p=Takao Suzuki, Masa-Toshi Yamamoto, Yoshihiro H. Inoue
Dros. Genet. Res. Ctr., Kyoto Inst. Tech.

P-059
CYTOGENETIC ANALYSIS OF A COHESION DEFECTIVE MEIOTIC MUTANT mei(3)M20.
p=KIMURA, Mai, and YAMAMOTO, Masa-Toshi
Dros. Genet. Res. Ctr., Kyoto Inst. Tech.

P-060
Orbit, the CLASP orthologue of Drosophila, is required for cell divisions to generate 16-cell cyst and development of fusome and the polarised microtubule network during gametogenesis.
Endre Mathe-1, Takao Suzuki-2, Masa-Toshi Yamamoto-2, David M. Glover-1, p=Yoshihiro H. Inoue-2
1) Dept. Genet., Cambridge Univ., Cambridge: 2) Dros. Genet. Res. Ctr., Kyoto Inst. Tech., Kyoto

P-061
Molecular analyses of the meiotic gene, mei(3)1223, in Drosophila melanogaster
p=Hiroshi Matsubayashi, Masa-Toshi Yamamoto
Dros. Genet. Res. Ctr., Kyoto Inst. Tech.

P-062
A trace amine, tyramine, affects behavior of the crayfish, Procambarus clarkii.
p=Akira Komatsu-1, Fumi Sano-2, Takashi Nagao-2
1) Dept. Physiol., Sch. Med., Tokyo Women's Med. Univ., Shinjuku-ku, Tokyo: 2) Human Inform. Sys., Kanazawa Inst. Technol., Matsuto-shi, Ishikawa

P-063
Enhancer trap screen to identify genes specifically expressed in the developing optic lobe
p=S. Murakami-1, D. Umetsu-1, T. Awasaki-2, K. Ito-2, T. Tabata-1
1) Lab. of morphogenesis and 2) Lab. of Structural Information, Inst. Mol. Cell. Biol., Univ. Tokyo, Tokyo

P-064

The enhancer trap screens for genes involved in formation of the fly visual center

p=D. Umetsu-1, S. Murakami-1, T. Awasaki-2, K. Ito-2, T. Tabata-1
1) Lab. of Morphogenesis and 2) Lab. of Structural Information, Institute of Molecular and Cellular Biosciences, The University of Tokyo, Japan

P-065

Male sterile mutation ms(3)236 fails to have the sperm stored in the female

p=Tomaru, M., Ohsako, T., Sato, H., and Yamamoto, M.-T.
Drosophila Genet. Res. Ctr., Kyoto Inst. Tech.

P-066

Genes that regulate morphogenesis of gastric caeca

p=Chie Hosono-1, Ryo Matsuda-2, Kaoru Saigo-3
Dept. Biolophys. and Biochem. Graduate School of Sci. Tokyo Univ.

P-067

Transcriptional activation mechanism of human estrogen receptor in Drosophila

p=Saya Ito-1,Ken-ichi Takeyama-1,2,Shun Sawatsubashi-1,Alexander Kouzmenko-1,Yuko Shirode-1,2,Eriko Suzuki-1,Akio Maki-1,Etsu Zyue-1,Kaoru Yamagata-1,Tetsuya Tabata-1,Shigeaki Kato-1,2
1)IMCB, Univ. of Tokyo, Tokyo : 2)SORST,JST

P-068

Glial cell proliferation induced by programmed cell death and neural injury in the Drosophila adult brain

p=Kentaro Kato-1, Takeshi Awasaki-1, -2, Kei Ito-1,-3
1) IMCB, Univ. of Tokyo: 2) PRESTO, JST: 3) BIRD, JST:

P-069

Towards understanding the basis of regulation of the activity of ADF/cofilin phosphatase Slingshot

p=Junichiro Yonekura-1-2, Ryusuke Niwa-1-3-5, Kyoko Nagata-Ohashi-4, Kensaku Mizuno-4, Tadashi Uemura-1-2-5.
1) Inst. for Virus Research, Kyoto Univ.: 2) Grad. School of Biostudies, Kyoto Univ.: 3) Grad. School of Frontier Science, The Univ. of Tokyo: 4) Grad. School of Life Sciences, Tohoku Univ.: 5) Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology

P-070

Analyses of arit1/danr and arit2/dan, which are involved in the antennal formation in Drosophila

p=Takuya Tsubota-1, Takuya Tsuji-1, Tetsuya Kojima-1, Ryu Ueda-2,3, Kaoru Saigo-1
1)Univ of Tokyo,Gra.Sch.Sci.,Dept.Biophys.& Biochem: 2)NIG, Invertebrate Genetics: 3)MITILS, Genetic Networks

P-071

HOM gene function in the development of Drosophila indirect flight muscles

p=Hideaki Arai, Hiroka Aonuma, Hidenobu Tujimura
Developmental biology, Tokyo university of agriculture and technology

P-072

Guidance mechanism of tracheal branch migration in Drosophila

p=Kagayaki Kato-1, Shigeo Hayashi-1.
1) RIKEN, CDB.

P-073

Intrinsic sub-axonal patterning in Drosophila neurons

p=Takeo Katsuki-1, -2, Masaki Hiramoto-1, -3, Yasushi Hiromi-1, -4
1) NIG, Mishima: 2) Div. Genetics, Univ. Advanced Studies: 3) PREST: 4) CREST

P-074

Transcriptional regulation of the Drosophila homeobox genes, aristaless and clawless, required for patterning along proximodistal axis of leg

p=Takuya Tsuji, Tetsuya Kojima, Kaoru Saigo
Dept. Biophys. & Biochem., Gra. Sch. Sci., Univ. of Tokyo

P-075
Analysis of mutations affecting neural stem cell morphology
p=Mai Saito, Yasushi Izumi, Takao Igo, Fumio Matsuzaki
Center for Developmental Biology, RIKEN ,KOBE

P-076
Are you interested in Drosophila species other than D. melanogaster? (National Bio-resource Project)
p=Masayoshi WATADA
Ehime University

P-077
Genome-wide analysis of vesicle transports in Drosophila.
p=masato abe-1,reiko kuwahara-2,shuka haraguchi-3,isamu kusaka-4,satoshi goto-5
Mitsubishi kagaku institute of life science, Mchida, Tokyo

P-078
Characterization of newly established Drosophila cell lines from embryonic mesoderm and larval gonadal mesoderm.
p=Shinzato, M.-1, Sato, T-1., Okada, T.-1, Miura, T.-1, Yamaguchi, T.-1, Niki, Y.-1 and Mahowald, A. P.-2
1)Dept. Biol. Univ. Ibaraki 2)Univ. Stanford Sch.Med.

P-079
Expression of Replication Protein A during development of D.melanogaster
p=Kaori Shimanouchi, Kei-ichi Takata, Kengo Sakaguchi
Dept. Applied Biological Science, Faculty of Science and Technology, Tokyo University of Science, Chiba

P-080
In vivo imaging of calcium dynamics within identified target cells during synaptogenesis
p=Hokto Kazama-1,-2, Takako Morimoto-Tanifuji-1, Akinao Nose-1
1) Dept. Phys., Univ. Tokyo, Tokyo: 2) JSPS Fellow

P-081
Sexual dimorphism in the brain of Drosophila melanogaster
p=Ken-ichi Kimura-1, Jun Maeyama-1, Rie Matsuda-1, Kazue Usui-Aoki-2, Yuki Watanabe-3 and Daisuke Yamamoto-4, 5
1) Hokkaido Univ. of Edu. Iwamizawa Campus, Iwamizawa: 2) Advanced research institute for science and engineering, Waseda Univ., Tokyo : 3) School of Human Sciences, Waseda Univ., Tokyo : 4) Dept. of Mol. Neurobiol., Inst. of Med. Sci., Univ. of Tokyo, Tokyo 5) School of Science and Engineering, Waseda Univ., Tokyo

P-082
Functional analysis of glycans in Drosophila melanogaster using genome-wide RNAi screening
p=Miki Hino-1, Reiko Kuwahara-1, Shuka haraguchi-1, Isamu kusaka-1, Shouko Nishihara-2, Satoshi Goto-1
1) Mitsubishi kagaku institute of life science, Tokyo: 2) Institute of life science, Soka Univ., Tokyo

P-083
Genome-wide screening for genes related to oxidative stress in Drosophila melanogaster
p=Toru Togawa, Taro Kaneuchi, Takashi Matsuo, Toshiro Aigaki
Dept. Biol. Sci., Tokyo Metropolitan Univ., Tokyo

P-084
Rap2l regulates gonadal morphogenesis in Drosophila
p=Naomasa Miyata, Takashi Matsuo, Toshiro Aigaki
Dept. Biol. Sci., Tokyo Metropolitan Univ.

P-085
Effort at constructing the artificial restriction-modification genetic system with selfish behavior in Drosophila
p=Qiang Xia-1, Tamas Lukacsovich-1, Yukiko Hirose-1, Ken Matsumoto-1, Minoru Tateno-1, Naoto Juni-2, Daisuke Yamamoto-1
1) Waseda University, School of Science and Engineering, Tokyo, Japan:2)KYOTO Inst. of Tech. Univ., DGRC:Un

P-086

Quick and versatile method of ectopic gene expression in the Drosophila embryo

p=Takahiro Shinjo, Takashi Hamaguchi, Ryutaro Murakami
Department of Physics, Biology, and informatics, Yamaguchi University,

P-087

An attempt to culture of male germline cells of Drosophila

p=Kodama, T.-1, Niki, Y.-1, Yamamoto, M.-2 and Mahowald, A. P.-3
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P-088

Exploring possible roles of a putative fruitless suppressor in Drosophila embryogenesis

p=Seigo SHIMA-1, Shunzo KONDO-2, Kazue USUI-AOKI-1, Toshiro AIGAKI-3, Naoto JUNI-4, Daisuke YAMAMOTO-1
1) Sch. of Sci. and Engin., Waseda univ.: 2) Mitsubishi kasei inst. Life Sci.: 3) Tokyo Metro. Univ., dept. Biol.: 4) Kyoto Inst. of Tech. Univ., DGRC:

P-089

A revision of the Zygothrica samoaensis species group (Diptera, Drosophilidae), with descriptions of seven new species.

p=Stephane Prigent-1,-2, Masanori J. Toda-2
1) Dros. Genet. Res. Ctr., Kyoto Inst. Tech.: 2) Inst. Low Temp. Sci., Hokkaido Univ.

P-090

Activity-dependent mechanisms for synaptic growth matched with postsynaptic muscle volume in Drosophila neuromuscular junctions

p=Hiroaki Nakayama, Takako Morimoto-Tanifuji, Akinao Nose.
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P-091

Analysis of Notch-mediated pattern formation in the Drosophila hindgut by Genome Object Net combined with genetic experiments

p=Naoyuki Yamasaki-1, Rie Yamane-1, Junko Umesaki-1, Haruka Yoshimori-1, Hiroshi Matsuno-1, Satoru Miyano-2, Ryutaro Murakami-1
1)Department of Physics, Biology, and Informatics, Yamaguchi University:2)Human Genome Center, Institute of Medical Science, University of Tokyo

P-092

Cellular and molecular mechanisms to achieve diversity of dendritic patterns

p=Kaoru Sugimura-1, Daisuke Satoh-1, Misato Yamamoto-1 , Satoshi Goto-2,3, Misako Taniguchi-2, Shigeo Hayashi-2,4, Stephen Crews-5 and Tadashi Uemura-1,6
1) The Institute for Virus Research, Kyoto University: 2) National Institute of Genetics: 3) Mitsubishi Kagaku Institute of Life Sciences: 4) RIKEN, CDB: 5)The University of North Carolina at Chapel Hill: 6) CREST, JST

P-093

neurotic, a novel maternal neurogenic gene, encodes an O-fucosyltransferase that is essential for Notch-Delta interactions

p=Takeshi Sasamura-1 Nobuo Sasaki-1, Fumiyasu Miyashita-1, Shiho Nakao-1, Hiroyuki O. Ishikawa-1, Mikiko Ito-2, Motoo Kitagawa-3, Kenichi Harigaya-3, Eric Spana-4, David Bilder-4, Norbert Perrimon-4, Kenji Matsuno-1.
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P-094

The regulatory role of Rab5 for vesicle-vesicle fusion of secretory granules and synaptic vesicles in the exocytotic processes

p=Hideyuki Shimizu-1, Satoru Kawamura-2, and Koichi Ozaki-2
1) Department of Biology, Graduate School of Science, Osaka University: 2) Graduate School of Frontier Biosciences, Osaka University

P-095

dtb encoding a Drosophila T-box protein is required for CNS and motor neuron guidance

p=Qing-Xin Liu Masaki Hiramoto Hitoshi Ueda Yasushi Hiromi Susumu Hirose
Department of Developmental Genetics, National Institute of Genetics and Department of Genetics, Graduate University for Advanced Studies, Mishima, Shizuoka-ken 411-8540, Japan

P-096

Scad67 is a SP-RING protein and participates in the SUMO conjugation

p=Makoto Nakamura
National Institute for Basic Biology, Okazaki

P-097

Preventing “roundabout” trajectory of longitudinal axons. : ROBO silences the responsiveness to the segmental boundary cue, Netrin presented by Frazzled.

p=Masaki Hiramoto-1,3 Yasushi Hiromi-2,3
1)PRESTO:2)CREST 3)NIG

P-098

Attenuation of sensitivity to trehalose by the expression of the IP3 absorbent IP3 sponge in a subset of gustatory receptor cells in Drosophila

p=Kazue Usui-Aoki-1, Ken Matsumoto-1, Sou Kohatsu-2, Hiroshi Matsubayashi-3, Kunio Isono-2, Masatoshi Yamamoto-3, Katsuhiko Mikoshiba-4, Daisuke Yamamoto-1,5.
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P-099

Genetic screening for mutations that affect the targeted axonal projection of the olfactory sensory neurons in Drosophila

p=Keita Endo-1, Yuka Yoda-1, Ken-ichi Kimura-2, Chihiro Hama-1
1) CDB, RIKEN, Kobe: 2) Hokkaido Univ. of Education, Iwamizawa

P-100

Drosophila Damaged DNA Binding Protein 1 (D-DDB1) is an Essential Factor for Development

p=Kei-ichi Takata1, Hideki Yoshida2, Masamitsu Yamaguchi3, Fumiko Hirose4, Kaori Shimanouchi1, Shizuka Murakami1, Gen Ishikawa1 and Kengo Sakaguchi1
1) Dept. Applied Biol. Science, Tokyo University of Science, 2) Venture Lab., Kyoto Institute of Technology, 3) Dept. Applied Biol., Kyoto Institute of Technology, 4) Div. Biochem., Aichi Cancer Center Research Institute

P-101

Overexpressing TRX genes enhance oxidative stress resistance in Drosophila

p=Y. UMEDA-1, 2, C. Ohkura-2 , S. Nakayama-2 , Y. Namba-1, Y. Ouchi-1 , T. Aigaki-2
1) Dept. Geriatric Medicine, Univ. Tokyo, Tokyo: 2) Dept. Biol., Tokyo Metropolitan Univ. Tokyo

P-102

Analysis of a histone methyltransferase dG9a in Drosophila

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1) Div. of Biotech., Kyoto Inst. Tech., Kyoto: 2) Venture Lab., Kyoto Inst. Tech., Kyoto : 3) Inst. For Virus Res., Kyoto Univ., Kyoto

P-001

The comparison of intrinsic rates of increase among chromosome-substituted lines resistant and susceptible to organophosphate insecticides in Drosophila melanogaster

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To investigate the genetic basis of the seasonal fluctuations in resistance to three organophosphates, observed within the Katsunuma population of Drosophila melanogaster, we compared the intrinsic rate of increase, generation time and net reproduction rate, among chromosome substitution lines between a resistant and a susceptible line, obtained from this natural population. There was significant variation among substitution lines, and lines possessing the third chromosome from the resistant line, which confers resistance to the three organophosphates, generally showed lower mean values of these fitness measures. In fact, chromosomal analyses indicated significant negative contributions of the third chromosome from the resistant line. However, significant positive contributions of the interactions among chromosomes from the resistant line to these fitness measures were also detected. We further conducted a local stability analysis, in which each chromosome substitution line was assumed to be introduced at a low frequency into the initial susceptible population. It was demonstrated that the resistance factor(s) on the third chromosome tend to decrease in their frequency under both density-independent and juvenile density regulated conditions. Based on these results, possible explanation for the seasonal fluctuations in resistance to the three organophosphates observed in the Katsunuma population was proposed.

P-002

Functional identification of water gustatory receptor neurons and their projection pattern in Drosophila.

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1)Department of Biology, Graduate School of Sciences, Kyushu University, Ropponmatsu, Fukuoka: 2)INRA Station de Phytopharmacie et Mediateurs Chimiques, France.

In Drosophila, gustatory receptor neurons are located at the base of taste sensilla on the labellum, tarsi and wing margins. There are four gustatory receptor neurons in a typical sensillum, each of which responds to one of three modalities; sugar, salt or water. Water gustatory receptor neurons are specialized gustatory receptor neurons in insects that are thought to have evolved as a result of the need to detect water. While recent identification of putative gustatory receptor genes in Drosophila might help to reveal the neural mechanism of the sensory coding of taste, it is first necessary to identify each gustatory receptor neuron at a cellular level. We used a Gal4 enhancer trap strain in which GAL4 is expressed in a single gustatory receptor neuron in each sensillum on the labellum. We investigated the function of these neurons by expressing shibirets1 and a mutant form of the Shaker potassium channel. Results obtained by proboscis extension reflex tests and electrophysiological recordings suggest that the GAL4-expressing neurons respond to water. We show that the water receptor neurons project to a specific region in the subesophageal ganglion, thus reveal the taste sensory map for identified gustatory receptor neurons in Drosophila.

P-003

A Screen for cell adhesion molecules involved in proximodistal axis formation in the Drosophila leg

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It has been well appreciated that cell adhesion molecules play important roles in tissue segregation. Little is known, however, about the identity of position-specific cell adhesion molecules. In Drosophila, Proximodistal (PD) patterning in the leg is elaborated from the circular domains of the leg disc. We expect to reveal a mechanism that governs cell behavior during PD axis formation by searching for new cell adhesion molecules. We screened for cell adhesion molecules using Gene Search System (GS). As a first step, ORFs in the Drosophila genome that have N-terminal transmembrane domain were picked up from the Drosophila genome database as candidates. Approximately 900 GS insertions were found near these genes, and were misexpressed in the leg under the control of Dll-GAL4. capricious (caps) was identified in this screen as a gene causing cell affinity defects. caps encodes a putative transmembrane protein containing leucine-rich repeats. Previous studies suggest that CAPS contributes to axon guidance, however, the molecular function of CAPS protein is still unknown. Here we report that CAPS plays a key role in specifying cell affinity in imaginal discs. We also report our progress in revealing a structure-function relationship of CAPS protein by isolating EMS-induced mutations.

P-004

Divergent sensitivity for sugars, salts and bitter compounds in labellar chemosensilla of Drosophila

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Recent studies indicated that the Gr genes encoding putative taste receptors in Drosophila are expressed in a spatially restricted pattern among chemosensilla on the labellum. Labellar sensilla are classified into the three types, L, S and I, according to their length and location. A L- and S-type sensillum houses four gustatory receptor neurons (GRNs), while an I-type houses only two GRNs. Response profiles of the I- and S-type have been unknown. We demonstrated that each type of labellar chemosensilla exhibits divergent taste sensitivity in response to sugars, salts and bitter-tasting compounds. L-type sensilla give good response to sugars among the three types. Two GRNs in the I-type sensilla showed novel response properties: one of the two GRN responds to both sugars and salt at low concentration, and the other responds to bitter compounds and salts at high concentration. It seems that phagostimulatory compounds are detected by one GRN type and deterrent compounds by the other in the I-type. These two novel GRNs provide a unique model to elucidate how taste receptors are differentially expressed and how the signals from two different taste modalities are integrated into a common transduction pathway.

P-005

How to localize regulators of planar cell polarity: what drives the biased sorting of Fz::GFP particles?

p=Yuko Shimada-1,2 David I. Strutt-3, Tadao Usui-2, Tadashi Uemura-1,2,4
1) Graduate School of Biostudies, Kyoto Univ.: 2) The Institute for Virus Research, Kyoto Univ.: 3) Biomedical Science, University of Sheffield: 4) CREST, JST

Each wing cell localizes actin bundles to its distalmost vertex and produces a single prehair. This planar polarization most likely requires redistribution of Frizzled (Fz), Flamingo (Fmi) or Dishevelled (Dsh), preferentially to proximal and/or distal (P/D) cell boundaries. How are these asymmetric patterns generated? In cells that express GFP-tagged Fz (Fz::GFP), intracellular Fz::GFP particles were most frequently found for several hours prior to initiation of prehairs. Those particles were hardly found in dsh[1] or fmi[RNAi] cells. Our time-lapse recording analysis suggested that sorting of Fz::GFP particles was biased towards distal cell boundaries, and that was not the case with Fz[P278L]::YFP or DE-cadherin::GFP, which were uniformly localized to all cell boundaries. These results suggest that the movement of Fz::GFP particles is relevant to making the asymmetrical pattern of Fz. To explore what drives this biased sorting, we applied several drugs to pupa. The distribution of Fz::GFP or Fmi at P/D boundaries was severely disturbed by Latrunculin-B (actin polymerization inhibitor), but not vinblastine (microtubule distabilizing drug). We also examined the effects of Breferdin A, which inhibits some of Arf GEFs, or of BDM (myosin inhibitor). From these data, we discuss how Fz::GFP is targeted to distal boundaries of wing cells.

P-006

Distinct functions of Rac1 and Cdc42 during axon guidance and growth cone morphogenesis in Drosophila

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Rho family small GTPases are thought to be key molecules in the regulation of cytoskeletal organization, especially for actin filaments. In order to examine the functional differences between Rac1 and Cdc42 in the morphogenesis of growth cones in Drosophila, we used primary embryonic cultures to observe neurite formation closely. We showed in vitro that activation of Rac1 and Cdc42 has distinct effects on neurite formation, particularly on growth cone morphology and the actin filaments within. We also showed in vivo that the phenotypes of Cdc42 activation and Rac1 inactivation in the CNS are similar to that of roundabout (robo) mutant, in that many extra axons cross the midline. We found that Rac1 inactivation is almost completely dominant over Robo receptor activation, suggesting that Rac1 activity is necessary for Robo signaling activation. Our observations indicate that Rac1 and Cdc42 have distinct functions in downstream events triggered by Slit-Robo signaling.

P-007

Ggmma1 subunit of heterotrimeric G proteins is required for neuroblast asymmetric division

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Drosophila neuroblasts (NBs) undergo typical asymmetric divisions into a larger neuroblast and a smaller ganglion mother cell, to which neural cell fate determinants exclusively segregate. To understand the mechanisms for the asymmetric division of NB, we have conducted a mutational screen for the 2nd chromosome, using Miranda as a maker. By screening 4,404 EMS-induced lethal mutations, we identified novel loci as well as known ones, which affect NB asymmetry. We are currently focusing on a mutant line N159 among them. In N159 germline clone embryos, all known apical components localize abnormally in mitotic neuroblasts, although Miranda localizes as the crescent. Interestingly, a majority of the mutant NBs divide into nearly equal-sized daughters. These phenotypes are essentially the same as those shown for the loss of function mutant of Gbeta13F, an effecter subunit of heterotrimeric G proteins. It turned out that N159 is a mutation of the Ggamma1 gene. In the wild-type NBs, Gbeta13F mainly distributes throughout the cell cortex. In contrast, it accumulates at the cytoplasm in N159. These results indicate that Ggamma1 is required for recruiting the Gbeta13F to the NB cortex suggesting that cortical Gbeta/gamma signaling is essential for the creation of cell size difference.

P-008

Molecular mechanism for the dynamic switching of axonal transport in the developing Drosophila brain

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The Drosophila HIG (Hikaru genki) protein is synthesized in neuronal cell bodies, transported through axons, and finally secreted to synaptic clefts. Intriguingly, HIG is transported to synapses at mid-pupal stage, when neurites begin to contact each other, but stays in the cell bodies at late-pupal stage for the maturation of synapses, and is eventually transported to synapses again at adult stage. This dynamic switching of axonal transport may be a novel mechanism controlling neural development. We performed a domain analysis of HIG to reveal its molecular basis. We made a series of transgenic flies expressing deletion proteins of HIG and examined their localization. We also examined their rescuing ability of the hig mutant phenotype (sluggishness). The results revealed that a domain was responsible for the mid-pupal facilitation of transport, whereas another domain was responsible for late-pupal retention to cell bodies, suggesting that localization of HIG is regulated by a different way in each stage. The results also showed that the HIG protein consisting of N-terminally half portion and lacking the domains controlling localization still possess the rescuing ability, implying that the function and regulatory signal of localization are segregated in the HIG molecule.

P-009

Expression of Wnt and Frizzled family genes in the developing Drosophila optic lobe

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During the development of the adult Drosophila visual system, individual photoreceptor axons in each ommatidium project towards the specific targets in the optic lobe of the brain. The highly ordered guidance of photoreceptor axons is thought to require the guidance cues in the optic lobe, and their reception and interpretation by the photoreceptor axons. The guidance cues must be precisely distributed in the developing optic lobe under the control of genes that pattern the optic lobe field. We are currently screening genes that are specifically expressed in the developing optic lobe in a hope to understand what the guidance cues are and how their expression is regulated. As a pilot screening, we investigated gene expression of widely conserved gene families. Members of the Wnt gene family encode secreted proteins that signal through the Frizzled family of receptors to function in a wide variety of developmental processes. The Drosophila genome project revealed the presence of seven Wnt and four Frizzled genes. Interestingly, two Wnt and two Frizzled genes showed specific expression patterns in a part of the optic lobe. We will discuss their possible roles in the photoreceptor axon projection and in the optic lobe development.

P-010

B type lamin plays crucial roles during late development in Drosophila.

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The nuclear envelope creates distinct nuclear and cytoplasmic compartment in eulkaryotic cell. It consists of two concentric lipid membranes, nuclear lamina, and nuclear pore complexes. The nuclear lamina lines the nucleoplasmic surface of the inner nuclear membranes and is directly concerned with proper cell cycle progression and nuclear organization. To understand molecular functions of the nuclear lamina, we analyzed Drosophila B type lamin (lamin Dmo derivatives) with molecular genetics method, since B type lamin was a major structure protein of nuclear lamina. We established independent 2 deletion mutant lines for lamin Dmo by P-element mediated excision. These showed almost same abnormal phenotypes, and lethality appeared at late pupal stage (just before emergence). In one mutant,deltaDm14-5 animal, the 2nd exon of lamin Dmo which contained initiation codon was completely deleted, and protein was not detected by means of western blotting with several lamin Dmo specific antibodies. Thus deltaDm14-5 is B-type lamin null mutant. At the lethal point, the morphological observation exhibited that the exterior body structures of lamin Dmo mutant were almost normal, but internal structures were different from those of wild type. To define reasons of null mutation, we are further analyzing deltaDm14-5 animal.

P-011

Antagonistic interaction between Notch and EGFR signaling establishes the joint of the Drosophila leg

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Drosophila legs have 9 segments separated by flexible joints at precise positions along the proximodistal axis. Notch signaling plays fundamental roles in the segmentation and growth of the leg. big brain (bib) is a target gene of Notch signaling pathway, and the expression of a GAL4 enhancer-trap line, bib[NP4281], is observed in the presumptive joint structure at late pupal stage. We show that a homeodomain protein, Defective proventriculus (Dve), is transiently expressed in the bib[NP4281]-expressing region. Forced expression of dve or the inhibition of Notch signaling in the bib[NP4281]-expressing region caused a jointless phenotype, suggesting that a Notch-mediated dve repression in the bib[NP4281]-expressing region is crucial for joint formation. A distal-to-proximal gradient of Epidermal Growth Factor Receptor (EGFR) signaling is required for the distal leg patterning. Interestingly, the elevated levels of EGFR signaling in the bib[NP4281]-expressing region also caused a jointless phenotype as observed for Notch inhibition. These results suggest that antagonistic interaction between Notch and EGFR signaling establishes the precise positions of leg joints.

P-012

Brain drain (Bdr) is a novel regulatory factor of EGFR signaling pathway

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Egfr/Ras/MAPK signaling pathway plays central roles in various developmental processes in Drosophila. The signaling activity is under control of complex network including positive- and negative- regulators. A P-element insertion mutant l(2)k15512 shows developmental defects in germ band retraction and dorsal closure during embryogenesis, similar to those observed in mutants of EGFR signaling components. Molecular analysis of the mutant revealed that l(2)k15512 has a P-element insertion in 5' UTR of MESR4 gene, which was previously identified as a misexpression suppressor of Ras signal. MESR4 gene encodes a protein containing nine C2H2 type zinc fingers and a PHD zinc finger motif. We named it brain drain (bdr) for its embryonic phenotype. The bdr mutant embryo had reduced levels of phosphorylated MAPK and FasIII, one of the target of Egfr signaling pathway. Reduction of bdr function resulted in morphological phenotypes, such as extra crossvein and rough eye, resembling those of Egfr gain-of- function mutants. Genetic interaction studies with various components of Egfr signaling pathway suggested that Bdr functions as a negative regulator of the EGFR/Ras/MAPK signaling pathway.

P-013

Gain-of-function screening for circadian rhythm mutants using a gene search system

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Circadian rhythms of physiology and behavior are generated by endogenous oscillators. This time-keeping is maintained by clock genes in Drosophila. Because the classical loss-of-function screening might fail to identify all clock-related genes, we began a gain-of-function screening using a gene search (GS) system, in which forced gene expression is caused by GAL4 driver. An armadillo-GAL4 driver induces ubiquitous gene expression, and a timeless-GAL4 driver induces pacemaker-cell-specific overexpression. After crossing GS strains with these GAL4 drivers, the progenies were maintained at 25 degree and 12L-12D photoperiod, then adult flies were transferred into constant darkness for recording the free running behavior. Screening of 3,000 GS strains for effects on locomotor activity rhythms yielded several abnormal phenotypes including arrhythmic, long period rhythms. We could also detect known clock-related genes, fmr1 etc. Here, we report the results of gain-of-function screening and discuss the efficiency of this method to identify clock genes.

P-014

Comprehensive identification of neural circuits for olfactory processing of the Drosophila brain

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The antennal lobe (AL) of Drosophila is the insect equivalent of mammalian olfactory bulb. It has about 50 glomeruli, each of which is likely to receive information from a distinct type of olfactory sensory neurons. To understand the mechanisms underlying olfactory processing, it is important to reveal the connectivity between the AL and other brain areas. By genetical approaches, we visualized neural circuits for olfactory processing. In addition to the three antenno-cerebral tracts already known, we identified ten novel pathways that connect the AL with various regions. The neurons of each pathway terminate at characteristic zone within the secondary olfactory centers. These zones spatially correlate with the projection patterns of recipient neurons. Such zonal specialization is established during development and maintained even after deprivation of olfactory input.

P-015

Functional analysis of eyeless and homothorax genes in antennal lobe development in Drosophila melanogaster

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The antennal lobes (AL) are the primary association centers for odor recognition in the Drosophila brain. Different odors sensed by the olfactory receptor neurons are represented in the topological map of the AL glomeruli, which information is further decoded to higher brain centers. In order to understand the cellular and genetic possesses that control the development of the ALs, we have searched for neuronal markers and transcription factors expressed in the developing ALs. Here, we show that, a Drosophila Pax-6 homolog, eyeless, is expressed in ventral local-inter neurons whereas a homeobox gene, homothorax, is expressed in projection-neurons. Developmental studies have demonstrated that both genes are expressed in the AL neurons of the larval and adult stages, suggesting functional importances of the two genes in AL development through metamorphosis. Currently, we are investigating developmental consequences of loss-of- and gain-of-function mutations of the two genes in the formation of the AL neurons.

P-016

Ubiquitin ligase Dnedd4 regulates Notch signal transduction pathway

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Dnedd4 is a HECT class ubiquitin ligase which is widely conserved in invertebrates and vertebrates. It is implicated in intracellular trafficking of some membrane proteins but the full spectrum of its functions is not yet known. We performed a gain of function screen (Gene Search screening, Toba et al. 1999) for genes that alter Drosophila limb patterns and identified Dnedd4 as a modifier of Notch signaling pathway. We isolated Dnedd4 mutations and showed that they suppress the phenotype of Notch loss of function mutation. EMS induced point mutations clustered within HECT domain, suggesting an essential role of ubiquitin ligation in Nedd4 function. Immunohistlogical analysis showed that Dnedd4 is expressed ubiquitously in imaginal discs, and that it localizes to basal region of imaginal disc epithelium. Over-expression of Dnedd4 changed intracellular localization of Notch protein. These data indicate that Dnedd4 negatively regulate Notch signaling pathway by regulating intracellular trafficking of Notch protein.

P-017

Developmental Stage-dependent Effects of Postsynaptic Activation of CaMKII on Synapse Formation at Drosophila Neuromuscular Junctions

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We have been studying the role of postsynaptic CaMKII during development of synapses at Drosophila neuromuscular junctions by using the system which consists of two muscles innervated by the same neurons. Activity of CaMKII can be manipulated in one of the two muscles or both muscles by GAL4-UAS expression system. We have reported that postsynaptic activation of CaMKII promotes coordinated maturations of pre- and postsynaptic sites of the larvae just after hatching (0.5 hr larvae) in a synapse-specific manner, which was revealed by using electrophysiological and morphological techniques. Here we found that its promotive effects were reduced in the larvae 7 hr after hatching (7 hr larvae). Morphological studies showed that area of glutamate receptor (GluRIIA) was increased in 0.5 hr larvae and then reduced in 7hr larvae by postsynaptic activation of CaMKII, suggesting the localization of GluRIIA is possibly regulated by CaMKII. Interestingly, we also found that synaptic activity on the muscle cell in which CaMKII activity was not manipulated were affected in 7hr larvae. These results suggest that the action of CaMKII changes during synaptic development and the propagation of synaptic modification from one synapse to the other appears only after a certain developmental stage of synapses.

P-018

Interaction between male-killing spiroplasma and Drosophila immune system

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Drosophila has an effective innate immune system that can clear infections with bacteria and other microorganisms. Despite this ability, one group of endosymbiotic bacteria, the spiroplasmas, which is known to cause male killing in Drosophila species, can survive unharmed within the hemolymph of the hosts. We investigated the interaction between the spiroplasma and the immune system of its Drosophila host. Expression of antimicrobial genes in spiroplasma-infected flies did not differ from wild-type controls either in the naturally infected state, or after septic shock. We therefore conclude that spiroplasma infection neither induce nor suppress an immune response in its host. Further experiments revealed immune reactions induced ectopically did reduce parasite titer. These results suggest that this bacterium can be hidden from the host immune system, but is potentially suppressible by it. A variant of male-killing spiroplasma does not kill males and its titer within hemolymph is significantly less than that of male-killing ones. However, when the non-male-killing spiroplasma infected to the loss-of-function mutants of immune system, its titer increased to the same extent as male-killing ones. This result suggests that the non-male-killing spiroplasma lose the ability to be hidden from the host immunity.

P-019

Drosophila heterotrimeric G proteins regulate daughter cell size in neuroblast asymmetric divisions

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During development, cells often divide into daughter cells of distinct cell fates and cell sizes. Drosophila neuroblasts undergo such divisions and repeatedly produce a smaller ganglion mother cell (GMC) and a larger neuroblast. The cleavage site of cells generally depends on the configuration of mitotic microtubules. During neuroblast divisions, the basal spindle half becomes smaller than the apical half, which results in the production of a smaller basal GMC. Our genetic screen identified a mutant for the Gbeta13F gene, which encodes a beta subunit of heterotrimeric G proteins. The mutant neuroblasts divide into nearly equal-sized daughters, while the cell fate determinants are segregated into one daughter. In contrast to the wild type, the mutant neuroblasts maintain large symmetric spindles throughout mitosis, indicating that Gbeta13F is necessary for reducing the size of the basal spindle half. Over-expression experiments in embryos and cultured cells suggest that the Gbeta signaling indeed prevents microtubule development. We propose that heterotrimeric G proteins regulate asymmetry of microtubule structure to achieve unequal division of neuroblasts. Furthermore, the multiple equal cleavages of Gbeta13F mutant neuroblasts accompany neural defects, suggesting indispensable roles of unequal division in assuring the stem cell properties of neuroblasts.

P-020

How did the number of the MOL diverge in evolution?

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The number of sexually dimorphic abdominal muscle, the muscle of Lawrence (MOL), diverged recently in the D. melanogaster species group. We challenge to explain how the diversity developed. There are three types of species; i.e., those possessing no MOL (ex. teissieri), a pair of MOL (melanogaster) and two pairs of MOL (subobscura). The MOL development is controlled by the Abd-B and fru genes in melanogaster. Abd-B[Mcp1]/+, a dominant mutant of Abd-B, has duplicated MOL as in the case of wild-type subobscura. We assume that Abd-B positively regulates fruitless expression in the CNS, leading to the formation of MOL in the A5 segment. It is inferred that, in Abd-B[Mcp1]/+ and subobscura, fru expression in A4 has exceeded the level required for the MOL induction, resulting in the formation of extra MOLs. On the other hand, teissieri, a species without MOL, has a fru expression pattern in the CNS very similar to that of melanogaster. We postulate that some downstream elements required for the MOL formation have become refractory in this species during evolution. To evaluate these hypotheses, we are currently conducting experiments to identify the Fru-target genes and to examine whether Abd-B expression can modulate fru expression levels.

P-021

Specificity of olfactory receptor neurons responding to sex pheromones in a moth, Ostrinia furnacalis

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We have recorded responses to female sex pheromone components, (E)- and (Z)-12-tetradecenyl acetates (E and Z12-14:Ac) and behavioral antagonists (Z9-14:Ac, E and Z11-14:Ac) in single receptor neurons of male Ostrinia furnacalis (Lepidoptera: Crambidae) using a cut-sensillum technique. According to the electrophysiological survey and electron microscopic observations the sensilla houses 1-3 neurons. Dose-response and cross-adaptation studies showed that type 1 neurons responded equally well to the two pheromone components. Neurons (type 2-4) responding selectively to one pheromone component and neurons (type 5-7) responding to behavioral antagonists were also observed. Co-localization between neurons responding to pheromone and antagonist compounds was found in some sensilla. O. furnacalis has a unique coding system for two major pheromone components built in neurons responding to both the two components. O. nubilalis, a close species of O. furnacalis has two types of neurons responding to each pheromone component specifically.

P-022

Axon branch pruning of Drosophila mushroom bodies during metamorphosis

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Remodeling of neural circuits is a general phenomenon in the development of nervous systems. One of the prominent features of neural remodeling is axon pruning. In order to understand the cellular and molecular mechanisms regulating the axon pruning, we analyzed the larval mushroom bodies (MB) of Drosophila, which remodels their axon branches during metamorphosis with defined time course. Mosaic analysis of single neurons demonstrates that the pruning of larval axon branches is carried out by degeneration rather than retraction and degradation of synaptic boutons on the axon branches is a first step of the degeneration. Analysis of a mass of larval axon branches shows that clusters of the synaptic boutons are degraded successively in early pupal stages. Genetic studies shows that the degradation of synaptic boutons is regulated by reception of ecdysteroid through EcR-B/USP within the MB neurons. Furthermore, we found that selective inhibition of cellular function of glial cells also affects the degradation of synaptic boutons. These findings strongly suggest that not only cell-autonomous but also non-cell-autonomous mechanisms are required for the axon branch pruning of MB.

P-023

Dual Function of Src Proto-oncogene in Epitherial Morphogenesis in Drosophila.

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Cell migration and cell determination is tightly coordinated during establishment of epithelial organs. The Drosophila tracheal morphogenesis is a coordinated process of cell migration and cell determination and provides an ideal system to study the processes of organogenesis. In this work, we identified src42A as a candidate for a gene that controls both cell migration and cell determination during tracheal formation. We found that Src activity is increased transiently in early stage of tracheal development at apical cell-cell junction sites. Reduced or hyperactivation of Src activity changed localization of E-cadherin from adherens junction. Using DE-cadherin-GFP fusion protein to label newly synthesized protein, we showed that Src42A activity regulates DE-cadherin turnover. In addition, hyperactivation of Src42A activity caused increase the number of Esg expressiong cells which is usually limited to the tip of migrating cells. This phenotype is similar to the embryos with an increased level of Wingless signaling. Furthermore hyperactivation of Src42A caused increase of Arm, a key transducer of Wg signaling. Those results suggest a possibility that Src regulates Wg signaling by stabilizing Arm. From these analyses, we concluded that Src42A coordinates cell adhesion and cell determination by regulating E-cadherin mediated cell attachment and Wg signaling mediated cell differentiation.

P-024

Btk29A is required for normal development of male and female reproductive organs in Drosophila

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fickle[P] (fic[P]) is a mutant in the Drosophila Btk(Bruton's tyrosine kinase)29A gene. Deficits in human Btk are responsible for X chromosome-linked agammaglobulinemia (XLA), where B cell maturation is blocked. Sab has been identified as a negative regulator of Btk activity in mammals. prt is a Drosophila homologue of Sab, although its interaction with the Drosophila counterpart of Btk has not been demonstrated. In fic[P] mutant females, oogenesis is arrested in an early stage, accompanied by a failure in the development of germline components. In fic[P] males, the single apodeme holding penis is split into two halves. These two fic[P] phenotypes in two sexes are both alleviated by a reduction in the prt gene dosage. In Btk29A- null germ line clones, the size of ring canals that connect nurse cells with oocytes is reduced. Interestingly, the ring canals appear to overgrow in prt mutants. Overexpression of wild-type or the E41K mutant of human Btk partially restores the fic[P] male genital phenotype. These observations collectively suggest that Drosophila Btk29A shares certain aspects of regulatory mechanisms with human Btk, and thus Drosophila may serve as a useful in vivo model for the study of human XLA.

P-025

Homology of polytene chromosomal banding patterns of X chromosome among three species, D. ananassae, D. vallismai, and D. melanogaster

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Establishment of definitive chromosomal homologies throughout the genus Drosophila would be desirable for evolutionary considerations and for extending the knowledge of molecular genetics accumulating D. melanogaster to other species. Homology at the level of the polytene chromosome banding pattern between non-sibling species is, however, still almost impossible to establish by different processes such as inversion, transposition and so on. Using the technique of in situ hybridization to polytene chromosomes, we started mapping genes in D. ananassae, its close relatives, D. vallismaia of D. ananassae sp. subgroup in D. melanogaster sp. group and D. melanogaster. As for the hybridization probes, we chose mainly DNA clones of D. melanogaster, which were already known chromosome sites and distribute throughout the X chromosome of D. melanogaster. By the alignment of homologues, we will report structural changes of gene arrangements among three species.

P-026

Drob-1, a Drosophila Bcl-2 family member, regulates pro- or anti-apoptotic signaling depending on specific death stimulus: Genetic analyses using drob-1 RNAi-mediated 'knock-down' flies

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The Bcl-2 family of proteins, which includes both anti-apoptotic and pro-apoptotic members, plays key regulating roles in programmed cell death. We have identified a Drosophila Bcl-2 family member, Drob-1, which is most similar to a mammalian pro-apoptotic protein, Bok, and promotes cell death by inducing both caspase-dependent and -independent pathways. In some circumstances, anti-apoptotic activity of Bax and Bak, which are generally considered to be mammalian pro-apoptotic proteins, as well as Drob-1 has been detected. However, it remains unclear how Drob-1 functions in the physiological or pathological conditions. To address the roles of Drob-1, we established RNA interference-mediated drob-1 'knock-down' flies (UAS-drob-1-IR transgenic flies). Here we show that developmental stage-specific expression of UAS-drob-1-IR caused semilethal phenotype and a decrease in embryonic cell death, suggesting that Drob-1 is required for normal developmental apoptosis. Furthermore, to elucidate the pathological roles in Drob-1, we used Drosophila models of neurodegenerative diseases, which are observed late-onset neurodegeneration by the developing eye-specific expression of human neurodegenerative disease-related proteins. We show remarkable enhancement of the neurodegeneration by expression of UAS-drob-1-IR and suppression of the disease pathology by overexpression of Drob-1, demonstrating that endogenous Drob-1 functions as a survival factor. These findings suggest that Drob-1 promotes or prevents cell death depending on the specific death stimulus and the mechanism might be evolutionary conserved in flies and mammals.

P-027

G-protein gamma subunit 1 is required for the gustatory response to sugars.

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Drosophila offers several advantages to explore the mechanisms of gustation at different levels of an organism. Taste substances are recognized by bipolar gustatory receptor neurons (GRNs) housed in chemosensilla on the labellum and tarsi. A novel family of candidate gustatory receptors has been identified in the Drosophila genome. But little is known about molecules involved in the gustatory transduction pathway. We have performed a differential screening to discover genes expressed in the GRNs. We compared the expression level of genes between the pox-neuro mutant which has no GRN and the wild type using high-density oligonucleotide probe arrays. One of the signal transduction molecules identified was the G gamma1 (Gg1) encoding a gamma-subunit of a heterotrimeric G-protein GTPase. RT-PCR analyses indicated that the Gg1 gene is expressed in gustatory organs. In an enhancer-trap strain, the P[Gal4] element was inserted upstream of the Gg1 gene. We observed the GFP signal in GRNs in labellum and tarsi of the Gg1-Gal4/UAS-GFP strain. Electrophysiological recordings from labellar chemosensilla of the strain showed that nerve responses to sugars were reduced, while responses to salt and water were not altered. These results suggest that the Gg1 gene is involved in the gustatory signal transduction for sugar perception.

P-028

Development of Drosophila central complex during metamorphosis.

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The central complex (CC) is a characteristic neuropil of the adult insect brain, and its gross structure is highly consistent among species. The CC is thought to be a higher-order brain neuropil controlling various behaviors. It consists of four substructures: the protocerebral bridge, the fan-shaped body, the ellipsoid body and noduli. They are composed of columnar small field elements and tangential large-field neurons forming strata perpendicular to the column. Although the neuronal architecture of adult CC has been described in great detail and the behavioral role of adult CC has been investigated, the development of CC has poorly been understood. Since the characteristic structure of CC is not recognizable in the larval brain, a large part of the adult CC would develop during pupal stages. In order to understand the blueprints of CC, we analyzed the gross morphology of CC throughout metamorphosis. We tested various labeling methods to identify the developing CC and found several antibodies and enhancer-trap lines that label various components of the CC from late third-instar larval stage to late pupal stage. As a result, we identified the precursor of CC in the larval brain and, its dynamic morphological change during morphogenesis is documented.

P-029

Possible involvement of the synaptic molecules for the formation of arousal consciousness in Drosophila.

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Precise definition and identification of substantial entity of 'consciousness' or 'mind' are the major unsolved problem of the current neuroscience. Recently, fruit flies were found to have a sleep-like state, and therefore flies are supposed to have the 'arousal consciousness', which is the most basic form of consciousness. To reveal its neural entity and molecular mechanism, we focused on the behavioral phenotypes of two mutants, hikaru genki (hig) and still life (sif), both of which are the mutants of the genes encoding synaptic proteins. Our study revealed that the phenotypes of these mutants show some resembling features with sleep. (1) They stand still without movement, and their posture resembles the sleeping flies. (2) Their locomotor activity becomes increased by the administration of caffeine. (3) Responsive threshold of these mutant flies for the stimulus is elevated compared with that of the wild-type flies. (4) The responsive threshold of the mutant flies comes down by the administration of caffeine. These data suggest the possibilities that these mutant flies are in the state of impaired consciousness and some neural circuits for the arousal consciousness are out of function. This study might lead us to the exploration of the consciousness of flies and our humans.

P-030

Age-related accumulation of deletions in the mitochondrial DNA of Drosophila

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Reactive oxygen species (ROS) is generated as by-products of the electron-transport chain in mitochondria. Cumulative damage by ROS is thought to result in a decrease in mitochondrial respiratory function and to contribute to the age-related decline in the physiological function of organisms. The mitochondrial genome is also subjected to damage with age through deletions. In the present study, we examined the accumulation of deleted mitochondrial DNA (mtDNA) in D. melanogaster at various ages from 1- to 65-day-old adults by PCR and Southern hybridization. When divided into three parts of the body (head, thorax, and abdomen), deleted mtDNA signals were detected more frequently in the thorax than the other parts, and the accumulation of deleted mtDNA was age-dependent. The signals were various in sizes, and no common signal from young to old was observed. More than ten different nucleotide sequences of the PCR products were determined thus far, and the breakpoints of the deletions were identified. In many cases, direct repeats were detected at the breakpoints of the deletions. These results suggest that deleted mtDNA accumulates with age in tissue-specific manner in Drosophila.

P-031

Identification of Drosophila genes involved in the determination of organ identity using a gene search system

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How organ identity is determined is one of the fundamental questions in developmental biology. We have shown that the context dependent transformation from eyes to other organs such as antenna, leg or wing is induced by the activation of Notch signaling in Drosophila eye imaginal discs. In this study, we performed a systematic gain-of-function screening using GS (Gene Search) lines to identify the genes that are capable of changing the disc-specific identity in the combination of Notch activation. Until now, we screened 9700 lines and obtained 32, 20 and 3 lines to induce eye-to-antennae, -leg and -wing transformation, respectively. In one of the lines (GS15923) that induced eye-to-wing transformation, the GS vector is inserted in upstream of an uncharacterized gene, CG31151. Surprisingly, the over-expression of CG31151 itself induced ectopic wings in Notch independent manner, suggesting that CG31151 is a key regulator gene to determine the wing identity. At present, we are generating a loss-of-function mutant of CG31151 to analyze the function of the gene on wing formation.

P-032

Visualization of unconstrained negative supercoils of DNA on polytene chromosomes of Drosophila melanogaster

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Starting from analyses of DNase l hypersensitivity, lines of evidence have been accumulated for changes in the chromatin structure, such as DNA methylation, chemical modifications of histones and chromatin remodeling during regulation of gene expression. However, besides transcription-driven supercoiling of DNA, our knowledge on conformation of chromatin DNA is elusive due to the lack of proper probes for analyses of DNA topology in vivo. Psoralen is a planar, aromatic compound that intercalates into DNA. Upon exposure to 365 nm light the intercalated psoralen mediates crosslinking of opposite DNA strands via formation of covalent bonds. It has been demonstrated that the rate of psoralen photocrosslink to double stranded DNA is linearly related to its level of negative superhelicity. We introduced biotinylated psoralen into Drosophila salivary glands and visualized it on polytene chromosomes with fluorescent streptavidin to make genome-wide survey for such domains. We observed bright psoralen signals on many transcriptionally active interbands and puffs. Upon heat shock, the signals appeared on heat shock puffs. The signals disappeared by prior nicking of DNA or inhibition of transcription with α-amanitin. These data demonstrate that transcription-coupled, unconstrained negative supercoils of DNA exist in approximately 150 loci within the interphase genome.

P-033

Comprehensive identification of the neuronal target recognition molecules in Drosophila neuromuscular system using GeneChip

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For the formation of neural networks, it is necessary for a neuron to recognize its precise target(s) to be innervated. Drosophila neuromuscular junction is an excellent model system for the study of neuronal target recognition. The axons of a single motoneuron precisely recognize and project to a specific subset of muscle cells. In this system, several target recognition molecules such as Capricious have been identified. While the ectopic expression of those molecules significantly alters target specificity, their loss-of-function mutants show only minor defects. These results indicate that multiple functionally overlapping molecules are involved in the target recognition. To understand molecular mechanisms of the neuronal target recognition, we aimed to identify comprehensively those molecules in such redundant system. We assumed that these molecules express differentially in each target muscle. Using GeneChip, we tried to identify genes expressed specifically in muscle 12 or 13, which is innervated by distinct motoneuron(s). We collected 200 cells for each muscle type with micropipettes, extracted total RNA, and performed GeneChip analysis. By the comparison of gene expression data from two muscles, we identified several molecules including cell adhesion molecules predicted to show differential expression pattern. We are performing in situ hybridization experiments for some candidate genes.

P-034

Isolation and Analysis of novel deltex alleles

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Local cell-cell interaction plays essential roles in the development of multicellular organisms. Notch signaling is involved in cell-cell communications that regulate cell fate determinations in many aspects, such as differentiation, apoptosis and morphogenesis. In Drosophila, a set of genes encoding components of Notch signaling has been identified. Among them, deltex encodes a cytoplasmic protein regulating Notch signaling in a positive manner. Resulting, we showed that Deltex played a important role in the regulation of endocytic trafficking of Notch. Although involvement of deltex in Notch signaling has been aware more than a decade ago, it has been difficult to evaluate the functional importance of deltex because any null mutant allele of deltex has not been available. In this study, we generated several new alleles of deltex. We isolate a presumptive null allele of deltex, which lacks a genomic region corresponding most of deltex coding region including the first methionine. Our phenotype analysis of this mutant revealed that deltex is not an essential component of the Notch signaling cascade but rather potentiates the signaling efficiency of these factors. We will also present the results suggesting that deltex functions in a highly tissue specific manner.

P-035

Glomerular organisation of larval mushroom body calyx of Drosophila

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The first processing of olfactory sensory information by the Drosophila CNS occurs in the antennal lobes, which have a glomerular organisation of neuropil. Each glomerulus has convergent input from olfactory sensory neurons that express the same olfactory receptor, and in most cases is the sole location of the dendrites of each of the output neurons (projection neurons) that lead to the calyx of the mushroom body, and to the lateral horn. The mushroom body calyx of Drosophila and of other insects has a glomerular organisation similar to that of the antennal lobes but this appears to be much more complex and its relationship to both input and output of the Kenyon cells is unknown. Here we report a simpler glomerular organisation of the mushroom body calyx of third instar larvae of Drosophila. Here there are about 15 to 20 glomeruli which contain dendrites from progeny of each of the 4 mushroom body neuroblasts. Groups of Kenyon cells labelled by GAL4 enhancer trap insertions are innervated in all glomeruli. MARCM mosaic analysis revealed that individual Kenyon cells are innervated in about 4 different glomeruli. The functional organization of the calyx is discussed in relation to the input from the antennal lobes.

P-036

Drosophila larvae showed abnormal behavior when abd-A was mis-expressed in the nervous system

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HOM genes encode an evolutionarily conserved transcription factors that work for the specification of segmental identity along the antero-posterior body axis during development among diverse animals. Though they are expressed in the developing CNS, little is known about their role in the CNS. We are trying to define the role by analyzing the defects caused in the CNS when HOM genes are mis-expressed in Drosophila. We found that larvae showed an abnormal behavior when abd-A was mis-expressed with a GAL4 line which drove genes in mushroom bodies. Most of the 3rd instar larvae went down into the food along the test tube wall and died under the food. Small number of pupated ones did not eclosed to the adult. Other HOM genes had similar effect. Scr, Antp, Ubx, abd-A and Abd-B caused the same abnormal behavior in the larva and pupa. lab, Dfd, pb and Antp caused defects in adult behavior and posture: they only moved slowly and kept their wing open. To determine whether these behavioral defects were caused by the malformation of the mushroom bodies, we did HU ablation experiment. Even when most part of the mushroom bodies was lost, larvae showed the abnomal behavior.

P-037
Functional analysis of ik2 kinase in Drosophila tracheal tubulogenesis

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Branched tubular epithelial structures are found in most animals and function to transport gases and liquids in the body. The morphogenesis of three-dimensional branched structures involves specification of different cell types, cell migration and cell shape changes. The Drosophila embryonic tracheal system is an excellent system to analyze migration of individual cells and three-dimensional tubulogenesis. To investigate the genetic and molecular basis for the tubular network formation, we screened for genes that perturb tracheal morphogenesis when overexpressed in tracheal cells, and identified ik2 kinase, a member of IkB kinase family. ik2 was detected as a maternal factor and weakly and ubiquitously in late embryos. Overexpression of ik2 in tracheal cells disturbed organization of actin filaments and tracheal tubular formation. Moreover, overexpression of ik2 gene in S2 cells inhibited formation of pseudopodia and cell movement. These results imply that ik2 play a role in cell movement and tissue organization by regulation of actin cytoskeletal dynamics. We will present mutant analysis of ik2 in tracheal tubulogenesis and discuss the ik2 function in formation of the branched epithelial structure.

P-038
Dm RECQ5/QE DNA helicase and retrotransposon mdg3

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Werner's syndrome, Bloom's syndrome and a subset of Rothmund-Thomson syndrome are associated with the loss of function of the respective RecQ homologues (BLM, WRN and RTS). These RecQ homologues compose of C- and N-terminal region flanking a conserved helicase domain. Drosophila melanogaster RECQ5/QE is a homologue of RecQ and has a 3' to 5' DNA helicase activity. In contrast to BLM, WRN and RTS, RECQ5/QE possesses only short N-terminal region preceding the helicase domain and a unique C-terminal region. The function of RECQ5/QE is hitherto unknown. To address the function of RECQ5/QE, yeast two hybrid screening was performed using the C-terminal region as a bait. Drosophila melanogaster retrotransposon mdg3 nucleocapsid protein (mdg3NC) was obtained from early embryo cDNA library. In addition, GST pull down assay was performed using deletion series of RECQ5/QE. The mdg3NC bound whole RECQ5/QE, the C-terminal region and an acidic region within the C-terminal region. We showed that RECQ5/QE helicase displaced short RNA fragments from complementary DNA and that the strand displacement of RNA/DNA was stimulated by mdg3NC. These data suggest that Dm RECQ5/QE helicase interacts with mdg3NC through the acidic region, and that RecQ homologue might be involved in retrotransposition and genomic stability.

P-039
Molecular evolution and population genetics of the Drosophila PGRP-LE genes

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Peptideglycan recognition proteins (PGRPs) are a family of pattern recognition molecules and are required for activation of insect immune response. In Drosophila, they have at least 13 members of PGRP genes, which are highly diversified on their sequences. In order to infer the evolutionary pattern and the population genetic forces acting on Drosophila PGRP genes, we examined properties of polymorphism and divergence of the PGRP-LE gene in Drosophila melanogaster and D. simulans. Among 11 lines of D. melanogaster, nucleotide diversity was about 0.6% per synonymous sites and about 0.5% in the noncoding region. The level of nonsynonymous substitutions is estimated much lower than that of synonymous substitutions. Also, the level of variation is different between PGRP domain and non-PGRP. It is considered that a strong selective constraint is acting on the PGRP-LE, especially on the PGRP domain. On the other hand, phylogenetic analysis showed that Anopheles gambiae lacks the ortholog of Drosophila PGRP-LE. These results suggest that PGRP-LE was born after the divergence of fly and mosquito (about 250 Myr ago) and evolved in a conserved manner owing presumably to its functional importance of the Drosophila immune response.

P-040
Control of photoreceptor targeting by layer-specific expression of the LRR protein Capricious

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In many parts of the vertebrate and invertebrate central nervous system, inputs of distinct types confine their synapses to individual laminae. Such laminar specificity is an important but poorly understood determinant of neural connectivity in the CNS. During Drosophila visual system development, photoreceptors R7 and R8 connect to targets in distinct layers of the medulla, a ganglion of the optic lobe. We show here that CAPRICIOUS (Caps), a transmembrane protein with leucine-rich repeat motif, is required for R8 axons to select their appropriate targets in the medulla. During visual system development, Caps is expressed in R8 and its target layer but not in R7 and its recipient layer. In caps mutants, the regular spacing of R8 axons in the medulla is disrupted. Some R8 axons extend their axons to their neighboring R8 target area. Thus, Caps is required for R8 axons to select targets in the medulla. R7 axons ectopically expressing Caps terminate in the R8 recipient layer in which Caps protein localizes. When Caps is expressed throughout the medulla, termination sites of photoreceptor axons in the ganglion are altered. Together, these results suggest that Caps regulates layer-specific targeting in the visual system.

P-041

Analyses of a male-female-sterile mutant, mfs(3)GS7354: behavioral sterility in male and physiological sterility in female?

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mfs(3)GS7354 was isolated from a series of the Gene Search (GS) P element insertion lines for its sterility. A phenotypic observation indicated that the male sterility was primarily assigned to a deficit in mating behavior, whereas the female sterility appeared to be due to a defect in the reproductive system: the mutant males exhibited all the elements of courtship behavior including attempted copulation but were unable to copulate; the mutant females, on the other hand, copulated without problem and laid eggs but hardly had progeny. This suggests that the sterility of mfs(3)GS7354 is involved with a sexually bifunctional gene. To evaluate this notion, genetic analyses including linkage analysis with the GS insert, and complementation tests with deficiencies and other P inserts are now in progress.

P-042

EARLY STAGE OF WING DISC DEVELOPMENT IN DROSOPHILA: TIME LAPSE ANALYSES OF CELL MOVEMENT

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Drosophila limb development becomes evident during embryogenesis when a group of ectodermal cells start to express the homeobox gene Distal-less (DLL). The precursor cells of the wing discs derive from those DLL-expressing cells that migrate dorsally. However, the mechanisms that promote dorsal cell migration remain unknown. We observed cellular movement of the wing precursor cells in living embryos using time-lapse imaging, and found that 1) the spatial relationship between wing cells and surrounding ectodermal cells was conserved during germ band shortening (stage 11-12). This result indicates that wing cells are displaced along in ectodermal tissue movement, and that the separation of the limb primordium into the wing and leg cell is more passive that previously believed. 2) Before invagination, microtubules appeared to randomly oriente in each wing cell, but immediately after invagination they started to polarize and extend posteriorly. 3) Tracheal branches started to adhere to the presumptive wing disc cells before invagination. To test a possible involvement of wing-disc / trachea cells interaction in wing disc invagination, we are currently investigating the cellular movement and microtubule dynamics of wing primordial cells in mutant embryos lacking tracheae.

P-043
Possible roles of RecQ5 : Complementation study in yeast

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The DmRECQ5/QE is a member of RECQ5 subfamily and is expressed in early embryo. Although the DNA helicase activity of RECQ5/QE has been characterized in vitro, the in vivo function of RECQ5/QE was largely unknown. To investigate the role of RECQ5/QE in the cell, the potential of RECQ5/QE is evaluated by substitution of sole RecQ, Sgs1 in budding yeast. The RECQ5/QE complemented the several phenotypes of sgs1, including synthetic growth defect with srs2, methyl methanesulfonate and hydroxyurea sensitivities, elevated frequencies in homologous recombination and sister chromatid exchange, but poorly the suppression of slow growth in top3. These data suggested that RecQ function and the pathway involving RecQ was conserved between Drosophila and Saccharomyces. The possible roles of RECQ5/QE will be discussed.

P-044
Evolution of olfactory receptor gene family in Drosophila and other insects.

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Both in vertebrates and invertebrates, the initial steps in odor perception takes place at the olfactory receptor (OR) proteins localized in the olfactory sensory neurons. D. melanogaster has 61 OR genes and is capable of odor discrimination. We have previously determined the nucleotide sequences of several OR genes from D. melanogaster and its closely related species. Here, I will focus on gene duplication within two OR gene families (Or33a, Or33b, and Or33c genes and Or46a and Or46b genes). Together with the orthologous gene sequences obtained from D. pseudoobscura, mosquito and honey bee genomic data, I will discuss about the evolution of OR gene family in insects.

P-045

A genetic screen for mutations that affect the formation of the muscle of Lawrence in concert with fruitless.

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The male courtship behavior and the formation of the male-specific muscle, called the muscle of Lawrence (MOL) are controlled by the fruitless (fru) gene in Drosophila. fru acts downstream of transformer in the sex determination hierarchy. However little is known about other regulators and targets of the fru action. In an attempt at identifying such genes, we started a genetic screen for mutations that interact with the fru[sat] mutant, by examining their potential effects to induce malformation of the MOL, sterility, and/or lethality. We used the actin79B-GFP minigene as a convenient reporter to observe the MOL. The actin79B minigene is mainly expressed in the muscle of the adult legs and thorax, in addition to the dorsal abdominal muscles, especially the MOL. Actin79B-GFP allows us to observe the MOL without any tissue staining, because the dorsal abdominal musclature emits fluorescence. We screened a collection of the Bloomington deficiency Kit by placing each deficiency in trans to the chromosome carrying both actin79B-GFP and the fru[sat] mutation. Until now, we obtained several strains that showed the malformation of the MOL or the male-specific developmental retardation. It is possible that the genes included in these deficiencies are involved in fru-mediated signaling.

P-046

Musashi is required for asymmetric cell division of Drosophila sensory organ precursor cells, and possibly modified asymmetrically in non-neural precursor cell.

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A neural RNA-binding protein Drosophila Musashi is responsible for this asymmetric cell of the division of Drosophila extra sensory organ precursor (SOP) cells. The Musashi protein could inhibit translation of tramtrack69 by directly binding to its 3 -UTR only in IIb neuronal precursors, while tramtrack69 mRNA are expressed both in IIa and IIb (Nature vol 411: 94-98, 2001). Curiously, however, we found that Musashi protein was equally distributed between IIa and IIb cells after the division of the SOP cell, suggesting that the inhibitory action of Musashi on the translational regulation of tramtrack69 mRNA is selectively suppressed by some mechanisms in IIa cells. Here, we propose that atypical Protein Kinase C (aPKC) is involved in the suppression of Musashi s function in IIa cells. aPKC protein is asymmetrically localized in IIa precursor cell, not in IIb cells, and we found consensus phosphorylation sites by Protein Kinase C (PKC) within the RNA-binding domain of Musashi. Thus we examined roles of the PKC sites in translational repression activity of Musashi and found that the substitution of Threonine in PKC site of Musashi with Aspartic acid, mimicking the phosphorylated status, abolished the Musashi-repression activity of tramtrack69 translation in S2 cells, suggesting the above mentioned hypothesis.

P-047

Speculation of the relationship between fatty acids and DNA metabolism in Drosophila.

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It has been known that fatty acids (FA) have some bioactivity. On the other hand, as well known, DNA polymerases are concerned with 3R of DNA. We tried to find agents that inhibit rat DNA polymerase beta (pol beta) activity, and found. Some of the inhibitors were a FA, nervonic acid (NA) and a bile acid, lithocholic acid (LCA). We investigated the molecular action of these agents three-dimensionally. We made the amino acid-substituted proteins, and performed analysis. Agents bound to the mutant proteins weakly. Generally free forms of FA are not present in the cells. When FA metabolized by lipases from lipid, FA could be bioactive. Phospholipases mainly metabolize lipids in the cells, so we are absorbed in phospholipases ヤ action. We speculated that FA not only contributes to nutrition metabolism, but also has a role in DNA metabolism regulation systems.

P-048

Identification of Drosophila Laval Common Motoneurons and Analysis of their Neuromuscular Junctions.

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Previous studies reveal that 32 motoneurons innervate 30 body wall muscles in an abdominal hemisegment of the Drosophila larva. Most of them innervate 1 or 2 specific muscles, but a small number of them innervate multiple muscles and are called a common motoneuron (CMN). We identified dorsal and ventral paired common motoneurons (DCMN and VCMN) using a GAL4 line which drove genes in a small number of neurons, and described their anatomy, immuno-histochemistry, muscle innervation, and junctional synapse type. DCMN was anti-eve positive, extended an axon ipsi-laterally via ISNa, and innervated dorsal muscles (1,2,3,4,9,10,19,20). VCMN extended an axon contra-laterally via ISNa, and innervated ventral muscles (6,7,12,13,14,15,16,30). Both had type Is axon terminals. These data show that DCMN and VCMN are MNISN-Is and MNSNb/d-Is respectively, which have been identified on the larval stage, and suggest that DCMN is aCC or U motoneuron, and VCMN RP5 in the embryo.

P-049

A role of nuclear matrix protein, Slender lobes, in accelerated proliferation of mushroom body neuroblasts

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The neuroblasts of mushroom body (MB) continuously proliferate from the embryonic to late pupal stages. The proliferation rate gradually increases to culminate in the early pupae and decreases at the later stages. Interestingly, this dynamic change appears to positively correlate with the size of neuroblasts. Therefore, there must be a mechanism that coordinates cell growth and division to endorse accelerated proliferation of neuroblasts. During a screening for mutations that affect MB morphology, we became interested in the sle gene, which was associated with P-insertion in one of GS lines. In sle deficiency mutants, we found that the alpha and beta lobes of MB were more slender than those of wild type in the adult brain. BrdU incorporation experiments indicated that the sle MB neuroblasts failed to accelerate their proliferation rate and also to increase the cell size during larval and early pupal stages. Our preliminary experiment suggests that the sle neuroblasts have prolonged interphase. Interestingly, Sle protein distributes in an extrachromosomal space in interphase nuclei and dynamically changes the distribution pattern during cell cycles. We will discuss about a regulatory scheme how a nuclear matrix protein affects cell growth, cycle and division to achieve accelerated proliferation of MB neuroblasts.

P-050

Molecular analysis of Drosophila myelodysplasia/myeloid leukemia factor (dMLF) and the interacting protein dCSN3

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Human myelodysplasia/myeloid leukemia foctor 1(hMLF1) gene was identified as NPM-hMLF1 fusion gene produced by the chromosomal translocation, which is associated with myelodysplastic syndrome and acute myeloid leukemia. We have obtained a clone encoding a Drosophila protein homologous to hMLF1 and characterized the function of Drosophila MLF (dMLF). We have found that dMLF inhibits reaper-, hid-, and grim- induced apoptosis in Drosophila, and that dMLF also suppresses the toxicity of an abnormally long polyglutamine tract. To generate further insights into hMLF1 or dMLF functions, we have searched for hMLF1-interacting proteins by yeast two-hybrid screening and identified CSN3, a component of the COP9 signalosome. We comfirmed that Drosophila homolog of CSN3 (dCSN3) also interacted with dMLF. To understand the significance of this interaction, we determined the amino acid regions involved in interactions between dMLF and dCSN3 by GST pull-down assay. Deletion analysis of GST-dCSN3 fusion constructs revealed that the PCI domain of the dCSN3 protein is necessary for the interaction with dMLF. We also identified the interaction region in the dMLF polypeptide with dCSN3 as the N-terminal region of amino acids 1 to 95. Now we are analyzing the interaction between dMLF and dCSN3 in vivo.

P-051

Effects of over-expression of growth factors on the development of germline cells of Drosophila.

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In adult ovaries of Drosophila, Dpp is known to play an essential role for the maintenance and growth of germline stem cells. However, little are known about which factors control the development of larval germline cells. By over-expressing several known growth factors with GAL4/UAS system, we investigated whether these factors affect the development of germline cells at larval stages.

P-052

Mutational Analysis of the Drosophila neural zinc finger gene, dNZF-1 that is expressed in neuronal subsets.

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Neural zinc finger factor 1 (NZF-1), myelin transcription factor 1 (MyT1, also termed NZF-2) and NZF-3 are members of a neural-specific zinc finger gene family in mammals, with DNA-binding domains of a C2HC-type zinc finger. Xenopus MyT1 was shown to play a critical role in neuronal differentiation, although human MyT1 was first identified by virtue of its binding to cis-regulatory elements of a glia-specific gene, the myelin proteolipid protein gene. However, functions of the NZF/MyT gene family remain elusive. We identified the Drosophila neural zinc finger gene, dNZF-1 that contains only two C2HC zinc fingers similar to the homologue of C. elegans. Whole-mount in situ hybridization and immunohistochemical analyses showed that both dNZF-1 transcripts and proteins are expressed in neuronal subsets of the central and peripheral nervous systems. To elucidate dNZF-1 function, we generated three dNZF-1 mutants by the imprecise excision of a P-element upstream to the dNZF-1 gene (KG4506). Antibody staining revealed that these deletion mutants appear to lack the protein, suggesting that they are null mutants. Using the GFP balancers, we found that almost mutants die at the late pupal stage. Studies on these mutants using various neuronal markers would allow us to understand dNZF-1 function in Drosophila.

P-053

Molecular basis of distance-dependent variation of nonautonomous apoptosis mediated by Ras

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It is generally the case that signals leading to cellular growth/proliferation also serve to enhance cell survival, as with EGF, Insulin, Dpp and Wg. However, growth and apoptosis signals are both regulated in a complex manner not only intracellularly but also outside the cells themselves, eg. in a developing field. In one study, local elevation of Ras survival signals induced an apoptotic response in the surrounding cells as judged by acridine orange staining (Karim and Rubin, 1998). Here, we reevaluate this type of apoptosis, measuring JNK and Caspase-3 activation. In the wing clones where Ras is activated, JNK is autonomously activated but cell death is blocked probably due to repression of Hid. However, JNK is nonautonomously activated also along the clone boundary where Caspase-3 is subsequently and weakly activated. In contrast, JNK is weakly activated and Caspase-3 is highly activated in locations distant from the clone. These short-range and long-range apoptotic responses may represent a molecular and cellular basis for eliminating cancer cells and compensating for cell number in tissue size homeostasis, respectively. We will further focus on the relationship of this nonautonomous death and cell affinity regulation as well as the involvement of diffusible apoptotic factors.

P-054

GABA-mediated inhibitory neural connections in the Drosophila antennal lobe.

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There are many anatomically and functionally common features in the olfactory system between vertebrates and invertebrates. Due to a relatively smaller number of neurons, the Drosophila is a good model for revealing the fundamental mechanisms of olfactory information processing. Although inhibitory synapses play important roles for olfaction, for example the lateral inhibition and the oscillatory activities, and the GABA is the major inhibitory neurotransmitter, detail of synaptic connections mediated by GABA in the antennal lobe is still essentially unknown. Here, we analyzed the expression patterns of GABA synthesis enzyme and receptors. By combining fluorescent in situ hybridization and GFP-immunostaining, we examined the precise distributions of neurons that release and receive GABA. We found that most of the local interneurons use GABA for transmitting signals with each other. In the case of projection neurons, we found that majority of mACT neurons, but not iACT and oACT neurons, are expressing GABA synthesis enzyme. We also found that the local interneurons have output sites in the antennal lobe, whereas the mACT neurons rarely have such sites. These findings indicate that inhibitory inputs to the antennal lobe are only from the local interneurons.

P-055

TRAP240, as a component of the mediator complex, represses transactivation function of androgen receptor

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Androgen receptor (hAR) is a transcriptional factor in a ligand-dependent way through two types of coactivator complexes which are the histone acetyltransferase complex and the TRAP/SMCC mediator complex associating with general transcriptional factors. These complexes are recruited by independent and sequential manner. However, the components of these complexes and their functional roles are unknown. We have established so far a Drosophila models in which wild-type or mutated hAR is ectopically expressed together with a GFP reporter gene, and confirmed that hAR activates transcription and recruits known coactivator components1). We examined here to identify unknown components of AR coregulator complex. We found that hAR transactivation was markedly enhanced in dTRAP240 null mutants, suggesting that dTRAP240 acts as a corepressor of hAR. Human TRAP240 also repressed hAR transcriptional activity. Interestingly, this repression activity was reduced by tricostatin A, and then repression domain is localized on C-terminal region in both of TRAP240s. These results indicate that despite a component of mediator complex, TRAP240 has a role of corepressor of AR transactivation through regulation of histone deacetylation. To understand molecular basis of TRAP240 repressor activity, we are trying to identify and characterize protein interacting with TRAP240 repression domain.
1)Takeyama et al., Neuron 35, 855-864 (2002)

P-056

AN ANALYSIS OF THE MALE STERILE MUTANT ms(2)n55 IN DROSOPHILA MELANOGASTER

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Homozygous ms(2)n55 males produce motile sperm and transfer them to females during copulation, but the males gave few progeny (egg to hatch rate is about 1%). To understand the cause of this sterility, we examined the amount of sperm produced by the mutant males as well as the amount transferred and stored in the females after copulation. We took advantage of the dj-GFP transgene to estimate the amount of sperm by measuring the intensity of GFP fluorescence. The amount of sperm production in ms(2)n55 males and the amount of sperm which are stored in the female storage organs are equivalent to the control ms(2)n55/CyO or ms(2)n55/+ males. We further examined whether ms(2)n55 sperm are competent to penetrate into the eggs and inseminate to initiate the nuclear divisions. The entry of sperm into eggs was examined by immunostaining with the sperm-specific antibody DROP1.1 and initiation of embryonic development by DAPI staining. A low frequency (12.3%) of sperm entry suggests that the primary defect of ms(2)n55 is a failure of sperm penetration into eggs. The ms(2)n55 locus was mapped between 33F2 ; 34A1 by deletion mapping.

P-057

The misfire gene functions in male pronuclear formation at fertilization of Drosophila

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The male sterile mutation, misfire (mfr), of Drosophila melanogaster is a novel paternal effect, fertilization defective mutant that affects sperm head decondensation. mfr sperm are motile, appeared normal morphologically and were transferred to the female during copulation. However, less than 0.1% of eggs laid by females mated to mfr males hatched. Although mfr sperm entered eggs at a high frequency (93%), 99% of the inseminated eggs did not initiate the first nuclear division. Unlike normal fertilizing sperm, the position and shape of mfr sperm tails within the egg were not constant, but varied in a seemingly random manner. The heads of inseminating mutant sperm were always located near the surface of eggs just underlying the egg plasma membrane, and maintained their needle-like shape, indicating the failure of nuclear decondensation. Further observations revealed that plasma membrane of inseminating sperm appeared intact including the head region. These phenotypes were equivalent to those of sneaky (snky), another fertilization defective male sterile mutation. Our observations strongly suggest that mfr mutant males are sterile because their inseminating sperm fail to form a male pronucleus due to incapacity of the degradation of sperm plasma membrane.

P-058

A role of microtubule-associated protein, Orbit in chromosome segregation and cytokinesis in Drosophila.

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The orbit, initially identified as a Drosophila mitotic gene, encodes a conserved microtubule-associated protein required for a proper organization of mitotic spindles and for stabilization of the plus ends of microtubules in interphase cells. To assess the roles of Orbit in cell division, we investigated mitotic progression and cytokinesis in the orbit mutants. The time-lapse observations of nuclear division cycles in early embryos derived from orbit[R24] females found abnormalities in several mitotic processes such as chromosome congression and segregation. And the observations to visualize microtubules revealed that organization and movement of polar microtubules essential for the chromosome behavior were defective in the mutant embryos. In addition, cytokinesis frequently fails in the orbit mutant. The orbit[R24] spermatocytes contained fewer amounts of central spindles. This phenotype was associated with a failure of accumulation of the Pavarotti protein on spindle midzone during anaphase. Moreover, the anillin was not localized on central cortex region before actin ring formation. The mislocalization of these proteins required for cytokinesis eventually results in a failure of contractile ring formation in the mutant cells. Finally, we have established systems to express EGFP-Orbit fusion protein in Drosophila and cultured cells. We are currently investigating the subcellular localization of this protein.

P-059

CYTOGENETIC ANALYSIS OF A COHESION DEFECTIVE MEIOTIC MUTANT mei(3)M20.

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In meiosis, homologous chromosomes pair and segregate at the first division. Sister-chromatids of paternal and maternal chromosomes are tied together and maintained until anaphase of the second division, although homologous chromosomes have been segregated from each other. The meiotic mutant, mei(3)M20, of Drosophila melanogaster shows high frequency of nondisjunction of all chromosome complements at meiosis I and II in both males and females. In order to understand the role of the mei(3)M20 gene, we examined chromosome behavior at meiosis of the mei(3)M20 males. Cytological examination revealed that mei(3)M20 induced precocious separation of sister-chromatids at an early stage of meiosis I. In the metaphase I cells of wild type, sister-chromatids are tightly bound at the centromere, but in mei(3)M20 the cohesion is loose and centromeres are separated. A chromosome mass found on the metaphase plate at the first division suggests that pairing of homologous chromosomes is normal. We performed FISH to analyze chromosome behavior of mei(3)M20 in meiosis using 1.688 satellite known to exist in the most proximal region of the X centric heterochromatin as a probe to monitor sister-chromatids cohesion and separation. We will discuss the function of the mei (3)M20 gene in meiosis.

P-060

Orbit, the CLASP orthologue of Drosophila, is required for cell divisions to generate 16-cell cyst and development of fusome and the polarised microtubule network during gametogenesis.

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The Drosophila orbit encodes a microtubule-associated protein required for correct spindle organization and for the attachment of kinetochores to microtubules. The mammalian orthologue is known as CLASP that contributes to the stabilization of the plus ends of microtubules at interphase. We found the Orbit is required for stem cell division and another four rounds of division to generate 16-cell cysts during both oogenesis and spermatogenesis. The Orbit not only associates with the mitotic spindle and pole but also with the central spindle, ring canal and fusome during oogenesis. The mitotic spindles of those cystocytes are either diminutive or monopolar and do not make contact with the fusome in orbit[6] mutant. The Orbit appears to facilitate interactions of the fusome with mitotic spindles and ring canals and to ensure correct growth of the fusome that is pre-determinative of 16-cell cyst formation and oocyte specification. Moreover, the Orbit protein is required during mid-oogenesis for the organisation of the polarised microtubule network inside the 16-cell cyst that ensures oocyte differentiation. In addition, we observed the similar phenotypes during spermatogenesis. Integrity and growth of fusomes are perturbed in the mutant spermatogonial cysts. And ring canals also fail to differentiate correctly in the mutant cysts.

P-061

Molecular analyses of the meiotic gene, mei(3)1223, in Drosophila melanogaster

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The meiotic mutation, mei(3)1223[m144], was isolated from a natural population. Homozygous males exhibit almost random segregation of homologous chromosomes in the first meiotic division. The gene was identified by using new deficiencies in the 62A region. We further carried out recombination mapping assisted by SNP markers. Recombinant chromosomes generated within the interval of 160 kb were recovered and the sites of recombination were estimated by SNP markers. We narrowed down the gene locus to a 30 kb fragment. In the corresponding region, only a single gene, CG13916, is predicted. Transformants of this gene rescued the mei(3)1223[m144] mutant phenotype. Sequence analyses of the mei(3)1223[m144] allele revealed that a single nucleotide insertion at the 3' region of the coding region of CG13916, resulting in truncation of C terminus of the MEI1223 protein. The predicted MEI1223 protein have similarity to the Scc3 gene product, which is known to be a subunit of the cohesin complex.

P-062

A trace amine, tyramine, affects behavior of the crayfish, Procambarus clarkii.

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A G-protein coupled receptor, tyramine receptor (TyrR), has been cloned in Drosophila (Arakawa et al., 1990; Saudou et al., 1990). Tyramine receptor mutation shows defects in the adult olfactory behavior and in the modulation of the larval neuromuscular transmission (Kutsukake et al., 2000; Nagaya et al., 2002). Tyramine-containing neurons have been identified in the larval CNS by the anti-tyramine imunohistochemistry (Nagaya et al., 2002). These evidence indicate that a trace amine, tyramine, functions as a neuromodulator in Drosophila. Here we show that tyramine also affects behavior of the crayfish. Tyramine was injected into the body cavity of the male crayfish, and its behavior was monitored for 60 minutes. An up-down position of the large claw and a flexion-extension behavior of the abdomen were analyzed. The tyraminized animal showed a posture with an upper position of the large claw and with the extended abdomen. This posture was different from that of the animal which octopamine and serotonin were injected into. Immunohistochemical staining revealed tyramine-containing neurons in the CNS, and an HPLC-ECD detection revealed the tyramine content in the abdominal ganglia that is a similar amount to octopamine. These results suggest that the tyraminergic nervous system functions also in the crayfish.

P-063

Enhancer trap screen to identify genes specifically expressed in the developing optic lobe

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The visual system of Drosophila melanogaster is an excellent model to understand the fundamental mechanisms involved in the correct neuronal differentiation and the precise axonal pathfinding. Photoreceptor axons in the Drosophila retina project towards the specific targets in the optic lobe of the brain. However, neuronal differentiation and axonal guidance are only poorly understood in the optic lobe in contrast to the retinal neurons. To understand the neural patterning in the optic lobe, we performed enhancer trap screen to look for genes expressed in the developing optic lobe. So far, about 3,000 lines have been screened, and up to 30 optic lobe specific enhancer trap lines were obtained. In addition to their expression pattern, we will present the mutant phenotypes of several enhancer trap lines.

P-064

The enhancer trap screens for genes involved in formation of the fly visual center

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The critical step of the nervous system development is to generate precise connections between pre- and postsynaptic neurons. Interestingly, axons not only detect environmental signals that guide them to their proper destinations, but emit signals that are used to pattern their postsynaptic target field. The visual system of Drosophila offers a unique opportunity to investigate these kinds of axon target interactions. Different classes of photoreceptor neurons (R cells) project to different optic ganglia; the R1-R6 axons connect to the first optic ganglion, the lamina, and R7 and R8 axons pass through the lamina and connect to the medulla. R cells send signals that regulate lamina neuronal differentiation, such as Hedgehog, which induces lamina neuronal precursor cells to undergo their final cell division, and Spitz, which triggers the differentiation of lamina neurons. But as little is known about how lamina neuron precursors interpret the signals delivered by R axons, we are seeking the genes which are expressed in the optic lobe and may play an important role in its patterning by screening enhancer trap lines. We present the results of the ongoing screening.

P-065

Male sterile mutation ms(3)236 fails to have the sperm stored in the female

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Drosophila Genet. Res. Ctr., Kyoto Inst. Tech.

The male sterile mutant, ms(3)236, produces motile sperm and transfers sperm to females by copulation. The amount of ms(3)236 sperm in the uterus was the same level as the control (ms(3)236/TM3) five minutes after copulation, although the amount stored in seminal receptacle and spermatheca was significantly fewer than the controls 1 h after copulation. Females mated with ms(3)236 males laid inseminated eggs significantly less than the controls. It should be stressed that more than 90% eggs inseminated by the sperms of both genotypes initiated nuclear divisions. One day after copulation with ms(3)236 males, more than 60% of females accepted copulation with the second male whereas less than 10% in the controls. The gene ms(3)236 was mapped within 95F on the 3R. The present study suggests that a low efficiency of sperm entry to the female sperm storage organs should be the primary cause of the sterility.

P-066

Genes that regulate morphogenesis of gastric caeca

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Drosophila gastric caeca are four midgut evagination of precursors consisting of visceral mesoderm parasegment 3 (VM-PS 3) and immediately adjacent endodermal cells. decapentaplegic (dpp) expressed in the VM-PS3 has been known to be required for gastric caeca development. We show that hedgehog regulates gastric caecum development as a critical activator of dpp in the VM-PS 3. Also, preliminary results reveal that several genes including breathless and prickle are involved in the morphogenesis of gastric caecum.

P-067

Transcriptional activation mechanism of human estrogen receptor in Drosophila

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Human estrogen receptor α (ERα) is a member of the steroid hormone receptor superfamily of ligand-activated transcription factors. Phosphorylation significantly modulates transcriptional activity of human ERα. However, very little is known about mechanisms or enzymatic activities involved in this important modification. Serine 118 is shown to be the major site of estradiol-induced phosphorylation of the receptor. It has been demonstrated that in mammalian cells ERa can be phosphorylated at this position by ERK and by cyclin-dependent kinase 7, cdk7. Using Drosophila GAL4-UAS-ERα transgene, we generated an experimental system in which to study transcriptional regulation of ERα and to identify novel kinase activities capable to phosphorilate ERα in vivo. ERα expressed in third instar larva eye disc driven by GMR-GAL4 shows classical ligand dependent transactivation. This transactivation was significantly altered in several kinase mutant lines. The validity of this experimental system was particularly confirmed by demonstration that ERα ligand-dependent transactivation was reduced in cdk7 dominant negative mutant and that drosophila cdk7 enhanced hERα transactivation in vivo through phosphorylation at serine 118. Using this approach we have identified two novel Drosophila MAP kinases Bsk and p38 capable to phosphorylate and enhance ERα transactivation in vivo. Importantly, Bsk and p38 represent homologues of mammalian Jnk and p38 kinases respectively that have not been previously implicated in modulation of ERα transactivation.

P-068

Glial cell proliferation induced by programmed cell death and neural injury in the Drosophila adult brain

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To understand plasticity of the adult brain, we investigated cell proliferation in the adult brain of Drosophila melanogaster throughout their lifetime. We found BrdU-positive cells around the antennal nerve from zero to six days after eclosion. Analyses with BrdU-pulse labeling and MARCM system indicated that BrdU incorporation is due to cell division rather than endoreplication. After three-hour treatment of BrdU, immunohistochemistry with a glial cell marker, REPO, and a neuron marker, ELAV, showed that 98% of BrdU-positive cells were REPO positive. This strongly suggested that the mitotic cells were glia. Ten-day treatment of BrdU suggested that BrdU-positive cells differentiate to either glia or an unidentified cell type that are negative with both REPO and ELAV. TUNEL analysis showed that some neurons around the antennal nerve undergo programmed cell death after eclosion. In flies that ectopicaly expressed a programmed cell-death inhibitor p35 in neuron, we could not observe BrdU-positive cells. To investigate relation between lesion of neurons and glial cell proliferation, we ablated an antenna from these transgenic flies. BrdU-positive glial cells were observed around antennal nerve. These results suggested that, in adult brain, glial cell proliferation is induced in response to neural lesion caused by programmed cell death and injury.

P-069

Towards understanding the basis of regulation of the activity of ADF/cofilin phosphatase Slingshot

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The ADF/cofilin family proteins are stimulus-responsive mediators of actin cytoskeletal dynamics and their activity to depolymerize or sever actin filaments is abolished by phospholyration of Ser-3. We previously isolated slingshot (ssh) mutants that affect bristle morphology and showed that SSH encodes an actin filament-binding phosphatase towards phospho-Ser-3. ADF/cofilin undergoes rapid dephosphorylation in response to several stimuli that cause reorganization of actin cytoskeleton, which suggests a possibility that those stimuli control the activity of SSH. Besides the catalytic domain, the SSH family members have two amino-terminal conserved domains and the carboxy tail; and their functions are not known. As one approach to explore the potential mechanism of the activity regulation, we made a series of domain deletions and addressed 1) if each of those forms decreases in the level of phospho-cofilin (P-cofilin) when expressed in S2 cells, 2) if it dephosphorylates P-cofilin in our cell-free assay, and 3) if it rescues the lethality and the bristle phenotype of ssh mutants. We found that one of conserved Tyr residues may involve in the regulation of the SSH activity. We will also present results of our search for proteins that interact with SSH.

P-070

Analyses of arit1/danr and arit2/dan, which are involved in the antennal formation in Drosophila

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The antenna and the leg of Drosophila is thought to have arisen via duplication and diversification of an ancestral structure. From the analyses of an enhancer trap line P0044, we found two genes, arit1/danr and arit2/dan, which were expressed specifically in the antennal disc and encode proteins containing a DNA binding domain called Psq domain. Reduction of the arit1 and/or arit2 activity caused the transformation of the arista into the tarsus-like structure, and misexpression of either of them resulted in the converse transformation. Since spineless(ss) is known to have similar activity and the forced expression of arit genes can rescue the ss mutant phenotype, we examined the relationship between ss and arit genes. In the antennal disc, they are expressed in nearly identical patterns. arit expression was regulated positively by ss, and ss expression was also positively regulated by arit at least partially. In addition, arit repressed the activity of leg-specific enhancer of Bar genes, and we identified the binding sites for Arit proteins in this enhancer. These observations suggest that arit genes are involved in maintaining the ss expression and specify the antennal fate by regulating gene expression through binding to the leg-specific enhancer.

P-071
HOM gene function in the development of Drosophila indirect flight muscles

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Drosophila adult musculature, in contrast to larval one, has a remarkable segment specificity. In particular the mesothoracic musculature includes huge flight muscles and is quite different from other segment's one. How the segment identity of adult musculature is specified during development? HOM genes might play an essential role in this process. One possibility is that the identity might be laid down during embryonic development by HOM genes in the imaginal myoblasts located in each larval segment and these myoblasts originate the segmental identity of adult musculature. The alternative one is that HOM genes work in the developing adult muscles during metamorphosis. We tested the latter by ectopic expression of different HOM genes using Actin88F-GAL4 line, which can drive gene expression in the developing indirect flight muscles. Ectopic expression of HOM genes caused phenotypes such as complete loss of muscles, reduction of the muscle fiber number, and thin fibers. Different homoeotic genes had different developmental defects, but Antp showed the weakest phenotype among HOM genes. The reaction to HOM gene misexpression was different among subgroups of indirect flight muscles.

P-072
Guidance mechanism of tracheal branch migration in Drosophila

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The Drosophila tracheal network is formed during embryogenesis by a series of branching, migration, and fusion of tubular ectodermal epithelia. The dorsal branch is one of the 6 major branches that extend from each tracheal primordium, migrates toward the dorsal midline and fuses to the contralateral dorsal branch. We analyzed molecular basis of guided migration of the dorsal branch. Tracheal tip cells containing the terminal and fusion cells were attached to epidermal cells expressing Engrailed, and make filopodial contacts with Dpp-expressing cells during the extension of the dorsal branch. When the ectopic multiplication of terminal cells was induced by the misexpression of DAxin in trahchea, the ectopic terminal cells still respected to migrate into regions of En-expressing cells. Conversely, activation of the Hh pathway in tracheal cells by expression of the constitutive activate form of the Ci in trachea inhibited extension of the terminal branches along with the stripe of en. We observed dorsal branch misrouting when Dpp signaling was disturbed by the misexpression of either the constitutive active form of Tkv or Dad in trachea. The results of these experiments demonstrate that Dpp and Engrailed-expressing epidermal cells provide positional information for migrating tracheal tip cells.

P-073

Intrinsic sub-axonal patterning in Drosophila neurons

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In Drosophila embryonic CNS, several molecules are localized to the specific segments of axons. For example, an axon guidance receptor Robo and its family members are detected only at the longitudinal axons and excluded from the commissural axons. How is this sub-axonal localization of molecules created? We assumed the following two mechanisms: 1) localization is dependent on cell-cell contacts (an extrinsic mechanism), and 2) localized pattern is formed without cell-cell contacts (an intrinsic mechanism). To test these possibilities, we examined the distribution of molecules in isolated neurons by using a Drosophila primary cell culture system, and found that several molecules are localized to the specific parts of axons without cell-cell contacts. Robo3 and Robo2 were detected at the distal part of axons, whereas an axonal marker BP102 antigen was detected at the middle or proximal part of axons. This result indicates the existence of intrinsic mechanisms that create the sub-axonal localization of molecules in Drosophila neurons. We are now looking for the molecular basis of the mechanism.

P-074

Transcriptional regulation of the Drosophila homeobox genes, aristaless and clawless, required for patterning along proximodistal axis of leg

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During the development of multicellular organism, the gradual morphogen activity subdivides the corresponding developing field through instruction of region specific expression of genes encoding transcriptional factor. The genes involved in proximodistal patterning of the Drosophila adult leg are expressed in concentric regions in the leg disc and these expression domains are thought to be determined by gradual activity of Wingless(Wg), Decapentaplegic(Dpp), EGFR signaling. To clarify the mechanism by which these morphogen gradients determine region specific expressin of genes, we are woking on transcriptional regulatory regions of three homeobox genes, aristaless(al), clawless(cll)(these are expressed in a central region of the leg disc), and BarH1/BarH2(Bar)(this is expressed in a circular region surrounding al and cll expression domain). Here we report about al and cll regulatory regions. We have identified leg enhancer of al(358bp region 6 kb upstream of transcriptional start site) and cll(2.2kb region downstream of stop codon) by transgenic analysis. And we show this al leg enhancer contains in-vitro binding sites of downstream transcriptional factor of Wg, Dpp, EGFR signaling, dTcf, Mad/Brk, Pnt/Yan, and of these binding sites, at least one Brk binding site and one Pnt/Yan binding site are functional in vivo.

P-075

Analysis of mutations affecting neural stem cell morphology

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In Drosophila, embryonic neuroblasts undergo asymmetric divisions and produce a chain of ganglion mother cells, each of which divides once into a pair of neurons or glia. After several rounds of division, neuroblasts become mitotically quiescent entering a resting state until they restart division at larval stages. To investigate molecular mechanisms controlling asymmetric neuroblast divisions, we have conducted EMS mutagenesis screening. During our screening, we have identified a novel class of zygotic lethal mutants, in which neuroblasts show abnormal morphology. In the wild type, mitotic neuroblasts maintain a spherical shape, which become irregular with projections when they cease divisions. In contrast, neuroblasts stop division earlier in this class of mutants and their shape becomes more irregular with longer projections than wild type neuroblasts. We collectively call this phenotype "abnormal projection" by the characteristic shape of mutant neuroblasts. We have identified 18 complementation groups on the second chromosomes. They show similar but variable degrees of phenotypes. This class of mutants include zipper and rhoA , both of which are essential for maintaining cell morphology, mitosis or cytokinetic processes. We are systematically identifying genes responsible for this class of mutations and analyzing their phenotypes during embryogenesis.

P-076

Are you interested in Drosophila species other than D. melanogaster? (National Bio-resource Project)

p=Masayoshi WATADA
Ehime University

Drosophila species was collected in some localities of Japan for the Drosophila researchers in this project. A total 300 strains of 41 Japanese Drosophila species has been stocked and listed in our laboratory at present. All strains of the stocks are available for foreign Drosophila researchers as well as Japanese. Some Drosophila species other than D. melanogster have specific characters such as diapause, parthenogenisis and hybrid sterility between species, not found in D. melanogaster. Comparative genomic study seems to become important and interesting to melanogaster researchers. In this poster, some morphological characters such as head color and wing patches specific to “other species” are shown using Japanese Drosophila. For example, males of D. albomicans (subgenus Drosophila) and D. auraria (subgenus Sophophora) have white head, but the mechanism of the white head color are different between the two species. In addition, morphological variation of sex combs in “other species” is shown in the poster. I hope that melanogaster researchers find interest in “other species” gradually in their studies.

P-077

Genome-wide analysis of vesicle transports in Drosophila.

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Vesicle transports play critical roles to localize secretory and membrane proteins in a cell. The localizations of such proteins are tightly regulated in multi-cellular organisms for their functions in polarity formation, morphogen distribution, cell morphogenesis and so on. To reveal how such multi-cellular events are regulated by the vesicle transport machineries, we have listed about 400 genes related to the vesicle transports from the Drosophila genome sequences and have started to investigate their subcelluler localizations and their knock-down phenotypes using RNA interference (RNAi). In the analysis of subcelluer localization using S2 cells, 11 proteins were localized in ER, 27 proteins in the Golgi apparatus and, 14 proreins in endosome and/or lysosome. The knock-down of gene expressions in whole bodies using act-gal4 driver resulted in growth defects (179lines/199lines) and appendage malformations (8lines). To study in details, tissue specific knock-down experiments using sd-, Dpp-, pannier- and Dll- gal4 drivers are in progress. Preliminarily, wing defects such as extra veins, deleted margins and curled wings were observed in the sd-gal4 screening.

P-078

Characterization of newly established Drosophila cell lines from embryonic mesoderm and larval gonadal mesoderm.

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We established new Drosophila cell lines from dorsal mesoderm at early embryonic stage (EDM) and gonadal mesoderm at third instar larva mesoderm (LGM), respectively. We characterized these cell lines and examined whether these cell lines are useful for the maintenance and growth of germline cells when co-cultured as feeder cells. Furthermore, hormonal effects on the LGM are also reported.

P-079

Expression of Replication Protein A during development of D.melanogaster

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Replication Protein A (RPA) is a major eukaryotic single-stranded DNA binding protein which is indispensably involved in the DNA synthetic systems. In Drosophila, the RPA is a heterotrimeric protein composed of three subunits, which are designated as RPA70, RPA30, RPA8. We are interested in knowing the roles during the development of Drosophila melanogaster. In the connection, we investigated the RPA expression pattern during the development. Northern blot analysis implied that most of mRNAs coding RPA subunit proteins have maternally been synthesized during the oogenesis. The northern pattern of each of the subunits seemed quite similar or same among them. To know the distribution mode more precisely, we conducted glycerol gradient sedimentation to concern whether RPA 70 associated with RPA30 in the 0-24h embryos or not. The results suggested that RPA is present mainly in the complex during the 0-24h embryos, but some of the subunits seemed to be the monomer dissociated from each other. We also tried in situ double staining by using their antibodies against RPA70 and RPA30 during the spermatogenesis. In the mature primary spermatocytes, the localization patterns of RPA70 and RPA30 were not coincided well, suggesting that they are dissociated from each other at the specific stage during the spermatogenesis.

P-080

In vivo imaging of calcium dynamics within identified target cells during synaptogenesis

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Bi-directional communication between a neuron and its target cell(s) is essential for the development of synapses, although the actual role of target cells is poorly understood. To clarify the molecular mechanisms by which postsynaptic cells contribute to synapse development, we focused on the dynamics of calcium, a pivotal second messenger, within the postsynaptic cell during synaptogenesis. Drosophila embryonic neuromuscular junction allows us to trace the course of synapse formation in vivo. Fluorescent calcium indicators (Calcium Green-1, Oregon Green 488 BAPTA-1) were used to probe the intracellular calcium concentration ([Ca]i). We constructed a system for fluorescence detection comprising cooled CCD camera, argon/krypton laser and a confocal unit based on the Nipkow disc that captures full-frame images at high frequency. Indicators were loaded into a single muscle cell by electroporation. It enables us to examine [Ca]i within an identified target cell under low background level. As a first step, we have captured signals that seem to correspond to a rise in [Ca]i following action potentials. We are now investigating calcium dynamics in muscle cells during synaptogensis, particularly just before and after the initial contact of a synaptic pair.

P-081
Sexual dimorphism in the brain of Drosophila melanogaster

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Brain structure is known to show a sexual dimorphism in mammals and in some insects such as moths, honey bees and cockroaches. The sexual dimorphism should play an important role in control of sexual behaviors. However, it is still obscure how the sexual dimorphism is formed during brain development under the control of genes and how it functions to make a sexual behavior. In Drosophila, the sexual dimorphism in the brain has not been well investigated. Recently, several new approaches begin to reveal the existence of the sexual dimorphism in the fly brain. We screened enhancer-trap Gal4 lines which showed sexual differences in Gal4 expression patterns in an adult brain. One Gal4 line showed the male specific expression in small groups of neurons, in addition to common expressions in both male and female neurons. We will show the projection patterns of the male specific neurons in the brain, using MARCM cell-labeling system.

P-082
Functional analysis of glycans in Drosophila melanogaster using genome-wide RNAi screening

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Recent genetic studies demonstrate that N- and O-linked glycan structures and proteoglycans serve a variety of functions in morphogenesis, growth regulation and disease. To obtain systematic information about role of glycans, we have listed 80 Drosophila genes homologous to mammalian and yeast glycosyltransferases and have started to knock-down their expression by the RNA interference (RNAi) technique by the use of Gal4 lines under the control of act-, sd-, dpp-, pan-, Dll- and ey-promoters. Down-regulation of these genes resulted in various abnormalities such as wing margin defect, no bristle and rough eye. Some lines exhibited phenotypes resemble to that of defective Wingless signaling, which is known to depend on intact proteoglycan biosynthesis. Thus, the modification of proteins by the carbohydrate chains has emerged as a key feature of several developmental signal transduction pathways.

P-083
Genome-wide screening for genes related to oxidative stress in Drosophila melanogaster

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Oxidative stress is thought to be a major cause of aging and age-dependent diseases. In the present study, we performed a screen for mutations affecting oxidative stress resistance of adult flies as an approach to understand the molecular mechanisms of aging. To measure oxidative stress resistance efficiently, we established an effective method to measure active period under high oxidative stress automatically. Using this method, we are screening a collection of lines mutagenized with a P-element based Gene Search (GS) vector containing UAS. In F1 hybrids between GS vector-insertion lines and GAL4 lines, genes downstream of vector insertion site can be misexpressed under the GAL4 control. F1 males between a GS line and hs-GAL4 are reared at 25C and then transferred to 30C. After pre-heat induction at 30C for 4 days, flies were subjected to the assay with H2O2-containing media. So far we have screened about 2,500 GS-vector insertion lines, and identified several candidate genes whose overexpression conferred oxidative stress resistance. Furthermore, some of these lines also showed extended mean longevity at 25C comparing to control flies.

P-084
Rap2l regulates gonadal morphogenesis in Drosophila

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Rap2 is a member of the Ras family of small GTPases and shares 60% identity with Rap1. Mammalian Rap2 has been suggested to function as a modifier of Rap1 signaling cascade. However, no evidence is provided for its in vivo function. We previously identified a Drosophila homologue of Rap2 (Rap2-like, Rap2l) as a gene whose misexpression in eye imaginal discs causes rough eye phenotype. Rap2l is ubiquitously expressed during embryogenesis and in developing imaginal discs, with a high level of expression in genital discs. In adult stage, the highest expression was detected in the cyst cells of testes. Loss-of-function mutants of Rap2l generated by imprecise excision were semi-lethal. Escapers were sterile for both males and females, whose gonads were twice as large as wild-type ones with morphological abnormality. Both semi-lethality and gonadal defects were rescued by a P-element construct containing the genomic region of Rap2l gene, indicating that Rap2l is responsible for these phenotypes. Expression of dominant negative form of Rap2l results in testicular defects similar to those of the loss-of-function allele. In contrast, constitutive active form of Rap2l causes distinct structural abnormality. These results suggest that Rap2l signaling is crucial for gonadal morphogenesis in Drosophila.

P-085

Effort at constructing the artificial restriction-modification genetic system with selfish behavior in Drosophila

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The genetic elements that exert harmful effects on host cells are widespread. The best understood example is the Ccd A/B operon, a set of genes of a plasmid that exists in certain bacterial cells. The Ccd B gene encodes gyrase, activation of which may lead to distortions of chromosomes of the host cell. The Ccd A gene encodes an inhibitor of gyrase, which protects the host from the toxic effect of gyrase. The offspring of the host cell inherits the products of these two genes even when they do not carry the Ccd A/B operon itself. Since the toxic gene product endures longer than the antidote does, such daughter cells are unable to survive. As a result, only the plasmid-carrying (Ccd A/B-carrying) cells survive and dominate in descendant populations. We intended to generate similar selfish genetic elements artificially. We made the "RM" constructs carrying a restriction endonuclease (R) gene as the toxin and a ﾒ modification ﾓ methylase (M) gene as the antidote. The "RM" constructs inserted in the P-element vector were introduced into Drosophila melanogaseter by germline transformation. The RM transformants, however, did not exhibit "selfish behavior". We discuss about the possible obstacles that hampered the generation of artificial selfish genes.

P-086

Quick and versatile method of ectopic gene expression in the Drosophila embryo

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The GAL4-UAS system is the most powerful method of targeted gene expression in Drosophila. However, target tissues for the misexpression is limited by the properties of GAL4 strains used. FLP-FRT system is another technique for forced-expression of genes, but, it is not always possible to generate clones in various embryonic tissues. We have developed a quick and versatile method to generate cells for forced-expression of genes in embryos. By injecting a constitutive active GAL4 plasmid into the fertilized egg, GAL4-positive cells were generated. When fertilized eggs of the UAS-lacZ strain were injected with the plasmid, beta-galactosidase-positive cells were observed, indicating that lacZ was successfully forced-expressed. In most cases, lacZ-positive cells did not form a homogeneous patch, but, they were spreading in blotchy patterns, which is good for the analysis of cell-to-cell interactions. Position and number of the positive cell clusters can be controled by the dose and site of injection. Since the number of UAS strains available in the Drosophilist ’s community is rapidly increasing, this method could be added to one of the choices for forced gene expression in the Drosophila embryo.

P-087

An attempt to culture of male germline cells of Drosophila

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In Drosophila, there are differences of patterns of division and differentiation between male and female germline cells. We are now going to culture male germline cells of Drosophila with various conditions to know which factors control the development of male germline cells.

P-088

Exploring possible roles of a putative fruitless suppressor in Drosophila embryogenesis

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The Drosophila fruitless (fru) gene encodes a neural sex determinant protein with a BTB domain and Zn finger motifs. Using the Gene Search (GS) system in which a gene can be overexpressed from the UAS-promoter randomly inserted in the genome, we screened for the genes that modify fru-induced eye roughness when expressed together with fru in the eye disc. In this screening, We found that the TBP Related Factor2 (trf2) gene suppresses the fru effect allowing the fly to have wild type-like compound eyes. Trf2 is a putative transcriptional regulator. The trf2 gene has been implicated to function in embryonic development. Although fru is known as a gene to regulate male sexual behavior, it is also involved in embryonic neural development, particularly in axonal fasciculation. We intended to examine the possible interaction of fru and trf2 in the developing embryonic neurons. In stage 9- 10 embryos, the fru gene is expressed in neuroblasts, midline cells and tracheal placodes, while trf2 is expressed ubiquitously including these cells. We are currently conducting experiments to see whether over-expression of trf2 in the CNS can produce embryonic phenotypes similar to those of fru mutants.

P-089

A revision of the Zygothrica samoaensis species group (Diptera, Drosophilidae), with descriptions of seven new species.

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The Zygothrica samoaensis group was erected and subsequently revised by Grimaldi (1987; 1990). Whereas most of the Zygothrica species are neotropical, the samoaensis group, as well as two monotypic groups, includes species exclusively from the Indo-Pacific area. Only two more Zygothrica species are known from Africa (Grimaldi, 1990; Tsacas, 1990). Z. samoaensis was described by Malloch (1934) from Samoa Islands and then was the first Zygothrica species known outside of Neotropics. Later, Z. fijiana from Fiji islands and Z. flavofinira from the Malaysian peninsula were described by Takada (1976). Finally, in his revision of the samoaensis group, Grimaldi (1990) added nine new species, rising up to twelve the number of known species in this group. The present study is based on collections made in recent years in the Indo-Pacific area. Therein, more than one thousand Zygothrica specimens were recovered. Their examination leads us to recognize and describe seven new species and to propose two synonymies, leaving the Z. samoaensis group with 17 species. Based on morphological characters, some phylogenetic relationships are recognized and we propose a subdivision of the group in three subgroups. The species are Indo-Pacific in distribution, occuring from Thailand (Westernmost) and Taiwan (Northernmost) to Fiji islands (Southernmost) and French Polynesia (Easternmost). As far it is known, all species of the samoaensis group are mushroom feeders.

P-090

Activity-dependent mechanisms for synaptic growth matched with postsynaptic muscle volume in Drosophila neuromuscular junctions

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In Drosophila larval neuromuscular junctions (NMJs), the distribution of synaptic boutons is normally coupled to postsynaptic muscle volume in order to ensure depolarization of the muscle fibers. To clarify the mechanisms for such synaptic growth, we investigated the relationship between muscle volume and synaptic size in longitudinal abdominal muscle fibers 6 and 7 (M6 and M7). These muscle fibers are innervated by the same neurons and the volume of M6 is about 1.5 times larger than that of M7. We found that in larvae 7 hours after hatching (7 hr larvae), the frequency of mESCs and area of synaptic sites were larger in M6 than M7, in proportion to their volume. However, in larvae just after hatching, the frequency of mESCs in M6 was similar to that in M7. Moreover, when sensory inputs were suppressed, the frequency of mESCs in M6 was the same as that in M7 even in 7 hr larvae. Finally, in a mutant of BMP type II receptor, which is defective in synaptic growth in NMJs, the synaptic size of M6 was larger than that of M7. These results suggest a contribution of activity-dependent mechanisms besides BMP signaling in forming synaptic size.

P-091

Analysis of Notch-mediated pattern formation in the Drosophila hindgut by Genome Object Net combined with genetic experiments

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The Drosophila hindgut is initially subdivided into dorsal and ventral domains, and a one-cell-wide boundary cell strand is induced between them. We have shown that Delta expressed in the ventral domain activates Notch signaling in abutting dorsal cells and triggers the boundary cell differentiation, and that Delta suppresses Notch processing cell-autonomously, preventing Notch activation in the ventral domain. The model is simple, but, it is yet difficult to predict what will be caused in this multicellular system if the amount of each component in the pathway is changed, because of the two different activities of Delta. We tested the validity of our model by Genome Object Net (GON, http://www.GenomicObject.Net/) combining with genetic experiments. We made genetic experiments in which expression of Delta and Notch were changed by use of mutations and forced gene expressions, and corresponding simulations were performed. Simulation results successfully represented the experimental results, and, in some cases, even could make predictions that were confirmed experimentally afterward. These results support the validity of our model, and, at the same time, demonstrate the usefulness of GON as a tool to assist thinking of experimental biologists, in particular, those who are studying pattern formation of multicellular organisms.

P-092

Cellular and molecular mechanisms to achieve diversity of dendritic patterns

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Little has been understood about mechanisms that generate the morphological diversity of dendritic trees. Drosophila dendritic arborization neurons (da neurons), which are classified into classes I- IV in order of increasing arbor complexity, provide an excellent model system to tackle this question. Class I neurons and class IV neurons employed distinct strategies of dendritic emergence from the cell body, branching in the embryo and branch elaboration in the larva. Class IV neurons were much more capable of responding to the severing of branches than class I neurons. In our attempt to reveal the molecular mechanisms underlying the distinct cellular behaviors, we found that Abrupt, a putative transcriptional repressor, was expressed only in class I neurons. When Abrupt was ectopically expressed in other classes, their arbor complexity and receptive field formation became very similar to those of class I neurons. Moreover, class IV neurons expressing Abrupt responded poorly to the severing of branches. In abrupt mutants, class I neurons showed an increase in the number of branch terminals and appeared to lose their characteristic comb-like arbor patterns. All of our data suggest that Abrupt controls class I-like dendritic morphogenesis.

P-093

neurotic, a novel maternal neurogenic gene, encodes an O-fucosyltransferase that is essential for Notch-Delta interactions

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Notch signalling, which is highly conserved from nematodes to mammals, plays critical roles in many developmental processes. In the Drosophila embryo, deficiency in Notch signalling results in neural hyperplasia, commonly referred to as the neurogenic phenotype. Here we identify a novel maternal neurogenic gene, neurotic, and show that it is essential for Notch signalling. neurotic encodes a Drosophila homolog of mammalian GDP-fucose protein O-fucosyltransferase, which adds fucose sugar to epidermal growth factor-like repeats and is known to play a crucial role in Notch signalling. neurotic functions in a cell autonomous manner, and genetic epistasis tests reveal that Neurotic is required for the activity of the full-length but not an activated form of Notch. Further, we show that neurotic is required for Fringe activity, which encodes a fucose-specific β1,3 N-acetylglucosaminyltransferase, previously shown to modulate Notch receptor activity. Finally, Neurotic is essential for the physical interaction of Notch with its ligand Delta, and for Fringe ability to modulate this interaction in Drosophila cultured cells. We present here an unprecedented example of an absolute requirement of a protein glycosylation event for a ligand-receptor interaction. Our results suggest that O-fucosylation catalyzed by Neurotic is also involved in the Fringe-independent activities of Notch and may provide a novel on-off mechanism that regulates ligand-receptor interactions.

P-094

The regulatory role of Rab5 for vesicle-vesicle fusion of secretory granules and synaptic vesicles in the exocytotic processes

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Rab5 is a key regulator of an endocytic vesicle transport from plasma membrane to early endosome. Function of Rab5 on synaptic vesicles is, however, speculated to be different from that on endocytic vesicles, since synaptic vesicles can be reformed directly from presynaptic plasma membrane without passing through endosomal intermediates. Previously, we reported that dysfunction of Rab5 induced homotypic fusion of synaptic vesicles in Drosophila photoreceptor cells. To further investigate a novel Rab5 function, we optically studied neuroendocrine-like cells that we found at the proximal region of the posterior Malpighian tubule of Drosophila. In the cells, EGFP-Rab5 was localized on the secretory granules, which is efficiently labeled with FM1-43. Using the dye, we visualized exocytotic release of secretory granules in the cells expressing either Rab5S43N (GDP form) or Rab5Q88L (GTP form). The results demonstrated that Rab5 is essential for regulating the fusion between granules, while it is not required for the fusion between plasma membrane and granules. Furthermore, Rab5S43N suppressed release of synaptic vesicles at the neuromuscular junction, whereas Rab5Q88L facilitated its release. We conclude that Rab5 regulates the homotypic fusion of synaptic vesicles as well as of secretory granules, which is probably a key step in the exocytotic process.

P-095
dtb encoding a Drosophila T-box protein is required for CNS and motor neuron guidance

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Neurons that extend axons along specific paths must recognize and interpret molecular signals that guide growth cones toward a target. We have identified a Drosophila gene dtb that encodes a novel T-box protein. Dtb was expressed in a subset of CNS neurons and the lateral monoscolopidial chordotonal organ (lch1), lateral pentascolopidial chordotonal organ (lch5) and multidendritic neurons (dda) on PNS. Dtb was also expressed epidermal patches. Mutations in dtb are defective in the formation of longitudinal tracts in the CNS. dtb mutations also disrupt sensory axon pathfinding in the PNS. When dtb was misexpressed using a rho-GAL4/UAS-dtb system, commissures became thin or absent in CNS and motor axons branched excessively or crossed segments. Our results suggest that dtb is required for the development of longitudinal axon tracts between segment of the developing embryonic CNS and by sensory growth cones as they navigate toward the embryonic CNS.

P-096
Scad67 is a SP-RING protein and participates in the SUMO conjugation

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Scad67 mutants were isolated as dominant suppressors of the Dpp-signal-dependent wing cell overgrowth phenotype. Scad67 encodes a novel protein with a SP-RING motif. Scad67 is an evolutionally conserved molecule and we found two Scad67 homologous proteins in human and in other vertebrates. Scad67 mutants show embryonic to pupal stage lethality. And the mutant animal at the late 3rd instar larvae shows immature imaginal discs development. Loss-of-function study by means of inducible double strand RNAi indicated that Scad67 function is required in the wing and external sensory organ development. SP-RING motif was originally found in PIAS family proteins including Drosophila PIAS homolog Su(var)2-10/Zimp. Recent studies have shown that PIAS family proteins function as E3-SUMO (Small Ubiquitin like MOdifier) ligase and enhance SUMO conjugation against specific substrates. We observed facilitation of SUMO conjugation by over-expression of the human Scad67-1 and Scad67-2 in the culture cells. We propose that Scad67 is a novel family of SP-RING protein having SUMO-E3 ligase activity and presumably regulate gene expression in the nucleus.

P-097

Preventing “roundabout” trajectory of longitudinal axons. : ROBO silences the responsiveness to the segmental boundary cue, Netrin presented by Frazzled.

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ROUNDABOUT (ROBO) is a receptor for secreted guidance molecule Slit. “roundabout” is named after the longitudinal axon trajectory in the mutant. Longitudinal axons abnormally cross midline even if they are running along “Roundabout”. It is established that Slit-expressing cells in the midline prevent abnormal midline-crossing by haptotropism at least. However, in robo mutants, longitudinal growth cones project towards midline abnormally from the position even when they are still distant from the midline cells. It has been an open question whether the “roundabout” phenotype indicates the balance between attractive and repulsive field centered on the midline in wild type. Here we show that the misprojection of longitudinal axons in robo mutant is due to the long-range effect of Netrin that is relocalized by Frazzled, not to the attractive gradient of Netrin made by diffusion. Besides, ROBO is required in longitudinal pioneer neurons for suppression of the responsiveness to Netrin that is localized repeatedly in each segment. In robo mutants, the longitudinal pioneer neurons do not stop tracking the commissure, which presents Netrin by expressing Frazzled. Consequently, these axons fail to traverse the boundary of segmental units, showing “roundabout” trajectory of longitudinal axons.

P-098

Attenuation of sensitivity to trehalose by the expression of the IP3 absorbent IP3 sponge in a subset of gustatory receptor cells in Drosophila

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IP3 sponge was developed as a recombinant hyper-affinity IP3 absorbent, which was constructed on the basis of the ligand-binding site of the mouse type-1 IP3 receptor. We used this IP3 sponge to investigate the possible involvement of IP3 signaling in gustatory transduction in Drosophila. The Tre gene encodes a gustatory receptor protein that is primarily responsible for trehalose sensitivity. We carried out the feeding preference assay, in which adult flies starved for 20 hours were given a choice to take either 2mM sucrose or 10mM trehalose. In another series of experiments, the flies were tested for their ability to distinguish solutions containing two different concentrations of trehalose in a similar two-way choice paradigm. The two solutions used for the assay were colored red or blue, respectively, and the relative amount of two solutions consumed by a fly was estimated by the coloration of the abdominal part of the fly. When the IP3 sponge was expressed by means of the Gal4-UAS system with the Tre-promoter-driven Gal4 transgene, acuity in trehalose sensitivity was significantly decreased in both types of assays mentioned above. These observations are in keeping with the hypothesis that activation of the Tre receptor initiates IP3 signaling.

P-099

Genetic screening for mutations that affect the targeted axonal projection of the olfactory sensory neurons in Drosophila

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In order to detect thousands of volatile chemicals, the olfactory sensory system in mammals involves a large number of odorant receptors, each of which has different affinity to different chemicals and is expressed in a small subset of the olfactory sensory neurons (OSNs). Since the OSNs of each subset project their axons to only two or three specific foci among thousands of targets in a brain, this system has become an attractive model to reveal mechanisms of the axonal targeting in a complex neural circuitry. Although several molecules have been shown to be involved in the process, little is known how the precise targeting is achieved. In Drosophila, there is a similar yet simpler olfactory sensory system; the OSNs expressing a given odorant receptor also project their axons to only one or two specific targets. We conducted a MARCM-based genetic screening for mutations that affect the targeted axonal projection of the OSNs in this animal. Among ~1200 independent lines mutagenized with EMS, we have isolated a number of mutants showing various defects in the axonal projection. In this presentation, we will show the typical mutant phenotypes and discuss about the possible mechanisms underlying the targeted axonal projection.

P-100

Drosophila Damaged DNA Binding Protein 1 (D-DDB1) is an Essential Factor for Development

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Damaged DNA binding protein complex (DDB), thought to recognize (6-4) photoproducts and related structures, has been implicated to have a role in global genomic nucleotide excision repair (NER) and E2F-1 mediated transcription. The complex consists of a heterodimer of p127 (DDB1) and p48 (DDB2), the latter also being known as XPE. We reported previously that in Drosophila expression of the DDB1 (D-DDB1) gene is controlled by the DRE/DREF system, and external injury to DNA is not essential for D-DDB1 function. In the present study of the function of D-DDB1 in a multicellular system, we prepared transgenic flies which were knocked-down for the D-DDB1 gene due to RNA interference, and performed immunocytochemistry to ascertain the distribution of D-DDB1 in the eye imaginal disc. D-DDB1 was found to be abundant in the anterior of the morphogenetic furrow (MF). Whole body over-expression of dsRNA of D-DDB1 in Drosophila using a GAL4-UAS targeted expression system induced melanotic tumors and caused complete lethality. When limited to the eye imaginal disc, a severe rough eye phenotype resulted. D-DDB1 therefore appears to be an essential development-associated factor in multicellular organisms.

P-101

Overexpressing TRX genes enhance oxidative stress resistance in Drosophila

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Thioredoxin (TRX), an antioxidant molecule and related family proteins may play important roles in aging process and age-related diseases. To elucidate in vivo functions of TRX family genes in Drosophila, we generated transgenic flies with enhanced or reduced level of expression, and examined for their oxidative stress resistance. DNA constructs with overexpression or dsRNA for TRX family genes introduced into the fly genome using standard techniques. Oxidative stress resistance was measured by using Drosophila Activity Monitor with food containing H2O2. Among the five TRX family genes that have a sequence homology to human TRX, one was dead head (dhd), a maternal gene, whose loss-of-function mutation has been shown to be embryonic lethal. The remaining four genes, named Sister of dhd (Sid), Brother (Bod), Cousin (Cod), Aunt (Aud), whose amino acid identities to Dhd were 43, 38, 33, and 30 %, respectively. Sid and Aud had a C-terminal extension of 52 and 182 a.a., respectively. We found that flies overexpressing Aud live longer by 21% comparing to control under oxidative stress condition. Enhanced resistance to oxidative stress in transgenic flies overexpressing Aud suggested that the gene could control aging process and progression of diseases that are related to oxidative stress.

P-102

Analysis of a histone methyltransferase dG9a in Drosophila

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Histone methylation is involved in chromatin remodeling and regulation of transcription. G9a which is a H3K9 specific histone methyltransferase has found in mammal. Based on amino acid sequences of human G9a (hG9a), we searched database of Drosophila and found the gene EG:BACR37P7.2 which shows significant homology to mammalian G9a. We named this gene Drosophila G9a (dG9a). We cloned the entire dG9a cDNA by RT-PCR using mRNA from Drosophila embryos, and determined its nucleotide sequences. The deduced dG9a gene product is 1637 amino acids. DG9a contains ankyrin repeat, PreSET domain and SET domain. Cystein residues are conserved in the region behind SET domain that corresponds to Post SET domain of mammalian G9a. We found the domain which is conserved between hG9a and dG9a. RT-PCR was carried out to determine levels of dG9a mRNA at various developmental stage. The dG9a mRNA was detected throughout development expect in embryos until 12hr after fertilization. To investigate whether dG9a has HMTase activity, we performed an in vitro methylation assay. The HMTase activity of dG9a was different from that of mouse G9a (mG9a) on its substrate specificity. In addition we report the results of developmental Western blotting analysis.

Invited Poster Presentation

July 29 - July 30
The 6th Japanese Drosophila Research Conference

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The 2nd Japan-Korea Symposium

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JK-1

Transcriptional regulation of the *Drosophila catalase* gene

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Reactive oxygen species cause oxidative stress and aging. The *catalase* gene is a key component of antioxidative defense network. However, the molecular mechanisms regulating of the *catalase* gene expression are poorly understood. We generated transgenic flies carrying the *catalase-lacZ* fusion gene, containing the *catalase* promoter region (-1030 to +18) fused to *lacZ*. Our data on expression patterns and putative transcriptional regulators of the *catalase* gene will be presented.

JK-2

Spatial and temporal regulation of *HexC* gene is critical for development in *Drosophila melanogaster*

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We previously reported that *Rbp9* mutation causes an ovarian tumor phenotype. In the process of searching for genes differentially expressed in *Rbp9* mutants, we found that *HexC* gene is up regulated in *Rbp9* mutants. Head, thorax and abdominal parts of the adult flies were dissected and examined for the *HexC* expression. We found that *HexC* is up regulated only in the abdominal regions. The up regulation phenotype of *HexC* transcription seems to be tightly linked to the ovarian tumor phenotype, as similar pattern of *HexC* up-regulation was detected in other ovarian tumor lines such as *bam* and *otu*. Up regulation of Hexokinase has been observed in many mammalian tumors. Therefore, the elevation of glycolytic rate seems to be the general phenomenon in tumor formation. We further characterized the *HexC* gene and found that its expression is temporally regulated during development. Expression of *HexC* gene during the developmental period when endogenous HexC activity is absent caused dramatic decrease in body size. Both cell number and cell size was affected. To place the *HexC* gene in the hierarchy of cell size regulation, we overexpressed *HexC* gene with other genes that has been reported to have roles in cell size regulation. The result will be presented.

JK-3

Regulatory DNA elements for the expression of the *GTP cyclohydrolase I* gene in *Drosophila melanogaster*

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GTP cyclohydrolase I (GTP CHI) catalyzes the reaction which converts GTP to dihydroneopterin triphosphate. This reaction is a rate-limiting step in the biosynthesis pathway of BH4. It has been known that two kinds of differentially spliced transcripts for GTP CHI are produced from two promoters, head-specific promoter and constitutive promoter. We have analyzed the 5'-flanking region of head-specific promoter of *Drosophila GTP CHI* gene. From the transient transfection experiment, we found the minimal 5'-flanking region required for the efficient expression of GTP CHI is located between -73 and +35. The deletions between -73 ~ -57 and -73 ~ -41 reduce the promoter activities by about 2 fold and 5 fold, respectively. The transgenic fly was constructed using the head-specific GTP CHI promoter (-73 ~ +35)-*lacZ* fusion plasmid and analyzed by β-gal assay. The very high β-gal activities were detected in adult eye, suggesting the *GTP CHI* gene is highly expressed in adult eye and the head-specific promoter may contain eye-specific promoter element. We have also cloned and sequenced the promoter region of *GTP CHI* gene in *Drosophila virilis* to find out the conserved sequences in the promoter region of the *GTP CHI* gene between two species. We find a conserved palindromic sequence between -73 and -41. Interestingly, this palindromic sequence is also present in the promoter region of human *GTP CHI* gene, suggesting it has an important role in the expression.

JK-4

Important amino acid residues of norpA-PLC and their functions in *Drosophila* phototransduction

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In *Drosophila*, the phototransduction is mediated by a phosphoinositide-specific phospholipase C (PLC) encoded by the *norpA* gene. In order to find the specific amino acid residues important in the functions of norpA-PLC, six *norpA* mutants were chosen and studied by a combination of biochemical, molecular and electrophysiological approaches. These mutants were selected from 40 available *norpA* mutants based on their detectable level of norpA-PLC protein in the photoreceptor. The mutants exhibit allele-dependent reductions in protein levels and *in vitro* PLC activity levels. The mutants also show allele-dependent reductions in amplitudes and retardation in kinetics. Three mutants carry a missense mutation and two carry a nonsense mutation within the *norpA* coding sequence. One mutant, that has been considered as a null mutant, did not carry any mutations in protein-coding region, suggesting that the mutant contains the mutations in promoter region. The missense mutations reduce the specific *in vitro* PLC activity as well as the level of norpA-PLC protein. However, the nonsense mutations did not affect the specific *in vitro* PLC activity although the level of norpA-PLC protein is reduced in these mutants. Electrophysiological studies of these mutants, together with molecular and biochemical studies, suggest that: (1) reduced amount of protein correlates with an increase in response termination time; (2) deactivation rate is most severely affected when PLC is reduced to around and less than 20% wild type level; (3) amplification of signal occurs at the G-protein level in mutants with less than 20% wild type amount of the PLC protein.

JK-5

Catecholamine determines favorable temperature of *Drosophila*

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Catecholamine and serotonin are known to be mediated in thermoregulation in animals but their exact roles *in vivo* are still poorly understood. Using our temperature-preference assay, we investigated the effects of catecholamine and serotonin on thermoregulation by analyzing the behaviors of flies genetically manipulated in catecholaminergic and serotonergic neurons. Wild type flies showed strong temperature preference at about 25°C, but pale heterozygote mutant flies having scarce dopamine showed temperature preference significantly lower than 25°C (cryophilic). Cryophilic phenotype became more severe of pale heterozygote mutants harboring pale-RNAi and blockade of synaptic transmission on dopaminergic neuron made flies extremely cryophilic. Meanwhile, dopamine-overflowed flies overexpressing pale and dopa decaborxylase (DDC) showed temperature preference significantly higher than 25°C (thermophilic). These data show that the level of catecholamine is strongly correlated with favorable temperature of flies and suggest that the catecholamine may function as a negative regulator driving animal seeking cold conditions in thermoregulation.

JK-6

GenExel *Drosophila* Genome Browser – A database for GenExel's fly stock library

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GenExel has generated a vast fly stock library comprising over 100,000 transgenic lines so far for rapid screening of whole fly genome. As the second round of its fundamental resource construction, the insertion positions of P-element of every single transgenic line of GenExel's fly stock library were determined by inverse PCR and sequencing. Integrating these sequence data above and data from publicly available Genome Annotation of *Drosophila*, GenExel has generated its own database which is unique and best-fit for its mission: "GenExel *Drosophila* Genome Browser".

The Genome Browser contains 3 parts; Gene Map Viewer, Genome Annotation Query, and Genome Blast Search. The first part of the Genome browser is the core of the database. It provides the gene map of each AE and shows the insertion position of transgenic lines. Also search by fly line number, AE number, and CG number is available. Genome Annotation Query searches genome annotations with function categories, links to the gene map for the search results. Search by gene ontology or protein domain of InterPro is available. Genome Blast Search runs NCBI BLASTN. The development of Genome Browser will make it possible for GenExel to analyze candidate genes and gene products more rapidly and precisely.

JK-7

Toll-8 negatively regulates Dpp signaling in the developing *Drosophila* wing

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In *Drosophila*, Decapentaplegic (Dpp) is a key regulator of wing development *via* induction of target genes, including *spalt*, *omb*, and vg^{Q}, in a concentration dependent manner. Here we show that Toll-8, a member of Toll receptor family negatively regulates Dpp signaling. The protein has a modular structure of a Toll/Interleukin I Receptor (TIR) domain in the intracellular region and leucine rich repeats (LRR) in the extracellular region. Expression of the *toll-8* gene is confined in the lateral region of the wing disc and is repressed by Dpp signaling. Expression of Toll-8 suppresses the phenotypic defects by expression of Dpp, Tkv, or Mad in the wing. Ectopic expression of Toll-8 in the medial region of the wing disc negatively regulates expression of a Dpp downstream target gene, *spalt*, and this repression is restricted in the cells that just abut mosaic clones over-expressing Toll-8. Interestingly, the extracellular region of Toll-8 is sufficient for *spalt* repression, suggesting the cell non-autonomous mode of its action. Taken together, a novel cell-cell communication via Toll-8 may play an important role for the regulation of Dpp signaling.

JK-8

Evidence that *auro*, the fly dihydropterin deaminase gene, is directly involved in the biosynthesis of aurodrosopterin

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Dihydropterin deaminase, which catalyzes the conversion of 7,8-dihydropterin to 7,8-dihydrolumazine, was discovered from the head extract of *Drosophila melanogaster*. Since it had been demonstrated that 7,8-dihydrolumazine and PDA[1)] could be converted into aurodrosopterin, a minor eye pigment of *Drosophila melanogaster*, in the acidic condition. We postulated that the deamination reaction might be a key step in the aurodrosopterin synthesis. To prove this, we purified the deaminase to homogeneity by combination of gel permeation, phenyl-Sepharose chromatography and chromatofocusing. The purified enzyme has a molecular weight of about 48,000, as analyzed by SDS-polyacrylamide gel electrophoresis. MALDI-TOF mass spectrometry followed by peptide mass database searches identified the purified protein as a product of CG18143. The sequence of CG18143 turned out to be highly homologous to human and rat guanine deaminases, raising the possibility that it encodes the dihydropterin deaminase. The product of CG18143 when expressed in *E. coli* actually exhibited dihydropterin deaminase activity. The flies in which a P element is inserted at 150 base upstream to CG18143 possess much less deaminase activity, ~25% of wild type, with brownish eye color and decreased amount of aurodrosopterin. These results provide an *in vivo* evidence that the dihydropterin deaminase is directly involved in the biosynthesis of aurodrosopterin. Here we named the CG18143 gene as *auro*.

1)PDA : 2-amino-4-oxo-6-acetyl-7,8-dihydro-3H,9H-pyrimido[4,5-b][1,4]diazepine

JK-9

The roles of intrinsic and extrinsic factors in MP2 interneuron identity determination of the *Drosophila* CNS

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Neural identity determination of the central nervous system (CNS) is known to be influenced by the intrinsic and extrinsic factors during development. However, it is not well established how these two intrinsic and extrinsic factors control neural identity determination of the CNS. *Drosophila* is an excellent model organism for the study of molecular mechanisms underlying the neural identity determination during CNS development. Our study focused on the simple cell lineage of the MP2 interneuron that results in two vMP2 and dMP2 after single cell division in the *Drosophila* CNS. The *spitz/Egfr* signaling genes are known to provide the ventral neuroectoderm with extrinsic cues. It was shown that the number of MP2s was reduced by 50% in the *single-minded* (*sim*) mutant that is defective in activation of the EGFR signaling. When *sim* gene was overexpressed in the midline cells and in each segment of the ventral neuroectoderm, the number of MP2s was increased by 25-57%. It was tested whether cell cycle genes, *cyclin E* (*cyc E*) and *string* (*stg*)/cdc25 phosphatase and cell differentiation gene, *dacapo* (*dap*), one of the Kip family members play important roles as intrinsic cues in MP2 identity determination. This analysis revealed that the MP2 interneuron identity is partially defective by 20-30% reduction of MP2 marker expression in cell division mutants *cyc E* and *stg* and by more than 90% reduction in the *dap* mutant. In addition, the overexpression of *dap* in the MP2s resulted in ectopic MP2s. This result indicates that the CNS midline cells provide extrinsic signals essential for the establishment of MP2 lineage by the EGFR signaling pathway. It was also revealed that differentiation gene *dap* affects the expression of MP2 lineage markers more severely than cell division genes *cyc E* and *stg.* This study will help understand molecular mechanisms for identity determination of the CNS that are influenced by intricate interplay between intrinsic and extrinsic factors.