The making of a worm: genes, cells and the organism

Zhirong Bao

Developmental Biology Program, Sloan Kettering Institute, USA

A major challenge in developmental biology is to understand how networks of gene interactions give rise to cellular behaviors and how networks of cellular interactions give rise to the organism. We have been taking a systems biology approach to address these question. We have built a powerful platform for automated and systematic single-cell analysis of development in the *C. elegans* embryo. It includes microscopy techniques for long-term 3D time-lapse imaging, image analysis software for automated cell tracking and cell lineage tracing, mapping gene expression onto individual, tracked cells, as well as systematic single-cell measurements of cellular behaviors during development that include proliferation, differentiation and morphogenesis. More importantly, we have devised methods to infer mechanistic models of development from image data. In this case, the model describes cell fate differentiation as trajectories of cell fates with a sequence of binary fate choices as well as predicted gene modules and cell-cell signaling events that regulate each binary choice. I will discuss our most recent progress in analyzing 200 conserved emb genes, constructing a multi-scale model involving genes, cells and the embryo, and the challenges ahead in understanding how higher level properties of cells and the embryo emerge.

Contact: baoz@mskcc.org

SSBD: a quantitative database approach for understanding spatiotemporal dynamics of *C. elegans* development

Kenneth H.L. Ho[1], Yukako Tohsato[1], Koji Kyoda[1], Shuichi Onami[1,2]

[1]RIKEN Quantitative Biology Center, Kobe, Japan
[2]National Bioscience Database Center, JST, Tokyo, Japan

Major advances in live cell imaging and computer simulation have allowed us to capture and model the dynamical nature of biological development of *C. elegans*. New insight and discovery in systems biology can be gained by analyzing observation/simulation data in spatiotemporal biological dynamics. The challenge is to have a systematic approach to share, analyze and visualize quantitative data, which are multi-scale, multi-type and containing many different experiments. We developed SSBD (Systems Science of Biological Dynamics) (http://ssbd.qbic.riken.jp) as a free open data repository for a variety of quantitative data of biological dynamics in BDML (Biological Dynamics Markup Language) format. BDML is a unified data format designed to represent 4D spatiotemporal numerical data from single molecule to multicellular organisms. It allows us to represent different observations from different experiments of biological dynamics of *C. elegans* during development in a unified format. These data are stored together in SSBD allowing direct analysis and comparison. SSBD also utilises OMERO (Allan et al., 2012) internally to manage their corresponding microscopic images. There are currently more than 180 sets of 4D quantitative cell division dynamics experimental data on *C. elegans* embryo (Bao et al., 2006; Kyoda et al., 2013) together with some 2.2 million microscopic images stored in SSBD. Additional quantitative data extracted from 2D time-lapse DIC movies in PhenoBank (Sonnichsen et al., 2005) are being added to SSBD. Quantitative biological dynamics of pronuclear migration data, which were generated by computer simulation of *C. elegans* (Kimura and Onami, 2005), are also available in SSBD. A web browser based 4D visualization tool allows easy access to data online. A RESTful API allowing 3rd party applications, such as R, to access all or selected set of data directly from SSBD. Additional standalone analytical and visualization tools, e.g. BDML4DViewer and phenochar, are also available for download. SSBD also contains experimental data on zebrafish (Keller et al., 2008) and *D. melanogaster* embryos (Keller et al., 2010) and simulation results on *E. coli* (Arjunan and Tomita, 2010).

Contact: kenneth.ho@riken.jp

Defining regulatory pathways coupling cell division timing and cell fate differentiation in *C. elegans* by automated lineaging

Vincy Wing Sze Ho[1], Ming Kin Wong[1], Xiaomeng An[1], Jiaofang Shao[1], Daogang Guan[1], Kan He[1], Jinyue Liao[2], Long Chen[3], Xiaotai Huang[3], Yiji Xia[1], Leanne Chan[3], King Lau Chow[2], Hong Yan[3], Zhongying Zhao[1]

[1]Department of Biology, Hong Kong Baptist University, Hong Kong Special Administrative Region of China
[2]Division of Life Science, the Hong Kong University of Science and Technology, Hong Kong Special Administrative Region of China
[3]Department of Electronic Engineering, City University of Hong Kong, Hong Kong Special Administrative Region of China

Coordination of cell division paces is essential for proper formation of various tissues and organs during metazoan development. Failure in the coordination may lead to tumorous growth or abnormal cell death. How cell division paces are regulated *in vivo* to accommodate cell fate differentiation and tissue growth remains poorly understood. *C. elegans* embryogenesis provides a unique opportunity to address the issue due to its invariant development. To identify the regulatory proteins coupling cell division pace and cell fate determination or tissue growth, we performed a high-content RNAi screening of the defects in embryonic cell lineage and cell fate differentiation for around 800 *C. elegans* genes using automated lineaging. Specifically, we first prioritized the gene list based on their conservation and their reported defects after perturbation. We next imaged three embryos per gene derived from the injected parents followed by lineaging analysis. The injected strain carries both a lineaging and a tissue marker, allowing us to simultaneously trace both cell divisions and cell fate differentiation for every minute of embryogenesis. We curated embryonic cell lineage up to 350 cell stage with two replicates for most of the perturbed genes. Our data indicated a tight coupling between cell division paces and cell fate differentiation or tissue growth. Quantitative analysis of the defects in cell division paces and cell fate differentiation or tissue growth allowed us to assemble regulatory pathway coupling the two biological processes, which is likely to have direct relevance to human biology. Gene inactivation coupled with lineaging analysis not only confirmed and extended the roles of well-studied genes, but also helped assign detailed cellular functions to uncharacterized ones. Our quantitative dataset with single cell resolution and high temporal resolution provided a rich resource for inference of genetic network governing spatial and temporal coordination of cell divisions during metazoan cell fate formation and/or tissue growth.

Contact: zyzhao@hkbu.edu.hk

Using cell-specific RNA-seq to study sex-specific gonadogenesis

Mary B. Kroetz, David Zarkower

University of Minnesota, USA

The *C. elegans* somatic gonad was the first organ to be lineaged and develops very differently in the two sexes, starting with the first division of the somatic precursors Z1 and Z4. However, despite much study, the genetic pathways that direct early gonadal development including its sexual dimorphism, remain largely unknown. To help define the genetic networks that regulate gonadal development in both sexes, I employed cell-specific RNA-seq. Single sex populations of animals were generated using sex determination pathway mutants and a highly specific GFP reporter was used to isolate Z1/Z4 or the Z1/Z4 daughter cells by FACS of dissociated larval cells from synchronized animals harvested just before and just after the division of Z1/Z4, when somatic gonad sexual differentiation begins. I identified transcripts present in both sexes in Z1/Z4 or Z1/Z4-daughter cells and for comparison I also identified the transcripts in both sexes in whole animals at both time points. Pairwise comparisons of samples identified several hundred gonad-enriched transcripts including most known Z1/Z4-enriched mRNAs, and reporter analysis has confirmed the effectiveness of this approach. Prior to the Z1/Z4 division only about 25 sex-enriched Z1/Z4 transcripts were detectable, but less than six hours later during development, I identified about 250 sex-enriched transcripts in the Z1/Z4-daughters, of which about a third also were enriched in the somatic gonad cells compared to whole animal. This blossoming of sex-specific gene expression indicates that a robust sex-specific developmental program, some of it gonad-specific, initiates in these cells around the time of the first Z1/Z4 division. Additional analyses of sex-enriched transcripts from Z1/Z4-daughters identified families of genes that are enriched in each sex. For example male-enriched transcripts identified in Z1/Z4-daugther cells include 7 homeobox genes and 3 Patched genes, which could indicate molecular modes by which male fates are imparted on the gonad primordium. Currently I am characterizing the molecular functions of the newly identified transcripts. Loss of function phenotypes are being determined individually and pairwise within gene families with an emphasis on male-enriched transcripts. This approach should not only help define the genetic networks driving gonadal development but will also provide an effective general approach to identify genetic networks driving development of other cell lineages in *C. elegans*.

Contact: kroet006@umn.edu

Genetics and the *C. elegans* Embryo: Past and Present

Bruce Bowerman

Institute of Molecular Biology, University of Oregon, USA

Over the past twenty some years, my laboratory has used classical genetics to investigate cell polarity and cell division in the early *C. elegans* embryo. We began by screening for non-conditional, maternal-effect, embryonic-lethal mutants with defects in cell fate patterning, scored by visual inspection of terminally differentiated mutant embryos. These early studies led us toward an interest in cytoskeletal dynamics and the isolation of temperature-sensitive, embryonic-lethal mutants with early embryonic cell division defects. The discovery of RNA interference then led to the widespread use of RNAi to knock down and investigate the early embryonic requirements for essential *C. elegans* genes. Thus RNAi presented a substantial challenge to the classical genetic analysis of early embryogenesis, given the labor and time required to positionally clone mutant loci identified in unbiased screens using chemical mutagenesis to alter genomic DNA sequences. In response to the transformative discovery of RNAi, we explored the use of RNAi in modifier screens (if you can't beat them, join them). These screens were designed to identify non-essential genes that when knocked down in function could restore embryonic viability to temperature-sensitive, embryonic-lethal mutants grown at semi-permissive temperatures. More recently the development of whole genome sequencing technologies, particularly Illumina-based DNA sequencing, has made it possible to identify the causal mutations in mutant strains much more rapidly than in days past. Thus we have revived our focus on the isolation and analysis of temperature-sensitive, embryonic-lethal mutants, and we are now exploring previously neglected categories of mutant phenotypes. My talk will touch on various highlights during this genetic journey, consider the strengths and weaknesses of the different genetic approaches we have explored over the years, and ponder how much value remains in being a classical geneticist interested in *C. elegans* embryogenesis.

Contact: bbowerman@molbio.uoregon.edu

Quantitative analysis of microtubule orientation and organelle movements during meiotic cytoplasmic streaming in *C. elegans* early embryos

Kenji Kimura[1,2], Tohru Sasaki[3], Kohta Sato[3], Ritsuya Niwayama[1], Seiichi Uchida[3], Akatsuki Kimura[1,2]

[1]Cell Arch. Lab., Natl. Inst. of Genet., Japan
[2]Dept. of Genet, SOKENDAI, Japan
[3]Grad. Sch. and Fac. of Info. Sci. and Elec. Eng., Kyushu Univ., Japan

During the oocyte-to-embryo transition, intracellular organelles move actively to remodel the oocyte. Such organelle movements are crucial for early embryogenesis. In *C. elegans* one-cell stage embryos, collective flows of organelles are generated during two distinct cytoplasmic streaming. The first streaming occurs during meiosis in a kinesin and microtubule dependent manner[1], and the second streaming occurs after meiosis in a myosin and actin dependent manner. The latter streaming is known to be initiated upon the symmetry breaking to establish the anterior-posterior polarity[2]. We have previously quantified the second streaming by using an image processing method and developed a computational modeling of the streaming[3]. However, it has not been fully understood how the collective movement of organelles in the first meiotic streaming emerges in the non-polarized embryo. To dissect the mechanism, we developed image processing methods to quantify microtubule orientation and organelle movements during the meiotic streaming. The quantitative analyses revealed that the orientation of cortical microtubules correlates with the flow direction of organelles. When the meiotic streaming was inhibited experimentally, the biased orientation of microtubules was lost. The results suggest that streaming itself has a role to align cortical microtubules to facilitate and stabilize the collective flow of the meiotic streaming. We are also constructing a computational model of the streaming.

1. Yang H. et al, 2003 Dev Biol 260, 245-259.
2. Munro E. et al, 2004 Dev Cell 7, 413-424.
3. Niwayama R. et al, 2011 PNAS 108, 11900-5.

Contact: kekimura@nig.ac.jp

Coordinated actomyosin kinetics in generating self-organized pattern formation in the cell cortex

Masatoshi Nishikawa[1,2,3], Sundar Naganathan[1,2,3], Guillaume Salbreux[3], Stephan W Grill[1,2,3]

[1]BIOTEC, TU Dresden, Germany
[2]MPI-CBG, Germany
[3]MPI-PKS, Germany

The cell cortex, which consists of crosslinked actin filaments and non-muscle myosin beneath the cell membrane, is responsible for cell mechanical processes such as deformation and maintenance of cell shape, cytokinesis, and rearrangement of intercellular geometry. These processes are driven by an active contractile stress generated by myosin motor proteins. In the highly contractile cortex, myosin is observed to accumulate in the characteristic aggregate. These myosin foci have finite lifetime, they first form and later disappear.Once formed, they exhibit complex behavior by mechanically interacting each other to move toward or away before disappears. Although a large number of studies have deciphered the molecular components and their regulation for activating actomyosin contractility, the overall dynamical behavior and the coupling between the mechanical and biochemical regulation for this self-organizing behavior has received little attention. We have studied actomyosin foci dynamics in the one cell stage *C. elegans* embryo. We developed a novel method to characterize turnover kinetics of cortical components in the comoving frame of reference, and have used this method to study, actin, myosin and RHO-1 kinetics. From mass conservation law, temporal change of the components is balanced by advective flux and turnover. Thus we can extract the kinetic change of cortical components by evaluating advective flux. This provides us with the kinetic landscape and the vector field of actomyosin kinetics in the phase space of cortical component concentrations. Interestingly, we observe actomyosin kinetics has a single stable fixed point. There is no biochemical instability in actomyosin kinetics to generate foci dynamics. Instead, foci formation is driven by a coordination between actomyosin mechanochemistry and RHO-1 kinetics. Our results revise our understanding of actomyosin self-organized pattern formation, a structure central to morphogenetic force generation processes.

Contact: masnishi@mpi-cbg.de

A conditional knockout system based on the combination of UV/TMP single-copy integration methods and deletion mutant strains in *C. elegans*

Eriko Kage-Nakadai[1,2], Rieko Imae[1,3], Osamu Funatsu[1], Sayaka Hori[1], Yuji Suehiro[1], Sawako Yoshida[1], Shohei Mitani[1]

[1]Department of Physiology, Tokyo Women's Medical University School of Medicine, Japan
[2]Advanced Research Institute for Natural Science and Technology, Osaka City University, Japan
[3]Graduate School of Pharmaceutical Science, The University of Tokyo, Japan

Single-copy integration of excisable transgenes enables the complete gene inactivation that is essential for creating conditional knockout (cKO) mutants. Many Let or Ste mutants isolated so far, remain to be stably analyzed. Currently, we established a Cre/LoxP-mediated gene excision system using the ultraviolet trimethylpsoralen (UV/TMP) single-copy integration (SCI) method. To visualize Cre/LoxP-mediated gene excision, tester strains possessing single-copy of floxed NLS::GFP were generated. Multi-copy transgenic strains that express Cre recombinase under the various promoters were crossed into the tester strains, resulting in the tissue-specific and heat-shock-dependent gene excision.

We had found that the RNAi knockdown of *pros-1*, which encodes *C. elegans* homolog of *Drosophila prospero* / mammalian *prox1*, affected several aspects of neural development and function. *pros-1* was selectively expressed in the glia, the excretory canal cell, and unidentified neurons. Because of the lethality of the *pros-1* deletion mutants, further genetic analysis had remained to be performed. Here we developed a cKO system based on the combination of the UV/TMP SCI method and pre-existing deletion mutants to generate *pros-1* cKO mutant strains. Briefly, the floxed *pros-1* SCI was generated using UV/TMP methods and crossed into *pros-1* deletion mutants. We confirmed that the phenotype of *pros-1* mutants was successfully rescued. The rescued animals were then crossed into multi-copy tissue-specific Cre transgenic animals. As a result, glia-specific *pros-1* cKO mutants exhibited defects in the neural function. These results showed that *pros-1* activity in the glia was involved in the neural function.

Contact: nakadai@life.osaka-cu.ac.jp

Chromosome dynamics during meiosis in *C. elegans*

Ofer Rog, Yumi Kim, Abby Dernburg

Department of Molecular and Cell Biology, University of California, Berkeley, USA
Howard Hughes Medical Institute, USA
Life Sciences Division, Lawrence Berkeley National Laboratory, USA
California Institute for Quantitative Biosciences (QB3), USA

C. elegans has emerged as an outstanding system to investigate the molecular mechanisms that govern meiosis, the specialized cell cycle that allows organisms to produce haploid germ cells for reproduction. In addition to the genetic advantages of the worm, the organization of the worm germline in a temporal gradient helps to reveal how genetic mutations or other perturbations affect meiotic progression. In addition, with the recent development of methods for expression of fluorescent transgenes in the germline, we can now observe the dynamic process of meiosis by fluorescence microscopy in living animals. This *in vivo i*maging approach has enabled us to better understand how homologous chromosomes pair, synapse, and eventually segregate to different daughter cells. We have investigated how special chromosome regions known as “pairing centers” interact with the microtubule cytoskeleton to mediate homolog pairing and synapsis. More recently we have focused on the formation of the synaptonemal complex (SC), a protein polymer that stabilizes interactions between homologous chromosomes and regulates meiotic recombination. I will present our recent findings about the regulation and dynamics of homolog pairing and synapsis.

Contact: afdernburg@lbl.gov

Protein phosphatase 4 promotes chromosome pairing and synapsis, and contributes to maintaining crossover competence with increasing age

Aya Sato-Carlton[1], Xuan Li[1], Asako Sugimoto[2], Peter Carlton[1]

[1]Institute for Integrated Cell-Material Sciences, Kyoto University, Japan
[2]Graduate School of Life Sciences, Tohoku University, Japan

Meiosis creates gametes by distributing diploid genomes containing homologous chromosome pairs into daughter cells that receive only one of each chromosome. To segregate correctly at the first meiotic division, chromosomes must pair and synapse with their homologous partners, and undergo crossover recombination, which requires breaking and repairing the DNA strands of all chromosomes during meiotic prophase. How chromosomes identify each other's homology and exclusively pair and synapse with their homologous partners, while rejecting illegitimate synapsis with non-homologous chromosomes, remains obscure. In addition, how the levels of recombination initiation and crossover formation are regulated so that sufficient but not deleterious levels of DNA breaks are made and processed into crossovers is not understood well. We have discovered that the highly conserved Serine/Threonine phosphatase PP4 plays essential roles in these events in *C. elegans*. We show that the PP4 homolog PPH-4.1 is required independently to carry out four separate functions involving meiotic chromosome dynamics: synapsis-independent chromosome pairing, restriction of synapsis to homologous chromosomes, programmed DNA double-strand break initiation, and crossover formation. Using quantitative imaging of mutant strains, including super-resolution (3D-SIM) microscopy of chromosomes and the synaptonemal complex, we show that independently-arising defects in each of these processes in the absence of PPH-4.1 activity lead ultimately to meiotic nondisjunction and embryonic lethality. Interestingly, we find that defects in DNA double-strand break initiation and crossover formation, but not pairing, become even more severe in the germlines of older mutant animals, indicating an increased dependence on PPH-4.1 with increasing maternal age. Our results demonstrate that PPH-4.1 plays multiple, independent roles in meiotic prophase chromosome dynamics and maintaining meiotic competence in aging germlines. PP4's high degree of conservation suggests it may be a universal regulator of meiotic prophase chromosome dynamics.

Contact: asato@icems.kyoto-u.ac.jp

LIN-41 regulates continuous centrosome inactivation during oogenesis through suppression of CDK-1 pathway in *C. elegans*

Rieko Matsuura, Tomoko Ashikawa, Yuka Nozaki, Daiju Kitagawa

Centrosome Biology Laboratory, Center for Frontier Research, National Institute of Genetics, Japan

Centrosome is a major microtubule organizing center (MTOC) in most animal cells. The MTOC activity of centrosomes is crucial for proper chromosome segregation in mitosis and meiosis during spermatogenesis. By contrast, during oogenesis, two successive meiotic cell divisions occur without centrosomes, since maternal centrosomes are inactivated gradually losing pericentriolar material components, and are thereafter eliminated during meiotic prophase I. It is believed that such elimination of maternal centrosomes during oogenesis and the inheritance of paternal centrosomes to progeny are crucial for maintaining the precise number of centrosomes in the fertilized zygote and for proper sexual reproduction. Despite being the widely conserved phenomenon in most metazoans, the mechanisms governing centrosome inactivation and elimination during oogenesis remain poorly understood.

Here, we conducted comprehensive RNAi screening in the *C. elegans* gonad to identify genes required for centrosome inactivation and/or elimination during oogenesis. We have thus far screened a whole set of genes (529 genes) required for proper meiotic events, using a direct visualization of GFP-γ Tubulin to monitor centrosomal behavior at all stages of oogenesis. Remarkably, we found that, upon RNAi-mediated depletion of LIN-41, there was a significant delay in centrosome elimination and also that centrosomes become activated and functional enough to nucleate microtubules affecting meiotic chromosome behavior around the late pachytene/diplotene stages of the meiotic cell cycle. We also observed that depletion of LIN-41 led to aberrant nuclear envelope breakdown and chromosome condensation during oogenesis. Furthermore, we demonstrated that such phenotypes provoked by depletion of LIN-41 were largely suppressed in the cyclin-dependent protein kinase 1 (*cdk-1*) mutant background. Taken together, our data suggest that the M-phase specific events including centrosome activation are strictly suppressed during oogenesis through the LIN-41/CDK-1 pathway to ensure the critical meiotic events and eventual generation of intact oocytes.

rmatsuur@nig.ac.jp

Acentrosomal spindle pole assembly in *C. elegans* oocytes

Amy Alexis Connolly[1], Valerie Osterberg[1], Sara Christensen[1], Chenggang Lu[2], Kathy Chicas-Cruz[1], Paul E Mains[2], Shawn Lockery[1], Bruce A Bowerman[1]

[1]University of Oregon, USA
[2]University of Calgary, Canada

As in many animals, *C. elegans* oocyte meiotic spindles assemble in the absence of centrosomes and yet are bipolar. While the positioning of the Meiosis I oocyte spindle in *C. elegans* has been studied in some detail, how the poles assemble in the absence of centrosomes, and how these spindles are limited to a bipolar state, remain poorly understood. In screens for temperature-sensitive (TS), embryonic-lethal mutants, we have identified TS mutations in multiple loci and have used these mutants to gain insight into oocyte meiotic spindle pole assembly. Thus far we have identified mutations in four genes: *or447*ts in *klp-18*, which encodes a kinesin 12 family member; *or1178*ts in *mei-1*, which encodes catalytic subunit of the microtubule severing complex katanin; *or645*ts in *aspm-1*, which encodes a calponin homology domain scaffolding protein; and *or1092*ts and *or1292*ts in *klp-7*, which encodes a kinesin 13/MCAK family member. By using live cell imaging of oocytes from transgenic strains expressing GFP and mCherry fusion to spindle-associated proteins, we have found that *klp-18* promotes spindle bipolarity (as suggested previously by other investigators), and that both microtubule severing by katanin, and the scaffolding protein ASPM-1 promote pole assembly. More recently, we have found that *klp-7* limits Meiosis I spindle assembly to a bipolar state. In *klp-7(-)* mutants, we observed an excess accumulation of microtubules and the assembly of extra spindle poles during oocyte Meiosis I. Moreover, reducing *klp-7* function can restore bipolarity in a monopolar spindle *klp-18(-)* mutant background. We also observed a more transient assembly of extra spindle poles in mutants lacking kinetochore function, and found that disruption of kinetochores in *klp-7(-)*mutants exacerbates the extra spindle pole phenotype. We suggest that in the absence of centrosomes both an increased accumulation of microtubules and a loss of microtubule attachment to kinetochores can promote the assembly of extra spindle poles during oocyte meiotic cell division.

Contact: amyc@uoregon.edu

Aurora A kinase AIR-1 is required for microtubule assembly of female meiotic spindles

Eisuke Sumiyoshi, Yuma Fukata, Satoshi Namai, Asako Sugimoto

Graduate School of Life Sciences, Tohoku University, Japan

In animal cells, mitotic spindles consist of microtubules (MTs) formed at centrosomes and around chromosomes. In contrast, female meiotic spindles are assembled independently of centrosomes, because centrosomes are eliminated during oogenesis. In *C. elegans*, female meiosis, MTs formed in oocyte nuclei are assembled into bipolar meiotic spindles at prometaphase. At anaphase, the bipolar spindle MTs shrinks, while parallel MT arrays are formed between chromosomes to be segregated. Assembly of these MTs of female meiotic spindles do not require γ-tubulin complex which is a major MT nucleator.

Previously, we reported that a kinase-independent activity of Aurora A (AIR-1) is essential for the assembly of chromatin-stimulated, γ-tubulin-independent MTs in mitosis of *C. elegans* early embryos, but the role of AIR-1 during meiosis has been unclear. Here, we examined whether AIR-1 is involved in the assembly of MTs constituting female meiotic spindles.

Live imaging analysis demonstrated that, in *air-1(RNAi)* oocytes, MTs were formed in oocyte nuclei and they clustered around chromosomes at meiotic prometaphase I; however, these MTs shrunk without assembling bipolar spindles, and MT arrays between chromosomes at anaphase were not formed. Similar MT behaviors were observed in meiosis II. Expression of the kinase-inactive form of AIR-1 in *air-1(RNAi)* oocytes/embryos did not rescue these meiotic phenotypes, suggesting that the kinase activity of AIR-1 is required for these processes. Consistently, AIR-1 localized to the female meiotic spindles throughout meiosis; the kinase-active form of AIR-1 detected with anti-phospho-AIR-1 antibodies localized on chromosomes and MTs near chromosomes at prometaphase, and on MT arrays between segregating chromosomes at anaphase. These results indicate that, in contrast to mitosis, the kinase activity of AIR-1 is required for the assembly and/or maintenance of centrosome-independent MTs that constitute female meiotic spindles.

Contact: esumiyos@m.tohoku.ac.jp

RNA-binding Protein ATX-2 Interacts with SZY-20 and ZYG-1 to Regulate Centrosome Assembly and Size

Mi Hye Song, Mike Stubenvoll, Jeff Medley, Miranda Irwin

Biological Sciences, Oakland University, MI, USA

Centrosomes are critical sites for controlling microtubule dynamics, and exhibit dynamic changes in size during the cell cycle. As cells progress to mitosis, centrosomes recruit more microtubules to form bipolar spindles. The *szy-20* gene encodes a centrosome-associated RNA-binding protein that negatively regulates ZYG-1, a key centrosome duplication factor. It has been shown that *szy-20* mutants possess enlarged centrosomes, causing abnormal microtubule processes and embryonic lethality. SZY-20 contains putative RNA-binding domains; mutating these domains perturbs RNA-binding by SZY-20 *in vitro* and its capacity to regulate centrosome size *in vivo*.

To further elucidate the roles of SZY-20 and RNA-binding in regulating centrosome assembly and size, we sought to identify factors associated with SZY-20. By proteomics, we identified an RNA-binding protein ATX-2 reproducibly pulled-down with SZY-20. Consistent with its physical association, depleting ATX-2 produces embryonic lethality and cell division defects seen in *szy-20(bs52)* embryos, including a failure of cytokinesis and polar body extrusion, and abnormal spindle positioning. Thus, ATX-2 is required for proper cell divisions in which *atx-2* acts synergistically with *szy-20*: Knocking down ATX-2 in *szy-20(bs52)* mutants enhanced the embryonic lethality and cell division defects.

Genetic analyses indicate that not only *szy-20* but also *atx-2* negatively regulates centrosome assembly. *atx-2(RNAi)* partially restores centrosome duplication to *zyg-1(it25)* embryos. Knocking down both *szy-20* and *atx-2* almost completely restored bipolar spindle formation to *zyg-1(it25)* embryos, suggesting a positive interaction between *atx-2* and *szy-20* in regulating centrosome assembly. Intriguingly, ATX-2-depleted embryos exhibit enlarged centrosomes as shown in *szy-20(bs52)*. Embryos partially depleted of ATX-2 possess significantly increased ZYG-1 and pericentriolar materials at centrosomes. Enlarged centrosomes are more evident in a null allele *atx-2(wm210)* embryos, suggesting an inverse correlation between the amount of ATX-2 and centrosome size.

Quantitative western and immunostaining analyses show that ATX-2 is almost absent in *szy-20(bs52)*, much similarly in *atx-2(wm210)*, but unaffected in *zyg-1* mutants. In contrast, SZY-20 levels are unaffected in *atx-2* or *zyg-1* mutants. Thus, SZY-20 acts upstream of ATX-2 and promotes the level of ATX-2, which in turn opposes ZYG-1 to limit centrosome size. In this study we show that an RNA-binding protein, ATX-2, plays a role in negatively regulating centrosome assembly and size.

Contact: msong2@oakland.edu

ATX-2, the *C. elegans* ortholog of human Ataxin-2, is necessary for cytokinesis, ER morphology and P granule segregation

Megan M Gnazzo, Ahna R Skop

University of Wisconsin-Madison, USA

Spinocerebellar ataxia type 2 (SCA2) is a devastating neurodegenerative disease caused by a polyglutamine expansion in human Ataxin-2. These mutations in Ataxin-2 lead to protein mis-folding and aggregation causing Purkinje cell degeneration and a loss of motor coordination or ataxia. Human Ataxin-2 localizes to stress granules, important stores for RNAs and associated RNA binding proteins under environmental stress. Although studies have suggested that Ataxin-2 may play roles in RNA metabolism and stress granule function, the cellular role of Ataxin-2 is unclear. Understanding the cellular functions of Ataxin-2 could provide new insights into SCA2 disease mechanisms. Our lab identified Ataxin-2 from isolated mammalian midbodies and the corresponding *C. elegans* ortholog, ATX-2, displayed defects in cytokinesis (Skop et al, 2004). To determine the cellular functions of Ataxin-2 during development, we are characterizing ATX-2 in the early *C. elegans* embryo using an *atx-2* ts allele and RNAi techniques. In *atx-2* RNAi and *atx-2* ts (ne4297ts) embryos, cytokinesis defects occur during meiosis and mitosis. In particular, polar body extrusion failures, spindle orientation and ER morphology defects are observed. In the absence of ATX-2, the ER structure collapses resulting in aggregates of ER throughout the cytoplasm, concentrating near the anterior cortex. This ER collapse phenotype is unique to ATX-2, as CAR-1 RNAi embryos do not display a complete collapse of the ER during mitosis. To determine where ATX-2 localizes during the cell cycle, we have created CRISPR and Mos-1 single copy insertion constructs for two of the identified ATX-2 isoforms (one full length and one N-terminally truncated). Lastly, P granule segregation defects have been observed in 70% of the ATX-2 depleted embryos suggesting that ATX-2 may play a role in RNA regulation and localization during development. We hypothesize that ATX-2 may play a role in mediating the targeting and regulation of RNAs localized in the midbody or mediating the local translation of these RNAs throughout the cell cycle. We will present our current understanding of the cellular functions of ATX-2 in the early *C. elegans* embryo.

Contact: gnazzo@wisc.edu

Morphology of actomyosin network is regulated by colocalization of RhoGAP RGA-3/4

Masashi Fujita[1], Shuichi Onami[1,2]

[1]RIKEN Quantitative Biology center, Japan
[2]National Bioscience Database Center, JST, Japan

Numerous processes in the cell resort to the proper morphology of the cytoskeleton. The central regulator of the actin cytoskeleton is Rho GTPases, which are inactivated by RhoGAP proteins. Interestingly, various RhoGAPs colocalize with the actin cytoskeleton *in vivo*. It is unclear whether these colocalization affects the spatial pattern of the cytoskeleton. RGA-3 and RGA-4 are the RhoGAP proteins that regulate cortical actomyosin network during polarity establishment and cytokinesis in one-cell embryos. Here we show that RGA-3 colocalizes with cortical actomyosin and this colocalization significantly affects the morphology of the actomyosin network. We observed that mCherry::RGA-3 colocalizes with GFP::Moesin, which is a maker of F-actin, in the cell cortex. In order to investigate the function of this colocalizatoin, we expressed a truncated form of RGA-3 that localizes to cytoplasm. This cytoplasmic RhoGAP rescued the hypercontractile phenotype of *rga-3/4*. However, there was a difference in the spatial pattern of the cortical actomyosin. The cortical F-actin in wild-type embryos has multiple foci, which repeatedly assemble and disperse. In contrast, the cortical F-actin in the cytoplasmic RhoGAP embryos formed stable large agglomeration. Myosin light chain (MLC-4) in the cell cortex also showed a similar trend. These differences could not be explained by the expression level of RhoGAP since neither partial depletion nor coexpression of RGA-3/4 did not cause the agglomeration. Instead it should be attributed to the colocalization because a Moesin fusion of the RhoGAP restored the cortical F-actin to a similar morphology to wild type. We propose a model in which the colocalization of RhoGAP constitutes a negative feedback that prevents the agglomeration of the contractile actomyosin network.

Contact: m-fujita@riken.jp

The Rho GTPase-Activating Protein RGA-7 controls the CDC-42 / WSP-1 pathway and filopodia formation during ventral enclosure in *Caenorhabditis elegans* embryos

Marie-Hélène Ouellette, Emmanuel Martin, Germain Lacoste-Caron, Karim Hamiche, Sarah Jenna

Chemistry department, UQAM, Canada

Ventral enclosure (VE) consists in the migration of hypodermal cells from their original dorsal location towards the ventral midline during late embryogenesis. Migration of hypodermal cells involves formation of actin-rich protrusions called filopodia, especially in the most anterior hypodermal cells, called leading cells. Cell migration and filopodia formation are controlled by parallel pathways leading to activation of the RAC- and CDC-42-specific effectors WVE-1/WAVE and WSP-1/WASP. RHO-1 has also been involved in VE in parallel with these two pathways. While the implication of Rho GTPases in the control of VE is clear, little is known about their regulation during this process.

We have identified a new gene coding for a Rho GTPase-Activating Protein (GAP), *rga-7*. *rga-7* mutant embryos exhibit various VE defects, including reduction of filopodia formation in leading cells and developmental arrest before elongation. *rga-7* codes for three transcripts: *rga-7l, -m* and *-s* controlled by two promoters. Generation of transgenic animals revealed that these transcripts are expressed in hypodermal cells during epidermal morphogenesis and that expression of *rga-7l/m* and to a lesser extend *rga-7s*, can rescue *rga-7* function when expressed in mutant animals. Expression of *rga-7l/m* under the control of the hypodermal cell specific *lin-26p* promoter also fully rescues *rga-7* function in mutant background, suggesting that *rga-7* functions in hypodermal cells during VE. *In vitro* characterization of RGA-7 GAP domain revealed specific GAP activity towards CDC-42 and RHO-1 but not towards CED-10, RAC-2, MIG-2 and CRP-1. To assess RGA-7 GAP activity *in vivo*, we tested genetic interactions between *rga-7* strong loss-of-function allele and reduction of function alleles of *wsp-1* and *let-502*, two *cdc-42* and *rho-1* effectors know to control epidermal morphogenesis. We showed that *wsp-1* allele fully suppressed *rga-7* VE-defects, while *let-502* allele enhanced them, suggesting that *wsp-1* functions downstream of *rga-7* and *let-502* in parallel. To confirm that down-regulation of CDC-42 by RGA-7 is required for VE, we overexpressed a CFP-fusion of CDC-42 in hypodermal cells and observed similar defects that in rga-7 mutant. We conclude that catalysis of CDC-42 GTPase activity by RGA-7 plays an important role for the generation of filopodia in leading cells and the migration of hypodermal cells during VE upstream of WSP-1.

Contact: jenna.sarah@uqam.ca

Dual mechanisms ensure PAR-1 cortical asymmetry in *C. elegans* zygote

Ravikrishna Ramanujam[1], Fumio Motegi[1,2,3],

[1]Temasek Life Sciences Laboratory, Singapore
[2]Mechanobiology Institute, Singapore
[3]National University of Singapore, Singapore

The transformation of an embryo to a mature adult relies on a precise sequence of highly coordinated events that converge towards establishment of cellular asymmetry. The hallmark of polarized animal cell is the establishment of an opposing gradient of two kinases, namely atypical protein kinase C (aPKC) and partitioning defective kinase PAR-1. These kinases are engaged in an antagonistic relationship and segregate into mutually exclusive domains at cell cortex, but the mechanisms that control their spatial asymmetry remains poorly understood.

Here we show that the asymmetric PAR-1 gradient is a consequence of dual mechanisms, in which PAR-1 adaptor protein PAR-2 functions to “protect” PAR-1 from cortical exclusion by aPKC and “stabilize” PAR-1 on the plasma membrane. The PAR-1 gradient arises after PAR-2 accesses the cortex, and the cortical level of PAR-1 shows a PAR-2 dose-dependency. PAR-1 kinase utilizes its conserved C-terminal KA domain to interact with PAR-2 and phospholipids. Phosphorylation by aPKC interferes with the ability of the KA domain from binding to lipids *in vitro* and cortical association *in vivo*. PAR-2 acts as a competitive inhibitor against PAR-1 phosphorylation by aPKC, protecting PAR-1 from cortical exclusion by aPKC. Furthermore, PAR-2 slows down and stabilizes dynamics of PAR-1 at the cortex in a manner independent of aPKC, thus supporting PAR-1 at the posterior cortex. These two mechanisms likely act in parallel to ensure the establishment of a proper gradient of PAR-1 complementary to aPKC.

Taken together, our findings illustrate the principles underlying the cellular gradients that rely on a spatial modification of the balance between two antagonizing kinases. We anticipate our findings will provide molecular insights for understanding PAR-1-related disorders, such as Alzheimer’s diseases and autism.

Contact: ravi@tll.org.sg

Regulation of asymmetric cell division by Wnt signaling

Hitoshi Sawa

National Institute of Genetics, Japan

In *C. elegans*, Wnt signaling regulates asymmetry of most somatic cell divisions. This asymmetry is regulated by the Wnt/β-catenin asymmetry pathway in which signaling components localize asymmetrically during divisions. For example, WRM-1/β-catenin localizes to the anterior side of the cell cortex and counter-intuitively to the posterior nucleus at telophase. We showed that APR-1 that is localized asymmetrically through cortical WRM-1 regulates asymmetry of spindle structure that is required for asymmetry of WRM-1 nuclear localization. In addition, we found that APR-1 is localized asymmetrically to the anterior cortex also in P0 zygote through the function of the PAR proteins but not WRM-1. APR-1 stabilizes spindle microtubules as in EMS. By comparing spindle movements and pulling forces in wild-type and *apr-1* embryos, we found that APR-1 decreases spindle pulling forces to regulate asymmetric spindle movements during the P0 division.

Because WRM-1 is localized to the posterior nuclei after most somatic cell divisions, these dividing cells are polarized in the same orientation along the anterior-posterior axis. We found that polarities of six epithelial stem cells are redundantly regulated by four Wnt genes, since quadruple Wnt mutants show nearly random orientation of polarity. In contrast, in the middle embryonic stages, cell polarity is grossly normal at least in triple Wnt mutants (*cwn-1 cwn-2 egl-20*) despite complete embryonic lethality of the embryos, suggesting that Wnts may not be required for polarity at this stage.

Similarly, we showed that Wnts are not essential for polarity of somatic gonadal precursors (SGPs), since polarity is normal even in quintuple Wnt mutants. However, we recently found that Wnt are required in the absence of LIN-17/Frizzled receptor. For example, *lin-17 cwn-1 cwn-2* mutants show about 90% gonadless phenotype due to loss-of-polarity of SGPs. I will discuss how Wnts and their receptors control SGP polarity.

Contact: hisawa@nig.ac.jp

BLMP-1/Blimp-1 regulates the spatiotemporal cell migration pattern in *C. elegans*

Yi-Ting Cheng[1], Tsai-Fang Huang[1], Chun-Yi Cho[1], Kiyoji Nishiwaki[2], Shih-Chung Chang[3], Yi-Chun Wu[1,4,5,6]

[1]Institute of Molecular and Cellular Biology, National Taiwan University, Taiwan
[2]Department of Bioscience, Kwansei Gakuin University, Japan
[3]Department of Biochemical Science and Technology, National Taiwan University, Taiwan
[4]Department of Life Science, National Taiwan University, Taiwan
[5]Research Center for Developmental Biology and Regenerative Medicine, National Taiwan University, Taiwan
[6]Institute of Atomic and Molecular Sciences, Academia Sinica, Taiwan

The spatiotemporal regulation of cell migration is crucial for animal development. Although many spatial signals guiding cell migration have been identified and characterized, little is known about temporal signals and the mechanisms integrating the two. In the *Caenorhabditis elegans* hermaphrodite, the two somatic distal tip cells (DTCs) undergo a stereotyped migration pattern. The heterochronic genes *lin-29*, *daf-12*, and *dre-1*, which, respectively, encode a zinc finger transcription factor, a steroid hormone receptor, and the F-Box protein of an SCF ubiquitin ligase complex, act together to promote the ventral-to-dorsal migration of the DTCs in the late third larval (L3) stage [1]. Guidance receptor UNC-5 is both necessary and sufficient for the dorsal migration of DTCs away from the ventrally concentrated netrin UNC-6. We found that *blmp-1*, which encodes a protein similar to the mammalian zinc finger transcription repressor Blimp-1/PRDI-BF1, prevented premature dorsalward turning of the DTCs by inhibiting premature *unc-5* transcription [2]. Interestingly, BLMP-1 protein was found to be present in the DTCs before, but not during or after, they underwent the dorsal turn. Constitutive expression of *blmp-1* at the time when BLMP-1 would normally disappear delayed *unc-5* transcription and caused retardation of the dorsal turn, demonstrating the functional significance of *blmp-1* downregulation. The correct timing of the disappearance of BLMP-1 was redundantly regulated by *daf-12*, *lin-29*, and *dre-1* and allowed the DTCs to make the dorsal turn. DAF-12 and LIN-29 were shown to repress *blmp-1* transcription, while DRE-1 was shown to bind to BLMP-1 and might mediate BLMP-1 degradation. We also found that FBXO11 (the human DRE-1 ortholog) bound to PRDI-BF1 and Cullin1 (a component of an SCF ubiquitin ligase complex) in human cell cultures. Consistently, a recent report shows that DRE-1/FBXO11 regulates the posttranslational level of BLMP-1/PRDI-BF1 [3]. We have therefore identified a gene regulation circuit that regulates the correct timing of the DTC dorsal turn. Our data suggest evolutionary conservation of these interactions and underscore the importance of BLMP-1/PRDI-BF1 degradation mediated by DRE-1/FBXO11 in cellular state transitions during metazoan development.

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2. Huang et al. (2014) PLoS Genet. (in press)
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Contact: prouyouh@hotmail.com

Cooperative control of cell cycle exit by G1/S inhibitors and SWI/SNF chromatin remodeling factors

Suzan A Ruijtenberg, Inge The, Sander van den Heuvel

Developmental Biology, Padualaan 8, Utrecht University, The Netherlands

Critical in development and tissue homeostasis is the transition of proliferating precursor cells to post-mitotic differentiated cells. The cell-cycle arrest associated with this transition involves the retinoblastoma (Rb) tumor suppressor and Cyclin-dependent Kinase Inhibitory proteins (CKIs). It remains unclear, however, to what extent these G1/S inhibitors are necessary and sufficient to coordinate cell cycle exit with differentiation.

Development of the nematode *C. elegans*, is characterized by tightly coordinated cell proliferation and differentiation, as illustrated by its reproducible cell lineage and lack of mutants with severe over-proliferation phenotypes. Combined inactivation of the *C. elegans* Rb-related gene *lin-35* and CKIs leads to extra cell division, but does not prevent formation of fully differentiated post-mitotic cells. To be able to identify what additional regulators promote cell cycle exit, we developed a CRE-LoxP based recombination-lineage tracing system for *C. elegans*. Using this system, we observed a remarkable tissue specific role for general cell cycle regulators. Moreover, combination with a candidate-based RNAi screen revealed an important contribution of SWI/SNF chromatin remodeling subunits in cell cycle exit. Cell-type specific inactivation of SWI/SNF alone resulted in weak over-proliferation of precursor cells in the mesoblast lineage, while combined inactivation of *lin-35* Rb, *cki-1* Kip1 and *swsn-1* BAF155/170 resulted in dramatically increased cell numbers. A similar tumor phenotype resulted from combined SWI/SNF and *lin-23* β-TrCP knockdown. Our data demonstrate lineage specific control of cell cycle exit through cooperation between SWI/SNF components and general cell cycle inhibitors. These observations are particularly relevant given the recently identified frequent mutation of SWI/SNF subunit genes in a wide variety of human cancers.

Contact: s.j.l.vandenheuvel@uu.nl

LEP-2/Makorin post-transcriptionally regulates LIN-28 in the juvenile-to-adult transition

R Antonio Herrera, Karin Kiontke, David Fitch

Center for Developmental Genetics, Biology, New York University, New York, USA

C. elegans and mammals share a developmental milestone: the juvenile-to-adult (J/A) transition, or puberty, respectively. In mammals, the timely onset of puberty requires two genes, LIN28b and Makorin-3 (Mkrn), and altering their levels results in early or delayed puberty. The *C. elegans* J/A transition is scheduled by heterochronic genes and *lin-28* is a key regulator. Here we show that the sole *C. elegans* Mkrn, *lep-2*, also controls the timing of the J/A transition and does so by repressing LIN-28 at the post-transcriptional level.

lep-2 was isolated in a screen for defects in male tail tip morphogenesis (TTM), a process by which, during the L4 stage, the tail tip changes shape from long and pointed to short and rounded. *lep-2* mutants display many retarded phenotypes: (1) TTM is delayed until adulthood. Due to this delay, young adult *lep-2* males have long larval-like tail tips that will undergo TTM as the male ages. (2) The adult cuticle of both sexes does not have the adult-specific collagen ROL-1. (3) Supernumerary molts occur in both sexes. (4) *lep-2(-)* adult males cannot perform wild-type mating behavior, although ray, fan and spicule morphology are unaffected. Interestingly, seam cell development and production of adult alae are wild-type in *lep-2* mutants. This suggests that *lep-2* acts redundantly or is not involved in regulating the timing of seam cell development. It also explains why screens for seam cell phenotypes failed to find *lep-2* as a heterochronic gene.

Genetic epistasis analysis shows that *lep-2* represses *lin-28* activity to promote the J/A transition. *lin-28* RNAi is sufficient to suppress the TTM defect in *lep-2* mutants. In wild type, LIN-28 is down-regulated during the L2 stage. However, in *lep-2* mutants this down-regulation does not occur to the same degree. *lin-28* mRNA levels are unaffected, demonstrating that LEP-2 regulates LIN-28 post-transcriptionally. We are using a LIN-28::Dendra2 fusion protein to see how LEP-2 represses LIN-28: by halting LIN-28 translation or by aiding LIN-28 degradation. LEP-2 and LIN-28 may interact directly; LEP-2/Mkrn is a putative E3 ubiquitin ligase and we are testing for LIN-28 ubiquitinylation and proteasomal degradation.

Together, our findings suggest deep conservation of genes involved in the timing of the J/A transition in animals. Because of this conservation, we suggest that *C. elegans* is a good model to study basic mechanisms of pubertal timing in mammals.

Contact: rah321@nyu.edu

How does the animal change its behavioral patterns during growth?

Manabi Fujiwara, Noriko Sato, Shinichi Maruyama, Takeshi Ishihara

Kyushu University, Japan

Animals adequately change their behavioral patterns depending on their developmental stage. We found that *C. elegans* larvae show a different preference in odorant from the adult; larvae show a chemotactic defect to a specific odorant, diacetyl. We also found that the germline loss, caused either by laser ablation of the germline precursor cells or by *glp-1* mutations, results in the diacetyl-specific chemotactic defect in adult animals. These results implicate that germline, which proliferates from 2 cells to 2000 cells through the larval stages and the adult, mediates the chemotactic behavioral change in *C. elegans*.

To identify molecular machinery for the regulation, we isolated a suppressor mutant of the diacetyl-specific chemotaxis defect of the germline-loss mutant, *glp-1*. We identified the suppressor mutation in *myo-1* gene, which encodes a myosin specifically expressed in the pharynx. The *myo-1* mutation causes a defect in the pumping movement of the pharynx. Since another pumping defective mutant, *eat-2*, also suppressed the chemotactic defect of *glp-1*, we concluded that feeding defects can mimic the effects of the germline proliferation, and enhance the chemotactic response to diacetyl in the germline-loss mutant. Interestingly, in the genome-wide RNAi screening, we found that the chemotaxis is restored in *glp-1* by RNAi knockdown of genes involved in mitochondria function including electron transport chain components and ATP synthase subunits. These results suggest that the germline proliferation may affect the neural function through the changes in energy states of animals.

Our data also suggest that ascarosides is involved in the regulation, since the mutation in *daf-22*, which encodes an enzyme for ascaroside synthesis, suppressed the chemotaxis defect to diacetyl of *glp-1*. We found that *glp-1* shows the decreased sensitivity to ascarosides. We hope that further analyses will reveal the mechanism how the animals change their behavior depending on the sexual maturation, energy states, and population density.

Contact: fujiwara.manabi.734@m.kyushu-u.ac.jp

The Tetraspanin TSP-21 positively modulates bone morphogenetic protein signaling in *Caenorhabditis elegans*

Zhiyu Liu, Lindsey Szymczak, Taner Aydin

Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY 14853, USA

The bone morphogenetic protein (BMP) pathway is critical for multiple developmental and physiological processes. In *C. elegans*, the BMP-like Sma/Mab pathway regulates multiple processes, including body size and male tail patterning. We have previously shown that loss-of-function mutations in the zinc finger-containing protein SMA-9 cause a dorsal to ventral fate transformation in the hermaphrodite postembryonic mesodermal M lineage, and that mutations in the BMP-like Sma/Mab pathway specifically suppress the *sma-9* M lineage phenotype (Foehr et al., 2006). Our *sma-9* suppression assay is highly specific and sensitive for identifying novel components in the Sma/Mab pathway. Using this assay, we have previously identified two evolutionarily conserved modulators of the Sma/Mab pathway, DRAG-1/RGM and UNC-40/neogenin/DCC (Tian et al., 2010, 2013). We have now carried out a large-scale *sma-9* suppressor screen and identified 40 new *sma-9* suppressors. A combination of complementation tests, snip-SNP mapping and whole genome sequencing showed that thirty-one of these suppressors are mutations in known Sma/Mab pathway components. Among the nine remaining alleles, two are mutations in *tsp-21*, which encodes one of the twenty-one tetraspanin proteins in *C. elegans*. Tetraspanins are a distinct family of transmembrane proteins with four transmembrane domains. Molecular genetic analyses showed that TSP-21 positively modulates Sma/Mab signaling at the ligand-receptor level, and that TSP-21 is expressed and functions in the signal-receiving cells to promote Sma/Mab signaling. They are known to organize membranes into the so-called tetraspanin-enriched microdomains (TEMs) that are also enriched in cholesterol and glycosphingolipids. While depleting cholesterol did not appear to affect Sma/Mab signaling, mutations in enzymes involved in glycosphingolipid biogenesis caused defects in Sma/Mab signaling. Our work provides *in vivo* evidence that both tetraspanins and glycosphingolipids are required to positively promote BMP signaling. Current research aims to determine mechanistically how they function to promote BMP signaling.

Contact: zl93@cornell.edu

TRIMming pluripotency

Cristina Tocchini[1,2], Jeremy Keusch[1], Sarah B. Miller[1], Susanne Finger[1,2], Heinz Gut[1], Michael Stadler[1], Rafal Ciosk[1]

[1]Friedrich Miescher Institute for Biomedical Research, Maulbeerstrasse 66, 4058 Basel, Switzerland
[2]University of Basel, Petersplatz 1, CH-4003 Basel, Switzerland

The cytoplasm of oocytes is capable of reprograming somatic nuclei to a pluripotent state, a process known as somatic cell nuclear transfer. Despite this property, oocytes do not undergo embryogenesis without a specific trigger (*e.g.* fertilization), suggesting that their reprogramming potential is kept at bay by repressive mechanisms.

In light of this, we conducted a genetic screen in *C. elegans* to understand how oocyte reprogramming potential is controlled. As a readout, we used a reporter for embryonic genome activation (EGA) to identify premature entrance into a pluripotent state. This screen allowed us to identify LIN-41, a TRIM-NHL protein expressed in the cytoplasm of developing oocytes, as a regulator of pluripotency. In the absence of LIN-41, developing oocytes not only induce EGA but also fully lose their germ cell identity. Rather than completing growth and meiotic arrest, LIN-41 mutants enter the mitotic cell cycle and undergo somatic differentiation, events that normally occur during the very next developmental stage, *i.e.* embryogenesis. Thus, LIN-41 emerges as a component of the timing mechanism that delays the onset of early embryonic events in oocytes, thereby regulating the transition between generations.

Previous studies have shown that LIN-41 is a member of the so-called heterochronic pathway in the soma and its mechanism of action is thought to involve mRNA regulation. To gain a better understanding of LIN-41 function in oocytes, we conducted structure-function experiments on its different domains and could identify and create mutants where the germline function could be uncoupled, at least in part, from the somatic one.

Our study is the first to identify a role for any TRIM-NHL protein in the maintenance of germ cell pluripotency. Furthermore this is the first example of a factor that regulates pluripotency specifically in oocytes.

Contact: cristina.tocchini@fmi.ch

The SET-2/SET1 histone H3K4 methyltransferase maintains pluripotency in the *Caenorhabditis elegans* germline

Francesca Palladino, V. Robert, M. Mercier, R. Ciosk, J. Merlet, S. Janczarski, C. Bedet

Ecole Normale Supérieure de Lyon, France
Université Lyon, France

Histone H3 Lys 4 methylation (H3K4me) is deposited by the conserved SET1/MLLmethyltransferases acting in multiprotein complexes including Ash2 and Wdr5. While individual subunits contribute to complex activity, how they influence gene expression in a specific tissue remains largely unknown. In *Caenorhabditis elegans*, SET-2/SET1, WDR-5.1 and ASH-2 are differentially required for germline H3K4 methylation. Using expression profiling on germlines from animals lacking set-2, ash-2 or wdr-5.1, we show that these subunits play unique and redundant functions to promote expression of germline genes and repress somatic genes. We further show that in set-2 and wdr-5.1 deficient germlines, somatic gene misexpression is associated with conversion of germ cells into somatic cells, and that nuclear RNAi acts in parallel with SET-2 and WDR-5.1 to maintain germline identity. These findings uncover a unique role for SET-2 and WDR-5.1 in preserving germline pluripotency, and underline the complexity of the cellular network regulating this process.

Contact: francesca.palladino@ens-lyon.fr

HTZ-1 and MYS-1 act redundantly to maintain cell fates in somatic gonadal cells through repression of *ceh-22* in *Caenorhabditis elegans*

Yukimasa Shibata[1], Hitoshi Sawa[2,3], Kiyoji Nishiwaki[1]

[1]Sch. of Sci. and Tech., Kwansei Gakuin Univ., Japan
[2]RIKEN CDB, Japan
[3]NIG, Japan

The stable maintenance of acquired cell fates is important during development and for maintaining tissue homeostasis. Histone modification, including methylation on lysine 27 of histone H3, is one of the major strategies used by cells to maintain their fates. We previously reported that *C. elegans* acetylated-histone-H4 (AcH4)-binding protein, BET-1, acts downstream of MYST family histone acetyltransferases, MYS-1 and MYS-2, to establish and maintain cell fates in multiple cell lineages.

We found that, in the *bet-1* pathway, the histone H2A variant HTZ-1/H2A.z and MYS-1 is required for the maintenance of cell fates in a redundant manner. BET-1 controlled the subnuclear localization of HTZ-1. HTZ-1, MYS-1, and BET-1 maintained the fates of the somatic gonadal cells (SGCs) through the repression of a target, *ceh-22*, which induced the formation of the leader cells of the gonad. Nuclear spot assay revealed that HTZ-1 localized to the *ceh-22* locus in SGCs. Therefore, *ceh-22* is a direct target of HTZ-1, and is repressed by HTZ-1, MYS-1 and BET-1.

We also found that H3K27 demethylase, UTX-1, had an antagonistic effect relative to HTZ-1 and BET-1 in the regulation of *ceh-22*. Genome-wide analyses revealed that stronger signals for HTZ-1 were observed in the region where H3K27 is less methylated, and vice versa. In addition, because UTX-1 enhances the co-localization between HTZ-1 and *ceh-22* locus, H3K27me3 prevents the deposition of HTZ-1. These results suggest that HTZ-1 and MYS-1 repress *ceh-22* when UTX-1 removes H3K27me on the *ceh-22* locus, thereby maintaining the fates of SGCs.

It is reported that HTZ-1 occupancy on promoter regions is similar to that of RNA polymerase II, however, less of a correlation between HTZ-1 occupancy and transcriptional activity. Together with the transcriptional repression by HTZ-1, we proposed that HTZ-1 regulates the pausing of RNA polymerase II. Because the poised state is ready-to-go for transcriptional activation, this mechanism may maintain fates in cells that are ready to respond to developmental signals.

Contact: yukshibata@kwansei.ac.jp

Sequential partitioning of histone methylation and demethylation activities ensures the robustness of natural transdifferentiation

Steven Zuryn[1], Arnaud Ahier[1], Manuela Portoso[2], Esther Redhouse White[1], Marie-Charlotte Morin[1], Raphaël Margueron[2], Sophie Jarriault[1]

[1]IGBMC (Institut de Génétique et de Biologie Moléculaire et Cellulaire), Strasbourg, France
[2]Curie Institute, Paris, France

Whereas postmitotic somatic cellular identity is generally a stable feature of multicellular organisms, natural interconversions between functionally distinct somatic cell types (aka transdifferentiation or Td) have been reported in species as diverse as jellyfish and mice. By contrast with experimentally induced transdifferentiation, often fairly inefficient and incomplete, some natural Td events occur with remarkable precision and efficiency. For example, our laboratory has recently shown that a rectal cell suddenly looses its differentiated identity and is reprogrammed into a motoneuron with invariant precision, in 100% of the wild type *Caenorhabditis elegans* animals (n > 2 200). As the molecular events that ensure such invariance are poorly understood, the efficiency and reproducibility observed in this defined reprogramming event represent a powerful and as yet unexploited avenue in which to probe the important mechanisms ensuring robust cell conversion.

Using unbiased and then targeted genetic screens affecting this well defined event, we have found that a conserved H3K27me3/me2 demethylase, JMJD-3.1, and the H3K4 methyltransferase Set1 complex act to ensure efficient transdifferentiation of post-mitotic *Caenorhabditis elegans* hindgut cells into motor neurons. This fascinating Td event proceeds through discrete steps: dedifferentiation and then re-differentiation into the new cell type, similarly to vertebrate examples of Td, such as newt lens regeneration. We found that invariant conversion requires precise, sequential organisation of each histone modifying activity into these discrete phases of Td within single converting cells. Functional partitioning of activities is achieved through temporary active nuclear degradation of JMJD-3.1 and phase-specific interactions with transcription factors (TF) that have conserved roles in cell plasticity and terminal fate selection. Our data suggest a model where the dynamic balance between different histone methylations promotes ultraprecise hindgut to motor neuron conversion, while TFs appears critical to drive the process. Furthermore, we found that these histone-modifying activities additionally play a critical role in insulating the process against variations when exposed to stressful environmental conditions, hence ensuring a robust outcome. Our *in vivo* results suggest that Set1 and JMJD-3.1 perform highly conserved roles across phyla to reinforce TF-driven de-differentiation and re-differentiation phenomena (particularly in suboptimal conditions), leading to robust cell conversion. These dynamic roles may be amplified in cell populations to ensure efficient regeneration of whole tissues and organs.

Contact: sophie@igbmc.fr

Essential roles of XRN2 and its novel binding partner PAXT-1 in RNA turnover and *C. elegans* development

Takashi Miki, Hannes Richter, Stefan Rueegger, Dimos Gaidatzis, Helge Grosshans

Friedrich Miescher Institute for Biomedical Research, Switzerland

XRN2 is an essential eukaryotic exoribonuclease that processes and degrades various substrates. We identified the previously uncharacterized protein R05D11.6/PAXT-1 as a subunit of an XRN2 complex in *C. elegans*. Targeted *paxt-1* inactivation through TALEN-mediated genome editing reduced XRN2 levels, decreased miRNA turnover activity, and resulted in worm death, which could be averted by overexpressing *xrn-2*. Hence, stabilization of XRN2 is a major function of PAXT-1. A truncated PAXT-1 protein retaining a predicted domain of unknown function (DUF3469) sufficed to restore viability to *paxt-1* mutant worms, elevated XRN2 levels, and bound to XRN2. This domain was found in additional metazoan proteins and mediated interaction of human CDKN2AIP/CARF and NKRF/NRF with XRN2. Thus, we have identified a bona fide XRN2-binding domain (XTBD) that can link different proteins, and possibly functionalities, to XRN2. In *C. elegans*, miRNAs are the only currently known targets of the XRN2-PAXT-1 complex. To identify additional physiological substrates and processes controlled by XRN2, we have performed genetic modifier screens and genomics analyses. The results of these approaches converge on an important but specific role of XRN2 in shaping the cellular transcriptome. Its mechanism and target specificity will be discussed at the meeting.

Contact: takashi.miki@fmi.ch

Trafficking of synaptic vesicle proteins

Sandhya P. Koushika

DBS-TIFR, Mumbai, India

Neurons communicate with each other through synapses. It is a challenging task to bring all components necessary for this process to the synapse from the distant cell body that can be up to a meter away in humans. The axon that connects the cell body with the synapses also provides a highway for the necessary cargo movement, enabling neurons to both develop and maintain synaptic connections. Synaptic vesicles are a prominent and essential axonal cargo. The molecular motor, UNC-104, picks up synaptic vesicles in the cell body, moves them along the axon and delivers them at their destination, namely synapses. I will discuss the regulatory mechanisms we have identified involved in (i) formation of a synaptic vesicle protein transport carrier and (ii) cargo movement towards in the neuronal process.

Contact: koushika@ncbs.res.in

Neurons and glia cooperate in assembly of the embryonic *C. elegans* nerve ring

Georgia Rapti, Shai Shaham

The Rockefeller University, New York, NY, 10065, USA

Central nervous system (CNS) assembly involves multiple cell-recognition events, including axon guidance and fasciculation. These events have been studied extensively in model systems, yet how a connected brain emerges from these selective-contacts remains mysterious. Glia have been suggested to act as guideposts for axons en route to their final destination, however, a detailed molecular understanding of this glial function is not currently at hand. To understand how glia and neurons cooperate to form a CNS, we have been studying assembly of the *C. elegans* nerve ring (NR), a neuropil consisting of ~170 axons enveloped by four astrocyte-like CEPsh glia and six mesodermal GLR glia. From a screen of fluorescent reporters expressed in subsets of embryonic neurons and glia, we determined that the NR is populated in a stereotypic and orderly manner, with glial processes extending early in conjunction with a small set of axons. Genetic ablation of CEPsh glia in embryos revealed that these cells are essential for the insertion of diverse axon classes into the NR, consistent with our imaging results. To identify genes regulating NR formation, we performed a forward genetic screen in which mutants with amphid neuron entry defects resembling those in CEPsh glia-ablated animals were sought. Amphid neurons enter the NR late, allowing both early and late NR assembly factors to be identified. Of the mutants identified, we have focused on one with a surprisingly penetrant (~70%) defect in NR axon entry. We demonstrated that axonal defects in this strain arise from two independent mutations. Each mutation alone results in mild NR entry defects (5-15%), but together the mutations synergize to block NR axon entry in more than 70% axons of amphid neurons or other interneurons. The lesioned genes encode conserved intracellular proteins: a GTPase regulator and a proprotein convertase, and our studies suggest that both function at the time of NR morphogenesis. Surprisingly, rescue of the double-mutant phenotype is only achieved by expressing either gene in both glia and early-entering NR axons. Our results open the door to uncovering previously unrecognized axon guidance genes that function in redundant pathways, and suggest that glia play a pivotal role in CNS assembly in *C. elegans*.

Contact: grapti@rockefeller.edu

Extracellular cues that pattern dendritic morphogenesis

Xintong Dong[1], Kang Shen[1], Chieh Chang[2]

[1]Stanford University, USA
[2]Cincinnati Children's Hospital, USA

Robust dendrite morphogenesis is a critical step in the development of reproducible neural circuits. However, little is known about the extracellular cues that pattern complex dendrite morphologies. In *C. elegans*, the sensory neuron PVD establishes stereotypical, highly-branched dendrite morphology. We have identified a tripartite ligand-receptor complex of membrane adhesion molecules that is both necessary and sufficient to instruct spatially restricted growth and branching of PVD dendrites. The ligand complex SAX-7/L1CAM and MNR-1 function at defined locations in the surrounding hypodermal tissue, while DMA-1 acts as the cognate receptor on PVD. Mutations in this complex lead to dramatic defects in the formation, stabilization, and organization of the dendritic arbor. Ectopic expression of SAX-7 and MNR-1 generates a predictable, unnaturally patterned dendritic tree in a DMA-1 dependent manner. A Furin-like proprotein convertase, KPC-1, functions in the PVD neuron to ensure that the signal is received at the right place and right time. Loss of KPC-1 results in a dramatic "wrapping" phenotype with most of the dendrites trapped around hypodermal-seam cell junctions where SAX-7 is also enriched. Our genetic and biochemical data support the model that KPC-1 is required to limit the activity of DMA-1 around the primary dendrite where the newly formed branches need to extend away, while allowing the SAX-7/MNR-1/DMA-1 signaling to occur at the hypodermal-muscle junction where tertiary and quaternary branches should form.

Contact: xdong1@stanford.edu

Spatial Control of Neurite Branching by Wnt-Frizzled/PCP Signaling

Chun-Hao Chen, Chun-Liang Pan

Institute of Molecular Medicine, National Taiwan University, Taipei, Taiwan

Spatial control of axon branching is crucial for the establishment of precise connectivity of the nervous system. However, the molecular mechanisms that specify axon branching sites remain incompletely understood. The Wnt family of secreted glycoproteins had been shown to direct neuronal migration and axon growth cone navigation. Here we examine their role in specifying axon branching pattern in the bilaterally symmetric PLM touch neurons. The PLM has an anterior neurite that generates a single synaptic branch at a fairly invariable location. Mutations in the Wnt genes *egl-20* and *cwn-1* shifted the PLM branching site proximally. In the *mig-14/Wntless* mutant where Wnt secretion was generally compromised, the PLM branching site became randomized along the PLM neurite. Wnt expression that mimicked the endogenous Wnt gradient rescued the proximal branching phenotype, whereas expression that reversed the Wnt gradient did not. We found that mutations in *mig-1*/*Frizzled* and *vang-1*/*Van Gogh*/*Strabismus* also caused proximal branching defects, and genetic analysis suggested that *cwn-1*, *egl-20*, *mig-1* and *vang-1* act in the same pathway. MIG-1 proteins were confined to the proximal PLM process in a punctate pattern, which was delocalized and became diffuse along the entire PLM neurite when *cwn-1* and *egl-20* were lost. We showed that a highly localized F-actin patch, which emerged from an initially diffuse distribution, preceded PLM branch outgrowth and defined the PLM branching site. The distribution of this F-actin patch seemed mutually exclusive to that of MIG-1, and it shifted proximally in the *cwn-1; egl-20* or the *mig-1* mutants. We hypothesize that Wnts specify PLM branching sites by spatially restricting F-actin assembly to the distal neurite through MIG-1 and VANG-1.

Contact: blackman0927@hotmail.com

The role of TDP-43 in axonal transport of mRNA

Justin C. Chaplin, Marie Mangelsdorf, Ramesh K. Narayanan, Robyn H. Wallace, Peter G. Noakes, Massimo A. Hilliard

Queensland Brain Institute, The University of Queensland, Australia

Amyotrophic lateral sclerosis (ALS) is a lethal neurodegenerative disorder causing progressive muscle weakness and wasting, and is characterized by ubiquitinated protein inclusions in the motor neurons of patients. A major component of these inclusions is the RNA binding protein TDP-43, and dominant mutations in TDP-43 have been reported as causative of ALS. Despite this, the function of TDP-43 in the nervous system and its role in the pathogenesis of ALS remains unclear. TDP-43 is known to be involved in RNA processing. A critical step in understanding its contribution to ALS is to identify the RNA transcripts it specifically regulates, and determine how mutation of TDP-43 can alter this regulation. We hypothesize a potential role of TDP-43 in the axonal transport of mRNA to the neuromuscular junction for local translation and synaptic maintenance.

In order to test this hypothesis we have generated a *C. elegans* model of ALS by expressing both the wild type, and an ALS-causing mutation, of human TDP-43 specifically in the GABAergic motor neurons. We found that while the wild type allele did not induce any significant changes in neuronal morphology or structural maintenance, expression of mutant TDP-43 produced clear axonal degeneration defects. The presynaptic loci of these neurons, visualized using a tagged vesicle associated protein RAB-3, were disrupted upon expression of the mutant allele, while remained largely intact in animals expressing wild type protein. Both these phenomena were adult onset and progressive in nature, recapitulating key aspects of ALS. To investigate cellular localization, a GFP::TDP-43 fusion protein was then expressed in the same GABAergic motor neurons. Localization was predominantly nuclear for both wild type and mutant alleles, with some expression in the cytoplasm and in puncta consistent with synaptic loci. This supports the theory that TDP-43 is shuttled between the nucleus and the synapse, and could play a part in axonal transport.

We are now using a molecular strategy that allows the visualization of intracellular mRNA transport in the neurons of *C. elegans* to directly determine the role of TDP-43 in axonal transport of specific target RNAs.

Contact: j.chaplin@uq.edu.au

A pair of RNA binding proteins modulates synaptic transmission, behavior, and alternative splicing in distinct neuron classes

Adam D Norris, John A Calarco

Harvard University, USA

Alternative splicing plays a key role in the function of the nervous system. However, little is known about the extent to which alternative splicing is regulated between distinct neurons, the factors controlling such splicing decisions, and the neurophysiological roles played by networks of isoforms. By monitoring alternative splicing at single cell resolution in *Caenorhabditis elegans*, we demonstrate that differential splicing within neurons is frequent and subject to highly specific regulation. We identify two conserved RNA binding proteins, UNC-75/CELF and EXC-7/Hu/ELAV, that regulate differential splicing between GABAergic and cholinergic neurons, and modulate extensive and overlapping networks of isoforms encoding neuronal proteins. We use the UNC-75 exon network to discover novel regulators of synaptic transmission, and to identify unique roles for isoforms of UNC-64/Syntaxin, a protein required for synaptic vesicle fusion. Our results indicate that combinatorial regulation of alternative splicing in distinct neurons represents a key evolutionary mechanism to specialize metazoan nervous systems.

Contact: adamnorris@fas.harvard.edu

Combinatorial expression of evolutionally conserved RNA binding proteins determines neuron-type specific alternative splicing of the *daf-2* insulin/IGF receptor in *C. elegans*

Masahiro Tomioka[1], Yasuki Naito[1,2], Hidehito Kuroyanagi[3], Yuichi Iino[1,2]

[1]Molecular Genetics Research Laboratory, University of Tokyo, Japan
[2]Department of Biological Sciences, Univeristy of Tokyo, Japan
[3]Medical Research Institute, Tokyo Medical and Dental University, Japan

The diversity of neuronal types is essential for development of the nervous system and physiological functions in the mature brain. It is known that the identity of neurons is determined by combinatorial expression of transcription factors which are controlled by regulatory networks of microRNAs and signaling pathways. Post-transcriptional regulation by alternative splicing (AS) is also important for specification of neuronal identities. Several studies have suggested that neuron-specific AS is regulated by neuronally expressed RNA-binding proteins that act as alternative splicing factors. However details of the mechanism and biological importance of neuron-type specific AS are largely unknown.

We have found that two isoforms of *daf-2*, a *C. elegans* insulin/IGF receptor homolog, are generated by alternative splicing of a cassette exon, exon11.5. Only the DAF-2 isoform with exon11.5-encoded residues localized preferentially to the axonal processes and can support starvation-induced salt chemotaxis learning. *daf-2* exon11.5 is located in a position analogous to that of a cassette exon, exon11, of the insulin receptor gene in mammals, implying the physiological importance of AS regulation for the insulin receptor functions.

Here we report that inclusion of *daf-2* exon11.5 occurs in a neuron-type specific manner. The neuron-type specific AS of *daf-2* was regulated by combinatorial expression of evolutionally conserved alternative splicing factors. Mutations of these factors caused various defects in salt chemotaxis learning according to combinations of the mutations. Neuronal expression of the *daf-2* isoform with exon11.5 partially rescued the learning defect of the splicing factor mutants. Thus these splicing factors may control the functions of learning-related genes including *daf-2* in restricted neuron types.

Contact: seidai@gen.s.u-tokyo.ac.jp

Pathways that modulate excitation-inhibition imbalance in *C. elegans* locomotor circuit

Seika Takayanagi-Kiya[1], Salvatore Cherra[1], Yingchuan B. Qi[1,2], Yishi Jin[1]

[1]Division of Biological Sciences, Section of Neurobiology, Howard Hughes Medical Institute, University of California San Diego, La Jolla, CA, USA.
[2]Institute of Developmental and Regenerative Biology, School of Life and Environmental Sciences, Hangzhou Normal University, Hangzhou, China

C. elegans sinusoidal locomotion requires coordinated activity of excitatory and inhibitory motor neurons in the ventral nerve cord. A gain-of-function mutation in the ACR-2 neuronal nicotinic acetylcholine receptor subunit, *acr-2(gf)*, disrupts locomotion and causes spontaneous convulsion behavior (Jospin et al. 2009). The ACR-2 channel is localized to the dendrites of the cholinergic motor neurons. Electrophysiology studies of the neuromuscular junctions show that *acr-2(gf)* mutants exhibit cholinergic overexcitation accompanied by decreased activity in GABAergic motor neurons, resulting in an imbalance of excitation (E) and inhibition (I) within the locomotor circuit. Interestingly, some human patients with Autosomal Dominant Nocturnal Frontal Lobe Epilepsy carry an identical missense mutation in the neuronal acetylcholine receptorβ2 subunit as in *acr-2(gf)* (Marini & Guerrin, 2007). Using *acr-2(gf)* mutant as a model to investigate the modulation of neural activity on circuit performance, we have discovered several pathways involving presynaptic neurotransmitter release (Zhou et al., 2013), ion homeostasis (Stawicki et al., 2011), and neuropeptide modulation that contribute to this E/I imbalance. In a recent study, we found that loss of neuropeptides encoded by *flp-1* and *flp-18* exacerbates convulsion, while homeostatic up-regulation of *flp-18* in *acr-2(gf)* animals suppresses convulsion (Stawicki, Takayanagi-Kiya et al., 2013). By analyzing a large collection of genetic modifiers of *acr-2(gf)*, we have recently identified several novel mutations in *unc-41/Stoned* and *unc-17/VChAT* that suppress convulsion, as well as a novel mutation in a putative chaperon that enhances convulsion. We will report molecular and genetic dissection of the underlying mechanisms. Given the similarity in molecular changes and behavioral consequences, our findings will lead to not only a deep understanding of E/I balance in neuronal circuits, but also will enhance the search for more effective strategies to manage neurological and psychiatric disorders.

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Contact: stakayan@ucsd.edu

Systematic reverse genetics approach to reveal molecular mechanisms of the odorant choice behavior.

Yuji Suehiro, Shohei Mitani

Department of Physiology, Tokyo Women's Medical University, Japan

Caenorhabditis elegans shows a variety of behavioral patterns such as forward movement, short reversal and omega turn. As sensory signals elicit the selection of the patterns, worms can appropriately respond to complex sensory inputs. The neural and molecular mechanisms of the selection, however, remain unclear. To investigate the mechanisms, we focused on two types of sensory neurons AWA and AWC. Both neurons project to common interneurons and elicit positive chemotaxis, but are activated by different kinds of odorants. Therefore, we tested the behavior when diacetyl (DAc) received by AWA and benzaldehyde (Bz) received by AWC were presented simultaneously.

As a result, each of worms chose either of the odorants when Bz and DAc were presented, while almost all worms chose to approach the odorants when either Bz or DAc and diluent (ethanol) were presented. This indicates that the simultaneous presence of odorants affected the selections of behavioral patterns during the assay, suggesting that the choice between DAc and Bz reflects the integration of the selections. Then, to reveal the molecules required for the normal choice, we performed screening using reverse genetics approach. In our laboratory, 5637 mutant strains have been already isolated, and we selected 1498 strains carrying mutations in genes, which are suggested to be involved in neural function. Using the strains, we performed the choice assay and screened strains, which showed abnormal choice. Next, to eliminate strains, which show chemosensory or motor dysfunction, we performed the choice assay using either of the odorants and diluent.

Finally, we obtained 95 candidate strains. After outcrossing with wild type worms, we performed rescue experiment by injection of genomic fragments including promoter region, coding region and 3' untranslated region of target genes after outcrossing with wild type worms. Because of the expression of the fragments, *mgl-1*(*tm1811*) and *sprr-2*(*tm3668*), which had shown excessive choice of DAc and Bz, respectively, displayed reverse phenotypes, suggesting that these genes regulate selections of behavior. Furthermore, expression of CED-3 in AIA and AIY interneurons downstream of AWA and AWC, resulted in variant choice. Because MGL-1 is reported to be expressed in the interneurons, MGL-1 may be involved in the selections of behavior by regulation of the activity of the interneurons. Now we are examining which types of behavioral patterns are affected by the mutation.

Contact: yujisue@research.twmu.ac.jp

Neuronal mechanisms of decision making in *C. elegans* olfactory behavior revealed by a highly integrated microscope system

Yuki Tanimoto[1], Akiko Yamazoe[1], Kosuke Fujita[1], Yuya Kawazoe[1], Yosuke Miyanishi[1], Shuhei J. Yamazaki[1], Keiko Gengyo-Ando[2], Junichi Nakai[2], Xianfeng Fei[3], Yuishi Iwasaki[4], Koichi Hashimoto[5], Kotaro Kimura[1]

[1]Dept. Biol. Sci., Osaka Univ., Osaka, Japan
[2]Brain Sci. Inst. Saitama Univ. Saitama, Japan
[3]Dept. Intel. Inf. Syst., Fac. Sci. Tech., Tohoku Bunka Gakuen Univ., Sendai, Japan
[4]Dept. Intel. Syst. Eng, Ibaraki Univ., Ibaraki, Japan
[5]Dept. Syst. Inf. Sci., Tohoku Univ., Sendai, Japan

The nervous system of animals transforms dynamically changing sensory information from the environment into appropriate behavioral responses. In particular, olfactory information plays critical roles in adaptive behaviors in the form of long- and short-range chemical cues that encode spatiotemporal information and chemical identity. To elucidate the neuronal mechanisms underlying olfactory behavior, it is desirable to quantify behaviors and neural circuit activities under realistic olfactory stimulus. However, reproducing realistic spatiotemporal patterns in odor concentrations is challenging due to diffusion, turbulent flow, and measurability of odor signals. We have developed an integrated microscope system that produces a virtual odor environment to quantify behaviors and neural circuit activities of the nematode *C. elegans*. In this system, *C. elegans* is maintained in the view field of a calcium imaging microscope by an auto-tracking stage using a pattern-matching algorithm. Simultaneously, odor stimulus is controlled with sub-second and subα-μM precision to reproduce realistic temporal patterns. Using this system, we have found that two types of sensory neurons, ASH and AWB play significant roles in decision making for navigation in a repulsive odor gradient. Calcium imaging and optogenetic analysis revealed that temporal increments of repulsive odor activate ASH to trigger turns that randomize the migratory direction, while temporal decrements of the odor activate AWB to choose proper direction for straight migration. Further mathematical analysis indicated that these sensory neurons are not only antagonizing, but also cooperate with each other by responding to stimulation changes at different time scales. Using this method will lead to comprehensive understanding of cellular mechanisms of decision making in a simple neural circuit.

Contact: tanimoto@bio.sci.osaka-u.ac.jp

To eat correctly: Phospholipid signaling in apoptotic cell recognition and internalization

Shiya Cheng, Kun Wang, Haibin Wang, Dongfeng Zhao, Xiaochen Wang

National Institute of Biological Sciences, Beijing, China

Apoptotic cells generated by programmed cell death are rapidly removed to prevent inflammatory and autoimmune responses and maintain tissue homeostasis. During this process, apoptotic cells expose “eat me” signals, which are recognized by phagocytes to trigger signaling cascades, leading to internalization and degradation of cell corpses. Phosphatidylserine (PS), which is normally restricted to the inner leaflet of plasma membranes, is exposed on the surface of apoptotic cells, thus distinguishing the dying cells from normal living cells and serving as a general “eat me” signal for engulfment. Using genetic approaches, we have identified two PS-binding proteins, TTR-52 and NRF-5, which are required for efficient clearance of apoptotic cells. Our findings indicate that TTR-52 acts as a bridging molecule to mediate cell corpse recognition by cross-linking the PS “eat me” signal on apoptotic cells with the CED-1 receptor on phagocytes[1]. NRF-5, a secreted LPS-binding/lipid transfer family protein, acts with TTR-52 and CED-7 to mediate PS transfer from apoptotic cells to engulfing cells and thus promotes recognition and engulfment by phagocytes[2]. Recently, we identified two additional TTR-52 family proteins that cluster specifically around cell corpses upon secretion. Interestingly, recognition of apoptotic cells by these two TTR-52 family proteins is affected by the mutation in *ced-7* but not *tat-1*, a feature similar to NRF-5 but distinct from TTR-52. Our study suggests that multiple secreted PS-binding proteins act coordinately to mediate recognition of apoptotic cells.

Phospholipids are building blocks of lipid bilayers and key regulators of membrane trafficking events. By using genetically coded reporters, we investigated the dynamics of various phospholipids during cell corpse clearance and analyzed their roles in phagosome formation and maturation. We found that internalization of cell corpses is regulated by coordinated actions of a couple of phosphoinositides.

In the meeting, I will present our recent work regarding phospholipid signaling involved in apoptotic cell recognition and internalization.

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Contact: wangxiaochen@nibs.ac.cn

Translational Regulators GCN-1 and ABCF-3 Act Together to Promote Developmental and DNA Damage-Induced Germ-Cell Deaths

Takashi Hirose, Bob Horvitz

HHMI, Dept. Biology, MIT, Cambridge, MA 02139, USA

In *C. elegans*, 131 somatic cells undergo apoptosis during wild-type hermaphrodite development. Extensive genetic screens have identified the BH3-only gene *egl-1*, the BCL-2 homolog *ced-9*, the APAF-1 homolog *ced-4* and the caspase gene *ced-3*, which together define an evolutionarily conserved cell-death execution pathway that drives developmental somatic cell deaths and DNA damage-induced germ-cell deaths. While the transcriptional induction of apoptotic genes is crucial to initiating an apoptotic program, less is known about translational controls in cell death.

From a genetic screen for mutations that cause a defect in the death of the sister of the pharyngeal M4 motor neuron, we identified the translational regulators GCN-1 and ABCF-3, which promote M4 sister cell death during embryonic development. GCN-1 and ABCF-3 are not obviously involved in the physiological germ-cell deaths that occur during oocyte maturation. By striking contrast, these proteins play an essential role in the deaths of germ cells in response to ionizing irradiation. The *S. cerevisiae* homologs of GCN-1 and ABCF-3 physically interact and are required for the phosphorylation of a conserved serine residue of eukaryotic initiation factor 2α (eIF2α), resulting in the specific activation of translation of select mRNAs. We found that the *C. elegans* GCN-1 and ABCF-3 proteins interact *in vivo* and are required for the phosphorylation of eIF2α, suggesting a conserved function in translational control.

To identify the translational targets of GCN-1 and ABCF-3, we performed mRNA-seq and ribosome profiling (Ribo-seq) analyses, which together generated a quantitative and comprehensive list of genes regulated by GCN-1 and ABCF-3 at the translational level. These analyses showed that *gcn-1* and *abcf-3* do not have major effects on the translation of the known cell-death genes but do control translation of several candidates that might be involved in the M4 sister cell death and in germ-cell deaths induced by ionizing radiation. We are testing if these candidates are involved in these cell deaths. We propose that GCN-1 and ABCF-3 act together to promote developmental and DNA damage-induced germ-cell deaths through translational control in a pathway distinct from the known cell-death pathway.

Contact: thirose@mit.edu

PGL-1 and PGL-3, a Family of Constitutive P Granule Components, Prevent Excessive Levels of Germline Apoptosis in *Caenorhabditis elegans*

Hyemin Min[1], Yhong-Hee Shim[1,2], Ichiro Kawasaki[1]

[1]Department of Bioscience and Biotechnology, Konkuk University, Seoul, 143-701, Republic of Korea
[2]Institute of KU Biotechnology, Konkuk University, Seoul, 143-701, Republic of Korea

Germ cell development is strictly regulated by several mechanisms including apoptosis to ensure high fidelity transmission of genetic materials to the following generations. Germline apoptosis is constantly observed in *C. elegans* adult hermaphrodite gonads under physiological condition, while the level of the apoptosis increases after DNA damage. Although the occurrence of germline apoptosis totally depends on the activities of core apoptotic machinery, how germline apoptosis is triggered during normal oogenesis without stress, or how the apoptosis level increases after DNA damage still remains elusive.

In this study, we revealed that PGL-1 and PGL-3, a family of constitutive protein components of P granules, the *C. elegans* germ granules, serve essential roles for controlling the levels of germline apoptosis. We first found that mutants of *pgl-1* and *pgl-3* showed increased levels of germline apoptosis under both physiological and DNA-damaged conditions. We also found that the number of germ cells lacking PGL proteins increased significantly following UV irradiation, and that only those PGL-absent germ cells were selectively engulfed by gonadal sheath cells in adult hermaphrodite gonads. We further revealed that, CEP-1, the p53 homolog, promoted elimination of PGL proteins from germ cells following UV irradiation. Furthermore, protein levels of CED-4, the Apaf-1 homolog, and cytoplasmic translocation of SIR-2.1, a Sirtuin homolog, significantly increased in *pgl* mutants and even more increased following UV irradiation. SIR-2.1 activity was essential for high levels of germline apoptosis in *pgl* mutants because the levels of germline apoptosis were completely suppressed in *pgl-1 sir-2.1* and *sir-2.1;pgl-3* double mutants to the level of *sir-2.1* single mutant under both physiological and DNA-damaged conditions. We conclude that PGL proteins prevent excessive levels of germline apoptosis by repressing both the protein levels of CED-4 and the cytoplasmic translocation of SIR-2.1, independent of CED-9, the Bcl-2 homolog. Our study has revealed previously unidentified important roles of PGL-1 and PGL-3 for the control of germline apoptosis achieved by the interaction with critical apoptosis regulators. This study was supported by grants NRF-2013R1A1A2009090, NRF-2013R1A1A2009820, and 2014 KU Brain Pool Program of Konkuk University.

Contact: mintmin0701@naver.com

C. elegans CED-3 caspase regulates centrosome asymmetry in an apoptotic death

Barbara Conradt, Sayantan Chakraborty, Samik Bindu, Eric John Lambie, Tamara Mikeladze-Dvali

Ludwig-Maximilians-University Munich, Germany

Mother and daughter centrosomes are differentially segregated during stem cell divisions in both insects and mammals; however, the functional significance of this and the underlying mechanisms remain unclear. We have found that *C. elegans* centrosomes are differentially segregated with regard to the amount of pericentriolar material (PCM) during the division of the NSM neuroblast (NSMnb) into the NSM, which is relatively large and fated to survive, and the NSM sister cell (NSMsc), which is smaller and undergoes apoptosis. Furthermore, both centrosomal asymmetry during NSMnb division and the inheritance pattern of 'small' and 'large' centrosomes by the NSMc and NSM, respectively, are dependent on the *C. elegans* caspase CED-3. After NSMnb division, the level of CED-3 rapidly decreases in the NSM, but increases in the NSMsc, where *ced-3* is required for both the induction of apoptosis and PCM dissolution. Finally, our results support the notion that *ced-3* activity itself is asymmetrically segregated during NSMnb division. The function of caspases in apoptosis is conserved from *C. elegans* to mammals. We speculate that the novel function of CED-3 in centrosomal asymmetry, segregation and dissolution in a cell lineage that generates an apoptotic death may similarly be conserved.

Contact: conradt@bio.lmu.de

Small GTPase CDC-42 promotes apoptotic cell corpse clearance in response to PAT-2 and CED-1 in *C. elegans*

Sheng Zeng[1,2], Lukas Jakob Neukomm[1,3], Andreas Philipp Frei[1,4], Philip Adrian Huegli[1,5], Michael Otmar Hengartner[1]

[1]Institute of Molecular Life Science, University of Zurich, Zurich, Switzerland
[2]Ph.D. Program in Molecular Life Sciences (MLS), University of Zurich/ETH Zurich, Zurich, Switzerland
[3]Department of Neurobiology, Howard Hughes Medical Institute, University of Massachusetts Medical School, Worcester, Massachusetts, USA
[4]Department of Microbiology and Immunology, Stanford University, Stanford, California, USA
[5]Department of Vector Biology, Max-Planck Institute for Infection Biology, Berlin, Germany

The rapid clearance of dying cells is important for the well-being of multicellular organisms. In *C. elegans*, cell corpse removal is mainly mediated by three parallel engulfment signaling cascades. These pathways include two small GTPases, MIG-2/RhoG and CED-10/Rac1. Here we present the identification and characterization of CDC-42 as a third GTPase involved in the regulation of cell corpse clearance. Genetic analyses performed by both loss of *cdc-42* function and *cdc-42* overexpression place *cdc-42* in parallel to the *ced-2/5/12* signaling module, in parallel to or upstream of the *ced-10* module, and downstream of the *ced-1/6/7* module. CDC-42 accumulates in engulfing cells at membranes surrounding apoptotic corpses. The formation of such halos depends on the integrins PAT-2/PAT-3, UNC-112 and the GEF protein UIG-1, but not on the canonical *ced-1/6/7* or *ced-2/5/12* signaling modules. Together our results suggest that the small GTPase CDC-42 regulates apoptotic cell engulfment possibly upstream of the canonical Rac GTPase CED-10, by polarizing the engulfing cell towards the apoptotic corpse in response to integrin signaling and *ced-1/6/7* signaling in *C. elegans*.

Contact: sheng.zeng@imls.uzh.ch

rab-35 and *arf-6* function together in linker cell corpse removal

Lena Kutscher, Nima Tishbi, Shai Shaham

The Rockefeller University, USA

Programmed cell death and subsequent cell clearance are necessary in the development and maintenance of organisms and tissues. Defects in cell clearance can result in inappropriate inflammatory responses and autoimmune disorders. Much is known about apoptotic corpse removal; however, our understanding of mechanisms driving the clearance of cells dying by non-apoptotic means is rudimentary. Linker cell death in *C. elegans* is an excellent model of non-apoptotic cell death. The linker cell is a male-specific cell that is born in the second larval stage, leads the elongation of the developing gonad, and then dies in a programmed manner independently of known apoptosis, autophagy, or necrosis genes. Importantly, linker cell corpses are robustly engulfed in mutants lacking the capacity to engulf apoptotic bodies, suggesting utilization of a novel engulfment program. To elucidate the mechanism of linker cell corpse removal, we carried out a forward genetic screen to identify mutants that are defective in this process. F2 mutant males were propagated by artificial insemination, as surviving linker cells or corpses may block spermatid exit and promote male sterility. We screened approximately 15,000 haploid genomes and uncovered six mutants with a strong defect in corpse degradation. One mutant carries a loss-of-function mutation in the gene *rme-4*, a RAB-35 guanine nucleotide exchange factor (GEF), and one mutant harbors a gain-of-function mutation in *arf-6*, a small GTPase. Subsequent analyses revealed that loss of *rab-35* also causes linker cell corpse degradation defects. We found that *arf-6* is required for *rab-35* action, as a deletion allele of *arf-6* suppresses the *rab-35* defects. *rab-35* and *arf-6* (gf) mutations cause a small but significant apoptotic corpse degradation defect, as these mutants have occasional persistent apoptotic corpses in first-stage larvae. Rab35 and Arf6 have been previously implicated together in the recognition and removal of foreign particles in mammals, but not in the degradation of programmed cell death corpses. Thus, *rab-35* and *arf-6* may work together to preferentially degrade corpses that die by non-apoptotic mechanisms.

Contact: lkutscher@rockefeller.edu

How do necrotic cells attract their phagocytes

Zao Li[1], Victor Venegas[1], Yuji Nagaoka[2], Eri Morino[2], Prashant Raghavan[1], Yushinibu Nakanishi[2], Zheng Zhou[1]

[1]Baylor College of Medicine, USA
[2]Kanazawa University, Japan

Necrosis is a type of cell death distinct from apoptosis in both morphology and mechanism. Like apoptotic cells, necrotic cells are swiftly removed from animal bodies to prevent harmful inflammatory and autoimmune responses. In the nematode *Caenorhabditis elegans*, gain-of-function mutations in certain ion channel subunits result in necrotic-like cell death of six touch neurons. Necrotic touch neurons are engulfed and degraded inside engulfing cells. It is unclear how engulfing cells recognize these necrotic cells. Phosphatidylserine (PS) is an important apoptotic cell surface signal that attracts engulfing cells. Using a GFP-tagged phosphatidylserine (PS)-reporter, we observed that necrotic touch neurons actively present PS, an “eat me” signal originally identified on the surfaces of apoptotic cells, on their surfaces. In addition, we found that the phagocytic receptor CED-1 recognizes necrotic cells and initiates their engulfment. We further discovered two distinct molecular mechanisms that act together to promote PS-exposure on necrotic cell surfaces. Our work demonstrates that cells killed by different mechanisms (necrosis or apoptosis) expose a common “eat me” signal that attract their shared phagocytic receptor. Furthermore, unlike commonly believed, PS is actively exposed onto the outer surface of necrotic cells rather than being leaked out of necrotic cells passively.

Contact: zhengz@bcm.tmc.edu

Novel binding partner of small GTPase RAB-11 regulates RAB-11 redistribution to Golgi after fertilization

Aisa Sakaguchi[1], Miyuki Sato[2], Keiko Gengyo-Ando[3], Katsuya Sato[2], Junichi Nakai[3], Ken Sato[1]

[1]Lab. of Molecular Traffic, Institute for Molecular and Cellular Regulation, Gunma University, Japan
[2]Lab. of Molecular Membrane Biology, Institute for Molecular and Cellular Regulation, Gunma University, Japan
[3]Saitama University Brain Science Institute, Japan

Fertilization is the starting point for development and triggers a dynamic conversion and redistribution of intracellular components. We have found that a multi-functional small GTPase RAB-11 changes its localization dynamically during oocyte-to-embryo transition in *C. elegans*. RAB-11 localizes on the recycling endosomes and functions in receptor recycling in growing oocytes. In matured oocytes, RAB-11 transiently changes its localization to the cortical granules which contain extracellular matrix components, and regulates their exocytosis after fertilization to re-establish the extracellular environment for embryos. After the cortical granule exocytosis, RAB-11 returns to the recycling endosomes and the Golgi in embryos. These observations suggest that spaciotemporal regulation of the RAB-11 activity is critical for proper development. In this study, we identified RAB-eleven-interacting protein-1 (REI-1) as a novel binding partner for the GDP-form of RAB-11 by yeast two-hybrid screening. We also found another gene encoding a protein similar to REI-1 in the *C. elegans* genome and named it *rei-2*. These genes are conserved in human and fruit fly. Many guanine nucleotide exchange factors for small GTPase have been shown to interact strongly with the nucleotide-free form and GDP-bound form of their target GTPase. We found that REI-1 interacts more strongly with the nucleotide-free and GDP-bound form than GTP-bound form of RAB-11 whereas REI-2 interacted with any of these forms. We also found that GFP-tagged REI-1 driven by its own promoter expressed predominantly in the germline and localized on the cortical granules in oocytes and partially co-localized with RAB-11 on the Golgi and recycling endosomes in embryos. To elucidate the physiological function of REI-1 and REI-2, we screened *rei-1* and *rei-2* mutants from TMP/UV deletion mutant library. In *rei-1* and *rei-2* mutants, the temporal GFP::RAB-11 localization on the cortical granules and the cortical granule exocytosis occurred properly. However, the localization of GFP::RAB-11 to the Golgi after fertilization was drastically impaired in *rei-1* mutant embryos. These results suggest that REI-1 functions in the redistribution of RAB-11 from the cortical granules to the Golgi after the cortical granule exocytosis.

Contact: aisa_sakaguchi@gunma-u.ac.jp

An evolutionarily conserved mechanism for PI3P turnover during early-to-late endosome conversion

Kai Liu, Xiaojuan Sun, Youli Jian, Zhiyang Gao, Yang Li, Xuezhao Liu, Chonglin Yang

Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, China

The intracellular trafficking of cargoes destined for lysosomal degradation requires the coordination of early and late endosomes as well as lysosomes, in which the early-to-late endosome conversion plays an essential role. Early and late endosomes are characterized by distinct Rab GTPases and phosphoinositides, with Rab5 and Phosphatidylinositol 3-phosphate on early endosomes, and Rab7 and Phosphatidylinositol-3,5-bisphosphate on late endosomes. It is now established that the Rab5-to-Rab7 switch enables the occurrence of early-to-late endosome conversion. However, it remains elusive how endosomal PI3P is turned over during this process. Here we report a mechanism for endosomal PI3P turnover that is shared between *C. elegans* and mammals. By genetic screen and gene cloning we identified two factors, SORF-1 and -2, as essential regulators of endosomal PI3P dynamics. In *C. elegans*, loss of *sorf-1* or *-2* function similarly increased the sizes of endocytic compartments in the macrophage-like coelomocytes. In the absence of Rab conversion, however, *sorf-1* and *-2* mutations lead to formation of huge organelles resulting from continuous fusion of endosomes. We found that PI3P and the RAB-5 effector RABX-5 persist on these huge endosomes, indicate a failure in endosomal PI3P turnover. The endosomal persistence of PI3P in double mutants of *sorf-1* or *-2* with genes required for Rab conversion contrasts to that in single mutants of these genes, in which endosomal PI3P is similarly turned over as in wild type. These findings suggest that SORF-1 and -2 cooperate with Rab switch to control the level of PI3P during endosome conversion. We further found that SORF-1 and -2 likely act in a complex, and they directly interact with BEC-1, the *C. elegans* homolog of Beclin 1. In *sorf-1* and *-2* mutants, BEC-1 is enriched on membranes or to close proximity of endosomes. Loss of *bec-1* or *vps-34* suppressed the formation of huge endosomes in double mutants of *rab-7* with *sorf-1* or *-2*. Collectively, these findings suggest that SORF-1 and -2 likely function through BEC-1 to suppress the generation of endosomal PI3P. Similar to that in *C. elegans*, inactivation of both Rab7 and WDR81, the human homolog of SORF-2, induced formation of large endosomes with PI3P persisting, and WDR81 interacts with Beclin1. Taken together, our findings reveal an evolutionarily conserved mechanism in which SORF proteins/WDR81 cooperate with Rab switch to regulate endosomal PI3P turnover.

Contact: kailiu@genetics.ac.cn

Compartmentalisation of the endoplasmic reticulum regulates the polarity in *C. elegans* embryo

Zuo Yen Lee[1], Monica Gotta[2], Yves Barral[1]

[1]ETH Zurich, Switzerland
[2]University of Geneva, Switzerland

In many cell types membrane lateral diffusion barriers help to separate distinct cellular compartments. For example, asymmetrically dividing yeast cells form a diffusion barrier between the mother and the future daughter cell. The *C. elegans* embryos are also highly polarised cells, particularly in their first division during which polarity markers and fate determinants are asymmetrically distributed. For example, the cytoplasmic polarity protein MEX-5 is asymmetrically localised in the anterior domain, and this localisation is crucial for proper embryonic development. However, how MEX-5 localisation is determined and maintained is not fully understood. In this study, we investigated the role of the ER in MEX-5 asymmetry maintenance. By using Fluorescence Loss In Photobleaching (FLIP) technique, we found that the endoplasmic reticulum (ER) is a continuous entity throughout the entire cell. Indeed, the ER luminal protein GFP-KDEL diffused freely between the anterior and the posterior domains. In contrast, the diffusion of the ER membrane protein SP12-GFP was restricted, suggesting the presence of a diffusion barrier in the ER membranes separating these two domains. We further identified the small GTPase *rap-1* as a regulator of barrier formation. *rap-1* mutant embryos showed a delayed yet stronger ER compartmentalisation compared to wild type. Interestingly, *rap-1* mutant embryos, despite being viable, showed a slight decrease of MEX-5 enrichment in the anterior domain. The PAR polarity gene *par-2* is known to regulate MEX-5 localisation. We found that the knockdown of *rap-1* in the *par-2* ts mutant background showed strong reductions of MEX-5 enrichment when compared to the mutant alone already at permissive temperature. Furthermore, this correlated with enhanced embryonic lethality in the same condition. Together, our data suggest that the compartmentalisation of the early embryo ER membranes by a *rap-1*-regulated diffusion barrier contributes to the asymmetric localisation of MEX-5 and thus the proper embryonic development.

Contact: zuoyen.lee@bc.biol.ethz.ch

Embryonic life under tension

Michel Labouesse

IGBMC, CNRS/INSERM/University of Strasbourg, Illkirch, France
IBPS, CNRS/University Paris-6, Paris, France

The importance of mechanical forces in biology is well accepted, yet an integrated view of their mode of action *in vivo* is lacking. In particular, a mesoscopic view of embryonic morphogenesis, with knowledge of the cellular processes influenced by mechanical forces, remains fragmentary. I will present our efforts to understand how *C. elegans* embryos elongate through three projects. First, while numerous reports have emphasized the central role played by seam cells in elongation, we do not know how the mechanical forces unleashed by myosin II are exerted and how their actions accounts for elongation. To address this issue, we are using laser nano-ablation to measure mechanical tension and modelling. Second, I will recap recent findings illustrating how the mechanical tension originating from muscles cells promotes and influences epithelial morphogenesis through a mechanotransduction pathway involving a GIT-1/PIX-1/PAK-1 module. I will go on to illustrate that this module plays a related role in vertebrates to promote hemidesmosome remodelling, and show that the vertebrate/nematode comparison is revealing novel aspects. Finally, I will touch on the issue of cell shape maintenance as epidermal cells become submitted to stretching and compression from the underlying muscles.

Contact: lmichel@igbmc.fr

The WAVE/SCAR complex interacts with the two *C. elegans* junctional complexes and regulates the levels and localization of α-catenin/HMP-1

Maria Agapito, Sailaya Mandalapu, Falshruti Patel, Yelena Bernadskaya, Maryam Honarbakhsh, Martha Soto

Rutgers - Robert Wood Johnson Medical School, USA

During *C. elegans* embryogenesis movements of cells and tissues depend on dynamic reorganization of the actin cytoskeleton. Genetic screens in our lab have identified a branched actin regulation pathway that includes Rac1/CED-10, the WAVE/SCAR/GEX complex and Arp2/3 as essential regulators of epithelial morphogenesis. The actin cytoskeleton is an integral component of the apical junction of epithelial cells. However, the nature of branched actin contributions to apical junction formation and maintenance is still not clear. Previous studies by our lab showed that WAVE/SCAR and Arp2/3 are needed for the membrane enrichment of proteins that regulate apical junctions in developing epithelial cells of *C. elegans*. We will present recent results addressing how Arp2/3 and its WAVE/SCAR nucleation-promoting factor help build and maintain apical junctions. Particularly exciting is the finding that WAVE/SCAR components associate with junctional complex components. In addition, loss of WAVE/SCAR components strongly reduces the apical enrichment of alpha-catenin/HMP-1, the protein that connects the cadherin junction to F-actin. These findings provide *in vivo* organismal evidence for the role of branched actin regulators in assembling and maintaining the apical junction during embryonic development.

Contact: sotomc@rwjms.rutgers.edu

Twitchin kinase interacts with MAPKAP kinase 2 in *C. elegans* striated muscle

Yohei Matsunaga, Hiroshi Qadota, Miho Furukawa, Heejoo (Helen) Choe, Guy M Benian

Department of Pathology, Emory University, USA

In muscle, giant polypeptides of the titin family contain protein kinase domains near their C-termini. These kinase domains are auto-inhibited but how they are activated has not been determined. Grater et al. (2005) hypothesized that small pulling forces occurring during muscle activity would be sufficient to remove an ~60 residue region (CRD) from the catalytic pocket and permit activity of titin kinase. Recently, the crystal structure of the largest segment of any giant kinase, the Fn-NL-kinase-CRD-Ig segment from *C. elegans* twitchin has been published (von Castelmur et al. 2012). *In vitro* kinase assays show that NL and CRD each inhibit kinase activity by one half. Molecular dynamic simulations indicate that the mechanically sensitive portion is NL and that the CRD remains attached to the large lobe of the kinase even after the small lobe has been unwound.

To determine what might remove this CRD and permit full activation, we used a region of twitchin containing the kinase to screen a yeast 2-hybrid library. One interactor is MAK-1, a nematode ortholog of MAPKAP kinase 2, first identified as a protein from rabbit skeletal muscle (Stokoe et al. 1992). The kinase domain of MAK-1 and the human ortholog are 53% identical. By yeast 2-hybrid analysis, interaction of MAK-1 with twitchin kinase is specific; the homologous regions from the other giant proteins in nematodes, TTN-1 and UNC-89 PK2, fail to interact. Both analysis of twitchin-TTN-1 chimeras in Y2H assays, and *in vitro* assays with purified proteins indicate that the CRD of twitchin is critical for interaction with MAK-1. A putative *mak-1* promoter is expressed in body wall muscle, hypodermis and intestine. Anti-MAK-1 antibodies localize to muscle I-bands, around and between dense bodies (Z-disk analogs), partially overlapping location of twitchin at the outer edges of A-bands. Nicotine is an acetylcholine agonist, and when wild type worms are exposed to nicotine they become paralyzed (sensitive). When *unc-22* (encodes twitchin) mutants are exposed to nicotine, however, they continue to move and twitch violently (resistant). *mak-1* mutants show an intermediate response. The double mutant, *mak-1*;*unc-22* (RNAi) shows complete resistance. That is, *unc-22* is epistatic to *mak-1*, consistent with the hypothesis that MAK-1 is upstream of twitchin. Therefore, MAK-1 probably interacts with twitchin CRD to regulate kinase activity of twitchin.

Contact: yohei.matsunaga@emory.edu

Attenuation of N-glycosylation causes polarity and adhesion defects in the *C. elegans* embryo

Julia Stevens, Anne Spang

Biozentrum, Growth and Development, University of Basel, Switzerland

Cell polarity establishment is a key mechanism in the development of any multicellular organism. The one-cell stage *C. elegans* embryo is highly polarized, requiring sequestration of cytoplasmic polarity factors at the plasma membrane and compartmentalization of the plasma membrane itself. Compartmentalization would aid asymmetric distribution of lipids and proteins, being partially responsible for the fates of the future daughter cells. Since most plasma membrane proteins are glycosylated, we determined the effect of reduction of N-glycosylation on early development. Albeit the polarity factor PAR-2 being recruited normally to the posterior cell cortex after fertilization of the oocyte, spreading of the PAR-2 domain over the posterior cortex was impaired, suggesting a role of at least one N-glycosylated protein in stabilization of PAR-2 at the posterior cortex. In addition, cell-cell adhesion was specifically lost between AB and P1 upon N-glycosylation attenuation. This loss-of-adhesion phenotype was rescued by interfering with polarity establishment, indicating that polarity establishment is upstream of plasma membrane segregation. Remarkably the epithelial adhesion complex components E-cadherin and MAGI-1 were found at contact sites at the two cell stage embryos. This localization was lost under reduced N-glycosylation, but was rescued by concomitant loss of the polarity factor PAR-2. Our data suggest a role of N-glycosylation in plasma membrane compartmentalization and the distribution of polarity and adhesion components.

Contact: anne.spang@unibas.ch

SPV-1, an F-BAR and RhoGAP domain protein, regulates spermatheca contractility

Pei Yi Tan, Bruno Pontes, Nils Gauthier, Ronen Zaidel-Bar

Mechanobiology Institute, National University of Singapore, Singapore

C. elegans ovulation involves the passage of an oocyte through an epithelial “pouch” termed the spermatheca, where fertilization takes place and egg shell synthesis begins, before the oocyte is pushed into the uterus. Actomyosin contractility is known to provide the force to squeeze the spermatheca, but how this contractility is regulated in a cyclical manner is not known.

We identified SPV-1, an F-BAR and RhoGAP protein, as a negative regulator of spermatheca contractility, as loss of spv-1 function resulted in overconstriction. Using a Rho-biosensor, we observed premature onset of RHO-1 activity in the spermatheca of *spv-1*(ok1498) mutants, consistent with the hyperconstriction phenotype. A translational fusion construct of SPV-1::EGFP revealed exclusive spermatheca expression and expression of deletion constructs in the mutant background pointed to an essential role for the RhoGAP domain in regulation of contractility and for the F-BAR domain in localization of SPV-1.

Importantly, SPV-1 localization at the apical cell membrane was transient. We observed detachment of SPV-1::GFP from the membrane upon stretching by an incoming oocyte, and speculated this to be due to the membrane curvature-sensing ability of its F-BAR domain. We tested this idea by expressing the F-BAR domain of SPV-1 fused to EGFP in fibroblasts subjected to repeated cycles of osmotic shocks to manipulate membrane tension. Using TIRF microscopy, we observed detachment of the F-BAR domain from the membrane during hypotonic shock while membrane localization was restored in isotonic buffer.

To investigate the consequence of constitutively localizing the RhoGAP domain of SPV-1 on the spermatheca membrane, we replaced the F-BAR with a PH domain. Strikingly, the transgenic worms harbour multiple embryos in the spermatheca, indicative of insufficient constriction.

In summary, our findings support a model in which SPV-1 regulates cyclical contraction of the spermatheca through tension-dependent localization to the apical membrane, downregulating RHO-1 when the spermatheca is collapsed and allowing RHO-1 levels to rise when the spermatheca is stretched. We predict such a mechanism for feedback between membrane tension and actomyosin contractility may be operating in other contractile systems.

Contact: biezbr@nus.edu.sg

Genetic analysis of epidermal cell mechanical properties during *C. elegans* embryonic elongation

Gabriella Pásti[1], Christelle Gally[1], Julien Pontabry[1], Huimin Zhang[2], Michel Labouesse[1]

[1]IGBMC Strasbourg, France
[2]Institute of Biology and Medical Sciences, SooChow University, China

The contribution of mechanical forces to development is gaining wider acceptance. But the detailed molecular mechanism by which cells sense and respond to forces remains elusive. To investigate those issues, we focus on *C. elegans* embryonic elongation, a process controlled by cell shape changes, transforming a ball of cells into a tube-shaped animal. *C. elegans* embryonic elongation consists of two phases. First, it is driven by the epidermal actomyosin. Second, muscles promote elongation through a recently reported mechanotransduction pathway involving CeHDs, which mechanically link muscles to the epidermis.

PAK-1 (p21-activated kinase) is a key regulator of both phases: it is a good starting point to further dissect the molecular landscape of elongation. To reach this aim, we have two approaches.

First, we carried out a systematic search for genetic interactions by an RNAi screen, targeting 356 genes in a *pak-1(O)* mutant.

Second, we looked for potential PAK-1 interactors by a yeast two-hybrid screen. We tested the *in vivo* relevance of the most interesting candidate, the alpha-spectrin SPC-1. We established a genetic interaction between the two genes, showing that double mutants display a novel elongation defect, whereby they retract to a lima bean-like shape after reaching the 1.5-fold stage. Retraction is not observed if muscles are defective, suggesting it is induced by muscle twitching. To define why embryos retract, we examined important elongation players and found them to be mostly normal. In contrast, spinning disk time-lapse analysis showed that the elastic properties normally displayed by epidermal cells in response to muscle twitching input are affected in double mutants. To further confirm these results, we are using laser ablations in the dorsoventral epidermis to measure epidermal viscoelastic properties.

Consistent with the genetic interaction, *in vivo* expression studies revealed similar localization between the two proteins at the level of CeHDs. Furthermore, we found that the SH3 domain of SPC-1 interacting with PAK-1 *in vitro* is essential for its function *in vivo*, and that the loss of SPC-1 disturbed PAK-1::GFP localization.

Altogether, we found that the SPC-1-PAK-1 interaction is important for *C. elegans* embryonic elongation. Moreover, we suggest it modulates the elastic properties of dorso-ventral epidermal cells submitted to external mechanical stress, helping to stabilize them between consecutive inputs.

Contact: biofiona@igbmc.fr

The microtubule minus-end binding protein PTRN-1 and other cytoskeletal proteins function in epidermal development

Marian Chuang[1], Tiffany I. Hsiao[1], Amy Tong[1], Shaohe Wang[2], Karen Oegema[2], Andrew D. Chisholm[1]

[1]Division of Biological Sciences, UCSD, USA
[2]Ludwig Institute, UCSD, USA

Wounding the *C. elegans* epidermis triggers innate immune and cytoskeletal responses that collaborate to allow animals to survive otherwise lethal damage. In *C. elegans* the death-associated protein kinase *dapk-1* negatively regulates epidermal wound responses. A gain of function allele *dapk-1*(ju4) causes hyperactive wound responses, including excessive secretion of cuticle and constitutive expression of antimicrobial peptides. To better understand how DAPK-1 regulates these processes we screened for suppressors of the *dapk-1*(ju4) morphological phenotypes. One suppressor causes complete loss of function in *ptrn-1*, which encodes the *C. elegans* member of the Patronin/Nezha/CAMSAP family of MT minus-end binding proteins. Patronin is thought to bind specifically to MT minus ends and protects them against kinesin-13-based depolymerization. Several recent studies have described the roles of MT minus-end dynamics in cultured cells, but their *in vivo* functions are not well understood. We find that loss of *klp-7/kinesin-13* function suppresses the *ptrn-1* suppression phenotype, consistent with the hypothesis that PTRN-1's minus-end protective function is important in the epidermis. We find that ptrn-1function is required in the epidermis for the *dapk-1*(ju4) hypersecretion phenotype. GFP-tagged PTRN-1forms puncta in the cytoplasm and at epidermal attachment structures, possibly the locations of MT minus ends in the epidermis. Intriguingly, loss of *ptrn-1*function does not have drastic effects on epidermal development or overall MT organization, suggesting PTRN-1 may act redundantly with other factors. In addition to *ptrn-1*, we have identified other suppressors of *dapk-1*(ju4) epidermal defects, including loss of function mutations in *dhc-1* (dynein heavy chain), *unc-44* (ankyrin), and *unc-33* (CRMP2), revealing roles for the cytoskeletal regulators in epidermal morphology. However, mutations in *dhc-1* cannot suppress the *dapk-1*(ju4) hyperactive innate immune response, whereas *ptrn-1*(lf) can, suggesting different mechanisms of suppression are involved. We will report on our investigations into the roles or PTRN-1 and other *dapk-1* suppressors in epidermal wound healing and in axon regeneration in the nervous system.

Contact: marian_chuang2004@yahoo.com

Morphogenetic roles of non-centrosomal microtubules

Masatoshi Takeichi

RIKEN Center for Developmental Biology, Japan

Microtubules play a number of critical roles in cell behavior and functions. There are two populations of microtubules at the interface of cell cycle, one polymerized from the centrosome and the other from non-centrosomal sites. In particular cell types, such as differentiated epithelial cells and neurons, their major microtubules are non-centrosomal at least in mammals. However, how the non-centrosomal microtubules are generated and stabilized, and what kinds of specific roles they play in cellular morphogenesis remain largely unknown. During our studies to analyze the molecular components of cell junctions in mammalian epithelial cells, we identified a protein named ‘Nezha’. This protein, which is also called CAMSAP3, binds and stabilizes the minus-ends of microtubules. CAMSAP3 and its relative CAMSAP2 are distributed as subcellular clusters not only at cell junctions but also at various cytoplasmic sites, and these clusters apparently serve for microtubule nucleation at these sites. When these proteins are depleted in epithelial cells, non-centrosomal microtubules are decreased and centrosomal microtubules are increased. As a result, various features of cells, including their overall morphology, are altered. Our resent studies are now revealing that CAMSAP3 is essential for maintaining the apicobasally-polarized organization of intracellular structures in epithelial cells. Thus, we are uncovering novel functions of non-centrosomal microtubules in cellular morphogenesis, to which less attention has been thus far paid in the fields of cell and developmental biology.

Contact: takeichi@cdb.riken.jp

The worm transcriptome: the past and the future

Yuji Kohara

National Institute of Genetics, Japan

Contact: ykohara@lnig.ac.jp

DAF-16/FOXO antagonizes age-related loss of germline stem cells in *C. elegans*

Zhao Qin, E. Jane Albert Hubbard

New York University School of Medicine, USA

Failure to maintain stem cells with age is associated with conditions such as tissue degeneration and increased susceptibility to tissue damage in many organisms, including humans. Here we use the *C. elegans* germ line as a model to study stem cell aging. The *C. elegans* germ line combines a well-established genetic model for aging studies with a well-defined, accessible stem cell system, providing a unique opportunity to dissect the effects of aging on stem cell dynamics.

We found that the stem cell pool in the *C. elegans* germ line becomes depleted over time. This depletion is far less severe in mutants with reduced insulin/IGF-like signaling (IIS). Maintenance of the germ line stem cell pool in these mutants is dependent on the activity of the downstream transcription factor DAF-16/FOXO. Surprisingly, in this context DAF-16/FOXO acts neither in the germ line itself nor in the intestine. These results suggest that DAF-16/FOXO regulates age-associated physiological changes in the germ line through a novel mechanism that is anatomically separable from its previously described germline-autonomous roles in larval germline proliferation and largely intestine-mediated lifespan regulation. In addition, neither neurons, muscle, nor the distal tip cells are implicated. Rather, we found that DAF-16/FOXO activity is required in a small set of cells in the somatic gonad. This result suggests that parts of the reproductive system that experience the transit of germ cells might signal to and ultimately influence the stem cell pool. Consistent with this hypothesis, we found that conditions that accelerate or delay flux of germ cells through the reproductive tract differentially lose or retain their stem cells, respectively. Our additional data support a model in which germ cell flux and germline stem cell maintenance are linked by DAF-16/FOXO activity.

Contact: jane.hubbard@med.nyu.edu

Sensory neuronal regulation of lifespan via modulating insulin-like peptides in *C. elegans*

Murat Artan[1], Dae-Eun Jeong[2], Dongyeop Lee[2], Young-Il Kim[1,2], Heehwa Son[2], Joy Alcedo[3], Seung-Jae Lee[1,2,4]

[1]Information Technology Convergence Engineering, Republic of Korea
[2]Dept. of Life Sciences, UK
[3]Department of Biological Sciences, Wayne State University, Detroit, MI, USA
[4]I-BIO, Pohang University of Science and Technology, Republic of Korea

Sensory neurons regulate the lifespan of several organisms, including *C. elegans* and *Drosophila*. Perturbation of sensory neurons prolongs the lifespan of *C. elegans* by activating the DAF-16/FOXO transcription factor, but the upstream regulators are poorly understood. Here, we show the mechanisms by which sensory neurons modulate lifespan through insulin-like peptides (ILPs). We found that mutations in *tax-2* and *tax-4*, subunits of a cyclic GMP-gated channel required for sensory neural functions, promoted the longevity of *C. elegans* in a *daf-16*-dependent manner. Mutations in *tax-2* enhanced the nuclear localization as well as transcriptional activation of DAF-16 in non-neuronal tissues suggesting a tissue non-autonomous regulation of lifespan by sensory neurons. Considering potential roles of neuroendocrine signaling via ILPs in this systemic lifespan regulation, we determined the expression of 34 ILP genes. We found that down-regulation of two ILP genes, *daf-28* and *ins-6*, mediated the longevity of sensory mutants. Further, overexpression of *daf-28* or *ins-6* reversed the elevated nuclear localization and transcriptional activity of DAF-16 in *tax-4* mutants. Thus, these ILPs appear to act as neuroendocrine factors that transmit the longevity signals from sensory neurons to other tissues by regulating DAF-16 activity.

Contact: murat@postech.ac.kr

daf-41/co-chaperone p23 regulates *C. elegans* lifespan in response to temperature

Makoto Horikawa, Christian Latza, Adam Antebi

Max Planck Institute Biology of Ageing, Germany

Temperature potently modulates various physiologic processes including growth rate, reproduction, and ageing. In *C. elegans*, longevity varies inversely with temperature and recent studies reveal that lifespan is regulated through distinct mechanisms at different temperatures. At warm temperature, it has been reported that the heat shock response mechanism and thermosensory system contribute to normal lifespan. On the other hand, it has recently been reported that calcium signaling, evoked by the *trpa-1* cold response calcium ion channel, regulates longevity at cold temperatures. However, it is still poorly understood how thermal regulation of longevity is regulated over a wide range of temperatures.

Here, we demonstrate that *daf-41*/ZC395.10, *C. elegans* homolog of co-chaperone p23, regulates adult lifespan in response to temperature. The *daf-41* is a novel Daf-c gene, involved in tolerance for several stresses and has a key role in chemosensory system. *daf-41* mutants also exhibited a surprising ageing phenotype: they lived longer than wild-type at 25^{o}C, had no remarkable ageing phenotype at 20^{o}C, but were shorter lived than wild-type at 15^{o}C. Our epistasis analysis revealed that long lived phenotypes of *daf-41* mutants at 25^{o}C were regulated through the chemosensory apparatus, insulin signaling and heat shock response mechanism, but acted in parallel to the thermosensory system and steroidal signaling. Moreover, quantitative RT-PCR analysis showed that *daf-41* inhibited transcriptional activities of DAF-16/FOXO and HSF-1. By contrast, at cold temperature, *daf-41* regulated lifespan through insulin signaling, thermosensory system and steroidal signaling, but in parallel to the chemosensory system and heat shock response mechanism. Our studies suggest that *daf-41*/p23 modulates key transcriptional changes in longevity pathways in response to temperature.

Contact: mhorikawa@age.mpg.de

SUMO modulates NHR-25/NR5A during *C. elegans* vulval development

Jordan Ward[1], Nagagireesh Bojanala[2,3], Teresita Bernal[1], Kaveh Ashrafi[1], Masako Asahina[1,2], Keith R. Yamamoto[1]

[1]University of California San Francisco, USA
[2]Biology Centre, Academy of Sciences of the Czech Republic, Czech Republic
[3]University of South Bohemia, Czech Republic

Precise temporal and spatial controls of gene expression and activity are vital to animal development. Transcription factors (TFs) regulate distinct sets of genes depending on cell type and developmental or physiological context. Failure to do so can cause severe developmental defects and pathology. The precise mechanisms by which regulatory information from ligands, genomic sequence elements, co-factors, and post-translational modifications are integrated by TFs remain challenging questions. We examined how a single regulatory input, sumoylation, differentially modulates the activity of a conserved *C. elegans* nuclear hormone receptor, NHR-25, in cell fate execution. Through a combination of yeast two-hybrid analysis and *in vitro* biochemistry we identified the single *C. elegans* SUMO (SMO-1) as an NHR-25 interacting protein, and show that NHR-25 is sumoylated on at least four lysines. Some of the sumoylation acceptor sites are in common with those of the NHR-25 mammalian orthologs SF-1 and LRH-1, demonstrating that sumoylation has been strongly conserved within the NR5A family. NHR-25 binds canonical SF-1 binding sequences to regulate transcription, and that NHR-25 activity is enhanced *in vivo* upon loss of sumoylation. During vulval development, sumoylation of NHR-25 is critical for maintaining 3° cell fate. Moreover, SUMO also confer formation of a developmental time-dependent NHR-25 gradient across VPC daughters. We conclude that sumoylation operates at multiple levels to affect NHR-25 activity in a highly coordinated spatial and temporal manner.

Contact: masako.asahina@ucsf.edu

Iron homeostasis in *C. elegans*: development and ageing

Gawain McColl

The Florey Institute of Neuroscience and Mental Health, Australia

Iron is an essential co-factor required for specific enzymatic function and respiration, and is involved in innate immunity and cell death processes. Homeostasis of iron is a carefully regulated cellular process; too little or too much iron can be deleterious. The free radical theory of ageing implicates iron as a causative factor for ageing through mechanisms of oxidative chemistry. We have employed x-ray fluorescence microscopy to explore iron homeostasis in *Caenorhabditis elegans* and to directly image the tissue and subcellular localization of iron. In addition, we are also identifying components of the (iron) metalloproteome. Normal storage of iron is surprisingly dispensable during development but is required for normal ageing. In post-reproductive adults dramatic intestinal-iron accumulation was observed, which correlated with increased reactive oxygen species. Insulin-like signaling modulates these effects, so that long-lived *daf-2* mutants are resistant to these age-related changes, while short-lived *daf-16* mutants show more marked effects. The longevity of *daf-2* mutants is sensitive to iron homeostasis. The deleterious redox activity of accumulated iron may be a targeted to extend lifespan, and represents an avenue to explore to new therapeutics.

Contact: gmccoll@florey.edu.au

Activated and inactivated immune responses in *Caenorhabditis elegans* against *Photorhabdus luminescens* TT01

Kazuki Sato[1,2], Toyoshi Yoshiga[1], Koichi Hasegawa[3]

[1]Laboratory of Nematology, Department of Applied Biological Sciences, Faculty of Agriculture, Saga University. Saga, 840-8502 Japan
[2]JSPS Research Fellow, Japan
[3]Department of Environmental Biology, College of Bioscience & Biotechnology, Chubu University, 1200 Matsumoto, Kasugai, 487-8501 Japan

The Gram-negative bacterium *Photorhabdus luminescens* TT01 which symbiotically associates with the entomopathogenic nematode (EPN) *Heterorhabditis bacteriophora*. EPN invades the insect hosts and release their symbiotic bacteria into the hemocoel to kill insect hosts by bacterial toxins. Even though the pathogenicity of several *P. luminescens* toxins is under active investigation, the underlying mechanism of their virulence remains unclear. We studied the role of two signaling pathway in the model organism *Caenorhabditis elegans*, involved in innate immune responses the p38 mitogen-activated protein kinase (MAPK) and the Insulin/IGF-1 like signaling pathway, against *P. luminescens*. Depletion of *pmk-1* by RNAi enhances susceptibility to *P. luminescens*, and downstream targets regulated by the p38 MAPK pathway were induced when fed on *P. luminescens*. On the other hand, knockdown of *daf-16* has no effects on *C. elegans* lifespan, but knockdown of *daf-2* dramatically increased resistance to *P. luminescens* in a *daf-16*-dependent manner. Several target genes controlled by the Insulin/IGF-1 like signaling pathway were suppressed and one of the *daf-2* ligands *ins-7* was induced by *P. luminescens*. Additionally, we revealed *ins-7* deletion mutant survived longer when fed on *P. luminescens*. These results suggest the p38 MAPK pathway is activated for the host defense against *P. luminescens*. The Insulin/IGF-1 like signaling pathway is highly effective for the resistance against *P. luminescens*, albeit DAF-16 is inactivated by the bacteria via INS-7 induction.

Contact: k.sato1406@icloud.com

The contribution of *C. elegans* transcription factors to fat storage and body size

Akihiro Mori, Albertha J.M. Walhout

Program in Systems Biology, University of Massachusetts Medical School, Worcester, USA

In genome-wide association studies, many genes that contribute subtly to complex human diseases such as obesity and type 2 diabetes are rapidly being identified. However, it is challenging to determine the precise contribution of each gene to each phenotype. We used *C. elegans* as a model to determine the contribution of transcription factor (TF) to phenotypes such as body size and fat storage at the level of the whole organism. So far, some studies of fat storage in *C. elegans* have used visual high-throughput methods that may be biased subjectively and lead to a misinterpretation of the functions of TFs that contribute to each phenotype. A few studies have used highly quantitative microscopy that provides accurate data but are not amenable to high-throughput settings.

In this study, we have systematically screened the ~1,000 worm TFs using RNA interference. We have employed Oil-Red-O staining to measure the fat content and developed a novel image analysis platform, IPPOME (Image Processing for Precise and Objective MEasurement), that enables the automatic detection of individual worms from high-magnification images to quantify fat storage and body size. We applied IPPOME to quantify the phenotypes and determined the contribution of TFs to each phenotype. Both RNAi and Oil-Red-O staining methods can cause experimental noise. To provide sufficient statistical power to measure even subtle phenotypes, each TF was tested in three independent biological experiments, and each experiment tested an average of 17 animals. Altogether, we capture known phenotypes and find that a large proportion of TFs play a subtle, but significant role in fat storage. We envision that this method can be used to determine the contribution of other types of genes, as well as other phenotypes in the future.

Contact: akihiro.mori@umassmed.edu

Age-Dependent Mitochondrial Fragmentation in *C. elegans* Touch Receptor Neurons

Hao-Ching Jiang, Jiun-Min Hsu, Chien-Ping Yen, Chun-Liang Pan

Institute of Molecular Medicine, National Taiwan University, Taiwan

Age-dependent behavioral and cognitive decline is accompanied by structural and functional deterioration in senescent neurons. Modeling neuronal aging in the mammalian brain is hampered by the relatively long life span of the common lab mammals. Robust genetics and the short life span make *Caenorhabditis elegans* a promising model to investigate the molecular and cell biological basis of neuronal aging. In our previous study, we found that characteristic age-dependent defects occur in the *C. elegans* touch neurons, and sensory evoked activity is required to maintain neuronal integrity during aging. Here we report that progressive mitochondrial fragmentation is a hallmark of touch neuron aging, and that sensory evoked activity is required for a balanced mitochondrial dynamics in these neurons. Our FRAP analysis suggested that mitochondrial fragmentation documented with fluorescent probes did indicate physical disconnection of this organelle. Small mitochondrial fragments colocalized extensively with lysosomes, suggestive of age-dependent mitophagy in these neurons. Mutations in *hsf-1*, which shorten *C. elegans* life span, markedly accelerated mitochondrial fragmentation in the touch neurons. A *daf-2* mutation dramatically extended life span and also delayed mitochondrial fragmentation. Similar to axonal integrity in senescent touch neurons, maintenance of mitochondrial dynamics required sensory evoked activity. We showed that mutations in *mec-4* and *egl-19*, which encode a Degenerin family sodium channel and a voltage-gated calcium channel, respectively, induced premature onset of mitochondrial fragmentation in the touch neurons, and the two genes acted in a common pathway. We also tested *drp-1*, which is a dynamin-related GTPase critical for mitochondrial fission, is regulated by neuronal activity and represent part of a cell biological mechanism to link mitochondrial dynamics to autophagic activity in the aging touch neurons.

Contact: r01448009@ntu.edu.tw

Identification of mechanisms underlying pheromone-mediated neural plasticity by analyzing wild type *C.elegans* isolates

Woochan Choi

Department of Brain Science, DGIST, Republic of Korea

Environmental signals generate proper animal behaviors in the context of internal metabolic status. The nematode *C. elegans* provides an excellent system in which to explore the neural and molecular basis of behavioral plasticity. Previously, we and others showed that adult hermaphrodites avoid the pheromone C9 that is detected by the ADL chemosensory neurons (Srinivasan et al., 2008; Jang et al., 2012). Now we found that when C9 was exposed to animals at 10-second intervals, animals showed avoidance response only to the 1st exposure to C9. Interestingly, when animals were starved for just 5 mins, now animals continuously responded to not only the 1st but following exposures to C9. *In vivo* calcium imaging experiments in the ADL neurons recapitulated the behavioral response, suggesting that C9 response of ADL is modulated by internal feeding status. To investigate the neuronal and molecular mechanisms underlying this plasticity, we are testing diverse wild type *C. elegans* isolates for C9 avoidance. Since polymorphic variations within wild type *C. elegans* isolates generate phenotypic diversity (de Vono and Bagmann, 1998), analysis of C9-mediatedd neural plasticity in various wild type *C. elegans* isolates will lead to identification of genes and molecules involved in behavioral plasticity.

Contact: dncks@dgist.ac.kr

A natural odor attraction between *C. elegans* and Lactobacillus bacteria

Jeaim Choi, Jin Lee

Yonsei University, Division of Biological Science and Technology, Republic of Korea

C. elegans is attracted to dozens of odors in laboratory behavior assay settings. However, natural sources of attractive odor are not known for worms. Moreover, how *C. elegans* finds bacteria, its favorite food source, is not understood. Previous studies have shown that *C. elegans* in nature can be found concentrated in soil containing rotten fruits (Barriere and Felix, 2005). The probiotic bacteria Lactobacillus can be found in many types of foods such as yogurts, cheeses, and other fermented foods. One of the odor components produced by Lactobacillus that may impart flavor to these foods is the chemical diacetyl, an odor that *C. elegans* is strongly attracted to (Sengupta and Bargmann, 1996). Diacetyl is produced when Lactobacillus is grown on media containing citric acid. Our preliminary data shows that worms are not particularly attracted to Lactobacillus, but when Lactobacillus is grown on citric acid, worms become attracted to Lactobacillus. We are currently studying whether this attraction is mediated by AWA odor sensory neurons and the diacetyl odor receptor, ODR-10. These experiments can lend evidence to how *C. elegans* may be sensing and navigating towards food bonanzas found near rotting fruit soils.

Contact: nekorasii@naver.com

Analysis of functions of LET-60Ras in regulation of exploratory behavior by a novel method, time- and cell-specific RNAi

Masayuki Hamakawa[1], Yuichi Iino[2], Takayuki Uozumi[1], Naoko Ueda[3], Takaaki Hirotsu[1,3]

[1]Graduate School of Systems Life Sciences, Kyushu University, Japan
[2]Department of Biophysics and Biochemistry, Graduate School of Science, The University of Tokyo, Japan
[3]Department of Biology, Graduate School of Sciences, Kyushu University, Japan

Exploratory behavior is essential for animals to gain food resources. *Caenorhabditis elegans* employs strategies in deciding to roam or dwell and to explore food resources efficiently in the presence of food. However, it is little known about the behavioral strategies that they use under environmental conditions far from food resources.

We found that under poor conditions, abnormal locomotion behavior in which animals continue to move in a loopy pattern, termed circular locomotion (CL), occurred in mutants of the Ras-MAPK pathway. CL resulted from impaired foraging behavior, which involves exploratory head movements and is controlled by a neural circuit consisting of IL1, OLQ, and RMD neurons. We inhibited activity of these neurons by *unc-103(gf)* and found inhibition of these neurons caused CL. The knockdown of *let-60*ras in these neurons by cell-specific RNAi induced CL. Moreover, expression of *let-60* in these neurons rescued the defects of locomotion in *let-60* mutants. These results indicate that LET-60Ras functions in the neurons to regulate the exploratory behavior.

We found that mutants of *glr-1*, which encodes an AMPA-typed glutamate receptor and is expressed in motor neurons including RMD neurons, also showed CL. Expression of *glr-1* by its own promoter reduced the fraction of animals showing CL in the *glr-1* mutants. To reveal the relation between the Ras-MAPK pathway and GLR-1, we analyzed the GLR-1 localization in the neurite of RMD neurons. In the animals with RMD-specific knockdown of *let-60*, abnormal localization of GLR-1 glutamate receptors in RMD neurons was observed. In addition, to investigate a temporal and cell-specific profile of the functions of *let-60*, we developed an RNAi method which enables to perform time- and cell-specific knockdown simultaneously. Using this technique, we found that the knockdown of the function of *let-60* in RMD neurons at the adult stage induced aberrant localization of GLR-1, while the knockdown at embryonic stage did not, suggesting that LET-60Ras is important for the control of GLR-1 localization in RMD neurons to regulate foraging behavior. We confirmed that this novel method can be applied to other genes.

Contact: beachriver14@gmail.com

Gravity perception and gravitaxis behavior in *C. elegans*

Tong Young Lee, Jin Lee

Yonsei University, Division of Biological Science and Technology, Republic of Korea

Gravity affects the behavior and development of almost all animals. Much like visual, odor, and other sensory cues are perceived by animals to navigate their environments, gravity perception is important for animals, especially birds, insects and fish that navigate a 3D environment. How gravity is perceived and processed in higher neuronal structures is not well known. We have decided to use *C. elegans* to understand gravity perception and neuronal mechanisms in the nematode. We have designed both solid media and liquid assays to study gravity behavior in worms. In agar-based assays, worms move down with the force of gravity in a behavior termed gravitaxis. Worms are not forced down by gravity: if an attractive odor is placed on the top of the assays, the worms choose to move up against the force of gravity to reach the odor. We are in the process of screening through several mutants including mechanosensory and other sensory mutants to identify genes important for gravitaxis behavior. In addition, we are developing a liquid gravity assay to observe gravitaxis in *C. elegans* swimming movement.

Contact: tylee1991@naver.com

Neural basis of plasticity and bidirectionality of klinotaxis.

Yohsuke Satoh, Hirofumi Sato, Hirofumi Kunitomo, Yuichi Iino

Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Japan

Klinotaxis is a behavioral strategy for chemotaxis in *C. elegans*: The animals detect dorso-ventral (D-V) chemical gradient during forward locomotion and gradually curve towards higher (or lower) concentrations. Tendency of klinotaxis is not fixed but is plastic depending on previous experience. For example, an animal curves to the side with higher NaCl concentrations after cultivation with a high NaCl concentration (positive klinotaxis to NaCl), and curves to the lower side after cultivation with a low concentration (negative klinotaxis to NaCl). Here, we report that *C. elegans* realizes this plastic bidirectional klinotaxis by differential use of at least two distinct neural circuits.

First, we reproduced bidirectional klinotaxis to NaCl by artificial phasic activation of ASER chemosensory neuron, which responds to NaCl concentration decrease, by optogenetics. Activating ASER in synchrony with head swing after cultivation with a high NaCl concentration induced repulsive curving in response to ASER activation, which corresponds to positive klinotaxis to NaCl, and the same phasic activation pattern after cultivation with a low NaCl concentration induced positive curving that corresponds to negative klinotaxis. Next, we examined involvement of the interneurons downstream of ASER by ablating or activating these interneurons. Phasic activation of AIY interneurons induced strong attractive curving in response to AIY activation regardless of previous experience. Ablation of AIY neurons abolished attractive curving induced by ASER activation. However, ablation of AIY failed to affect repulsive curving induced by ASER activation. These results suggest that plastic bidirectional klinotaxis to NaCl is regulated by switching between a microcircuit that contains AIY and those that do not contain AIY. We will further discuss about the involvement of other interneurons that are postsynaptic to ASER.

Contact: sunfish@bs.s.u-tokyo.ac.jp

Role of ASE left gustatory sensory neuron in worms' behavior of NaCl chemotaxis

Lifang Wang[1], Masahiro Tomioka[2], Hirofumi Kunitomo[1], Yuichi Iino[1,2]

[1]Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Japan
[2]Molecular Genetics Research Lab, Graduate School of Science, The University of Tokyo, Japan

Animals show various behaviors in response to environmental conditions, which are often plastic according to previous experiences. *C. elegans*, sensing chemicals with a limited number of sensory neurons, is an ideal model for analyzing the role of each neuron in innate and learned behaviors. ASE neurons (ASE-Left neuron and ASE-Right neuron) play major roles for salt chemotaxis but have lateralized functions: ASER neuron is stimulated by decreases in NaCl concentration and ASEL neuron is stimulated by increases in NaCl concentration. Until now, our lab has focused on the roles of ASER and its signaling pathways in salt chemotaxis and other behaviors, but whether and how ASEL regulates the behaviors in salt chemotaxis remains unknown. In this study, chemotaxis assays and optogenetics combined with behavioral quantification with worm tracking system were used to investigate the ASEL's roles in worm behaviors. By using transgenic worms in which channelrhodopsin (ChR2) was expressed in the ASEL neuron, the neuron was activated by blue-light illumination and the behavioral response was recorded. The result indicated that worms showed behavioral responses to ASEL activation after cultivation with NaCl, but no or small responses after cultivation in NO NaCl conditions. After cultivation in the presence of NaCl, the worms' turning rate decreased during ASEL stimulation and increased immediately after termination of ASEL stimulation. The behavior of ASER-ablated mutants was tested in chemotaxis assays. The chemotaxis index was almost zero for worms that had been cultured in NO NaCl conditions, which was consistent with the result of optogenetics and was possibly because activation of ASEL does not generate behavioral responses. On the other hand, when worms were cultured in the presence of NaCl, worms migrated to higher concentrations, which was also identified with above optogenetics' result and our lab's former data, and it was possibly explained by the behavior that worms cultivated with NaCl went forward when NaCl concentration increased, but turned when NaCl concentration decreased, both contributing to migration to higher concentrations.

Contact: lifangwang06@gmail.com

Analyses of the developmental changes in the odor preference of *C. elegans*

Takahiro Hino[1], Manabi Fujiwara[2], Takeshi Ishihara[2]

[1]Graduate School of Systems Life Sciences, Kyushu University, Japan
[2]Faculty of Sciences, Department of Biology, Kyushu University, Japan

Many animals change their behavioral patterns according to the developmental stages. The behavioral changes must be important, since the appropriate behavior should be different as animals grow and become sexually mature. However, the cellular and molecular mechanisms which give rise to the behavioral changes associated with development are unclear.

Here we explore the mechanism of the behavioral changes during the development with *C. elegans*. We have shown that *C. elegans* larvae show different odor preferences from adult animals. The adult animals show a strong preference for diacetyl, but the larvae do not show such a preference for diacetyl over other odorants. The increase in the chemotactic response to diacetyl during the growth may benefit the animals to locate food, as diacetyl was known to be a metabolite of bacteria.

To analyze the mechanism which regulates the developmental changes in the odor preferences, firstly we developed a new chemotaxis assay format, which is adequate to stably measure the larval stage chemotaxis. With this assay format, we confirmed that the larval stage animals show weaker chemotaxis to diacetyl than the adult stage animals. Secondly, we examined the possibility that the different odor preferences of the larvae are due to the slow moving speed of the larvae. We analyzed the chemotaxsis of several mutants with different moving speeds, and found no correlation between the odor preferences and the moving speeds. This result suggests that the behavioral change is more likely to be due to modulation in sensory circuits. Finally, for the purpose of revealing the molecular mechanism, we screened for mutant larvae which restore the chemotactic response to diacetyl like adult stage, and have isolated one candidate mutant. We hope to uncover the mechanism which gives rise to the behavioral changes associated with development, by analyzing the gene responsible for the phenotype.

Contact: 3SL14055W@s.kyushu-u.ac.jp

Optical neural silencing by novel light-driven proton pumps in *C. elegans*

Megumi Takahashi, Ayako Okazaki, Takashi Tsukamoto, Yuki Sudo, Shin Takagi

Division of Biological Science, Graduate School of Science, Nagoya University, Japan

Archearhodopsin-3 (Arch), a light-driven proton pump activated with green light (~550 nm), can be utilized for neural silencing in animals including, *C. elegans*. To control neural activity under various situations, it is desirable to have optogenetic tools with a variety of altered properties such as action spectrum or ion selectivity. Here we report application of two light-driven proton pumps to *C. elegans*. One is ArchT, whose light sensitivity is reported to be 3.3 times higher than that of Arch in mammalian neurons. The other is a variant of Arch generated through structure-guided mutagenesis, Mid-Arch, whose absorption maximum shifts to 500 nm. To examine their activity in worms' cells, each pump was expressed in the body-wall muscles or neurons of the entire body of worms, and the percentage of worms which are paralyzed with light was scored as an index of silencing of the cells. Worms expressing ArchT stopped locomotion when illuminated with a green light, though the silencing activity of ArchT was not significantly different from that of Arch. Worms expressing Mid-Arch also stopped locomotion with light. The 500 nm-light affected worms' locomotion more efficiently than the 550 nm-light, which is consistent with the results of *in vitro* studies. These pumps will be helpful to study the function of *C. elegans* neural networks. In addition to this, we have also confirmed that a novel proton pump derived from a eubacterium *Thermus thermophilus*, whose activity in eukaryotic cells had not been examined so far, can serve as a light-driven silencer. This is the first report on optogenetic application of "eubacterial" proton pump. Thus, locomotion of *C. elegans* is a convenient *in vivo* assay system to assess the utility of candidate molecules as optogenetic tools, which can then be applied to other higher animals.

Contact: takahashi.megumi@a.mbox.nagoya-u.ac.jp

Behavioral changes in *C. elegans* chemotaxis to alkaline pH

Takashi Murayama, Ichiro Maruyama

Okinawa Institute Science and Technology Graduate University, Japan

Monitoring of environmental and tissue pH is crucial for the survival of animals. The nematode *C. elegans* is an excellent model organism for the analysis of neural circuits that regulate animal behaviors. The animal is attracted to mildly alkaline pH, and avoids strongly alkaline pH. Our genetic dissection and Ca^{2+} imaging demonstrated that ASEL and ASH are the major sensory neurons responsible for attraction to mildly alkaline pH and repulsion from strongly alkaline pH, respectively. In ASEL, a transmembrane guanylyl cyclase, GCY-14, is activated by environmental alkalinization, and in turn, a cGMP-gated channel serves for Ca^{2+} influx into the sensory neuron. In ASH, TRPV channels are found to be required for the neural activation upon stimulation with strongly alkaline pH. To understand the animal's behavioral changes at molecular and cellular levels, we have analyzed behaviors of mutants defective in ASEL and/or ASH under various alkaline pH, and have found that activities of ASEL and ASH compete each other for the behavioral change. While mildly alkaline pH preferentially activates ASEL, strongly alkaline pH activates both ASEL and ASH, and ASH activity overrides the activity of ASEL. Neural circuits responsible for this behavioral change will also be discussed.

Contact: tmurayama@oist.jp

Chemotaxis assays reveal phoretic carrier sensing in *Caenorhabditis*

Gavin C Woodruff, Natsumi Kanzaki

Forestry and Forest Research Products Institute, Tsukuba, Ibaraki, Japan

Ecological specialization is ubiquitous throughout the diversity of life. However, although a great deal is known about *C. elegans* and other *Caenorhabditis* species, the biology of their ecological context is poorly understood. Proliferating *Caenorhabditis* are most commonly found on rotting fruit and plant material. *Caenorhabditis* are also associated with invertebrate phoretic carriers, which are thought to transport nematodes to new sources of food. Some *Caenorhabditis* species, such as *C. elegans*, are phoretic carrier generalists, and have been observed associated with a variety of different carrier species. Other *Caenorhabditis* species, such as *C. japonica*, are phoretic carrier specialists, and have only been observed with a single host. Here, an experimental system investigating the biology of phoretic host sensing in *Caenorhabditis* is proposed. It is shown using chemoattraction assays that *C. japonica* dauer larvae are highly attracted to homogenates of its host, the shield bug *Parastrachia japonensis*. Conversely, *C. japonica* adults are not attracted to *P. japonensis* homogenates. *C. elegans* dauer larvae demonstrate variation in attraction to *P. japonensis* homogenates, but all strains are less attracted to *P. japonensis* than *C. japonica*, consistent with these species' known carrier associations. Further chemotaxis experiments utilizing different *Caenorhabditis* and carrier species are proposed. Potentially, this system can be utilized to address the genetics and evolution of ecological specialization in the future.

Contact: woodruff@affrc.go.jp

flp-12 neuropeptide and acetylcholine orchestrate to generate proper head locomotion of *C. elegans*

Do-Young Kim

Department of Brain Science, DGIST, Daegu, 711-873, Republic of Korea

A multitude of neuropeptides and neurotransmitters orchestrate to execute numerous biological programs. However, mechanisms of how these components are coordinated to generate proper behaviors are not fully understood. In *C. elegans*, the SMB motor neurons consist of four neurons that innervate head neck muscles (White et al, 1986), and monitor the amplitude of sinusoidal movement (Gray et al, 2005). The SMB neurons has shown to express a single FMRFamide-neuropeptide FLP-12 and acetylcholine (ACh) neurotransmitters. To identify roles of FLP-12 and ACh in the head locomotion, we first observed phenotypic consequences of loss of FLP-12. We found that the average wave amplitude is increased in the *flp-12* deletion mutants, indicating that FLP-12 may regulate head movement. Since Ach is broadly expressed in *C. elegans* nervous system, it is challenge to study the function of ACh in a single neuron. We plan to express tetanus toxic light chain (TeTx) specifically in the SMB neurons to block Ach transmission. Additionally, we are searching for genes that have roles in function of SMB via gene expression profiling. From this study, we will reveal how neurotransmitters and neuropeptides regulate the SMB neurons to generate head locomotion.

Contact: doyoungkim@dgist.ac.kr

Dopamine-octopamine layered monoamine signaling modulate sensory response in odor learning

Fumie Hiramatsu, Kosuke Fujita, Yuki Tanimoto, Yukako Fujie, Kotaro Kimura

Dept. Biol. Sci., Osaka Univ., Osaka, Japan

Learning is the process of acquiring new information by the nervous system, which is reflected in behavioral activities. Although learning regulated by glutamate signaling (e.g. through NMDAR) has been well established, the involvement of neuromodulator in learning is unknown in details yet. By using *C. elegans*, we take the advantage of genetics, the wiring diagram, as well as the transparency of the animal for the ease of calcium imaging *in vivo*. We have shown previously that neuromodulation by dopamine and octopamine, a layered monoamine signaling, regulates non-associative enhancement of *C. elegans* avoidance behavior to a repellent odorant 2-nonanone (Kimura et al., 2010). When the animals are pre-exposed to 2-nonanone without food for one hour prior to the assay, they showed enhanced avoidance distance compared to control conditions. Since we have recently found that AWB as well as ASH sensory neurons are involved in 2-nonanone avoidance responses in control animals (Tanimoto et al., this meeting), we are currently analyzing learning-dependent changes in AWB and ASH activities. The cell activities are monitored using our integrated microscope system for *C. elegans*, where during calcium imaging, the worm is exposed to an odor flow reproducing temporal changes in odor concentration that a worm would experience in avoidance behavior in an odor gradient. We have observed several changes in the neural response after pre-exposure and are currently trying to analyze whether they are monoamine-signaling dependent. Our future finding may contribute to the studies of changes in sensory signaling regulated by neuromodulation in higher animals.

Contact: hiramatsu@bio.sci.osaka-u.ac.jp

Non-redundant function of two subtypes of octopamine receptors in food deprivation-mediated signaling in *C. elegans*

Satoshi Suo, Midori Yoshida, Shoichi Ishiura

Graduate School of Arts & Sciences, University of Tokyo, Japan

Amine neurotransmitters act primarily through G protein-coupled receptors (GPCRs). In many cases, there are multiple receptors that bind to the same neurotransmitter and activate the same intracellular signaling cascades. In a model animal *Caenorhabditis elegans*, four amine neurotransmitters, octopamine, tyramine, dopamine and serotonin function in neurons and muscles to modulate behaviors and metabolism in response to environmental cues. It has been previously shown that octopamine activates CREB (cAMP response element-binding protein) in the cholinergic SIA neurons during food deprivation. This activation is mediated through activation of the octopamine receptor SER-3 that is expressed in these neurons. We analyzed another subtype of octopamine receptor, SER-6, which is highly homologous to SER-3. As seen in *ser-3* deletion mutants, CREB activation induced by exogenous octopamine and food deprivation was decreased in *ser-6* deletion mutants compared to wild-type animals, suggesting that SER-6 is required for this signal transduction. Expression of SER-6 in the SIA neurons was sufficient to restore CREB activation in the *ser-6* mutants, indicating that SER-6 functions in these neurons as does SER-3. The response to exogenous octopamine and food deprivation was not different between *ser-3*/+;*ser-6*/+ double heterozygous animals and wild-type animals. In addition, overexpression of one receptor subtype did not fully restore CREB activation in the absence of the other receptor. Taken together, these results demonstrate that two types of similar GPCRs, SER-3 and SER-6, are required for normal signaling and function in the same cells in a non-redundant manner.

Contact: suo@bio.c.u-tokyo.ac.jp

Analyses of molecular mechanisms that negatively regulate forgetting of olfactory adaptation

Takahiro Ito[1], Tomohiro Kitazono[1], Akitoshi Inoue[1], Takeshi Ishihara[2]

[1]Graduate School of Systems Life Sciences, Kyushu University, Japan
[2]Faculty of Sciences, Department of Biology, Kyushu Universiity, Japan

Forgetting of memory is important for animals to survive in the continuously changing environments. If we cannot forget memory, we will encounter various kinds of problem. For example, new memories may stand in opposition to old memories, and a lot of memories filled in our brain may disturb learning new information. In this study, we analyzed the forgetting of the memory for the diacetyl adaptation. In *C. elegans*, TIR-1/JNK-1 pathway regulates the secretion of a neural signal from AWC neurons to accelerate forgetting. TIR-1/JNK-1 pathway regulates a neural signal that affects the AWA neurons sensing diacetyl. However, the downstream components of TIR-1/JNK-1 pathway remain unclear. To identify genes that negatively regulate the forgetting of the memory at the downstream of TIR-1/JNK-1 pathway, we focused *ceh-36* mutant, in which AWC neurons lose their specific function. The loss of the functional AWC neurons in *ceh-36* causes the longer retention of the memory, because the forgetting signals may not be secreted. We mutagenized *ceh-36* mutant by ethyl methane sulfate, and screened suppressor mutants of *ceh-36*, which show the normal retention of diacetyl adaptation. For the efficient screening of the suppressor mutants, we developed a new screening method, in which, we used Cu^{2+} as a repellent. By this method, we obtained some candidates of the suppressor mutants. These mutants will support the study of forgetting of memory. We plan to investigate this mutation for identifying the genes that affect forgetting.

Contact: 1sc10242m@gmail.com

Numerical approach towards quantitative understanding of neural network of *C. elegans*

Takuya Onuma[1,2], Hirofumi Sato[2,3], Yuichi Iino[2,3], Yuishi Iwasaki[1,2]

[1]Department of Intelligent System Engineering, Ibaraki University, Japan
[2]JST, CREST, Japan
[3]Department of Biophysics and Biochemistry, University of Tokyo, Japan

Calcium imaging is a powerful tool to visualize neuronal activity *in vivo*. On the other hand, membrane potential plays a key role in determining neural activity. In most cases in *C. elegans*, the experimentally observed value is not the membrane potential but the fluorescence intensity. Time scales of membrane potential dynamics and Ca^{2+} probe dynamics are different by two or three orders of magnitude. Furthermore, the correspondences between the membrane potential, Ca^{2+} and the fluorescence intensity are nonlinear with time delay. Keeping the above mentioned issues in our mind, we propose a minimal mathematical model of neural network whose result is quantitatively comparable to the experimental data.

Our model includes two dynamical variables: the membrane potential V and the Ca^{2+} concentration $[Ca^{2+}]$. Neural activity is described by a conductance-based model of V. That is, the electrical behavior of neuron is determined by the sum of currents through various ion channels, synaptic currents and an additional current as an external stimulation for sensory neuron. Instead of the detail of each ion channel, the electrical properties of a single neuron is integrated into a current-voltage curve (I-V curve) in our model. To evaluate $[Ca^{2+}]$ from V, we introduce a steady-state $[Ca^{2+}]$-V relationship which is bell-shaped curve as a function of V. Calcium imaging shows that there are two types of neurons: fast or slow response neuron to external inputs. To characterize the types of neurons, furthermore, we introduce the time constant parameter of Ca^{2+} response. Finally, the fluorescence intensity is calculated from $[Ca^{2+}]$ on the basis of affinity curve of Ca^{2+} probe. In addition, our model are able to consider the individual difference among worms in the expression level of Ca^{2+} probe.

We applied the neural model to NaCl chemotaxis circuit. In *C. elegans*, the main chemosensory neurons for NaCl are ASEL/R. As nearest "downstream" interneurons of ASE, AIAL/R and AIBL/R were taken into account. Numerical simulation was carried out to reproduce the imaging data under the condition that ASER was stimulated by a downstep of NaCl concentrations from 50mM to 25mM. We found that I-V curve (electrical properties of a single neuron), the steady-state $[Ca^{2+}]$-V relationship, and the polarities of chemical synapses were reasonably predicted. The time constant parameter of Ca^{2+} response was necessary to yield slow behavior in Ca^{2+} dynamics.

Contact: 13nm906a@hcs.ibaraki.ac.jp

A computational model of olfactory signaling in *C. elegans*.

Mamoru Usuyama, Yuishi Iwasaki

Department of System Engineering, Ibaraki University, Japan
JST, CREST, Japan

AWC neurons indicate the off-response to odor stimuli using cGMP as a second messenger. A transient Ca^{2+} increase is observed after odor removal. However, how an odor signal controls cGMP concentrations to modulate activity of AWC neurons is still unclear. In this study, we propose a numerical signal transduction model for the odor signal process to good understanding for olfaction in *C. elegans*. Methods for neuronal modeling can be divided into two categories, detailed modeling and simplified modeling. In detailed modeling, kinetic schemes of the intracellular molecules are described. When biochemical and molecular biological aspects in neuron have been evidenced, detailed modeling is useful for providing verification experiments with confidence. In simplified modeling, on the other hand, complicated molecular dynamics is described by the phenomenological equation with a small number of parameters. For signal transduction in a sensory neuron, simplified modeling is useful for investigating a neuronal response to a given external stimuli. However, it is difficult to discuss why sensory adaptation and learning occur. We adopt detailed modeling on the basis of physiological knowledge because our aim is to provide a numerical tool to discuss the intracellular signal transduction in an olfactory receptor neuron. We modify the previous model (Usuyama et al., PLoS ONE 2011) to be an essential model which consists of minimum molecular components to yield olfactory adaptation. The model includes a feedback system to replicate the off-response in olfaction. We assume that feedback process is driven by an intracellular calcium dependent manner to control the intracellular second messenger cGMP.

In vertebrates and insects, their olfactory systems have the fast and strong adaptation and learning mechanisms. *C. elegans* also shows an adaptation to odor stimuli. In AWC neurons, it has been reported that the transient response to odor stimuli is correlated with its exposure time. Early adaptation in vertebrate olfaction is caused by beta-arrestin and GRK. Sensory adaptation caused by beta-arrestin was also reported in *C. elegans* sensory neurons. However, it is unclear that beta-arrestin works in early adaptation. Therefore, we attempt to reproduce a correlation between stimulus exposure time and transient Ca^{2+} increase without adaptation mechanisms of beta-arrestin.

Contact: usuyama@mx.ibaraki.ac.jp

The homeodomain protein LIM-4 specifies a peptidergic and cholinergic motor neuron fate in *C. elegans*

Jihye Yeon, Jinmhan Kim

DGIST, Republic of Korea

The expression of specific transcription factors dictates the differentiated features of postmitotic neurons. However, the mechanism of how specific molecules determine or specify neuronal cell fate during development is not fully understood. In *C. elegans*, the cholinergic and peptidergic SMB neurons consist of four motor neurons that innervate muscle quadrants in the head, send processes posterior down the sub-lateral cords (White et al., 1986), and monitor the amplitude of sinusoidal movement (Gray et al., 2005). To identify the factors that specify the neuronal cell fate of SMB, we performed genetic screens and isolated several *lim-4* mutant alleles, in which *flp-12* neuropeptide gene expression was completely abolished only in the SMB neurons. Previously, it was shown that the LIM-4 LIM homeobox protein has a major role in specification of AWB chemosensory neuron identity (Sagasti et al., 1999). We found that the expression of other SMB markers, *odr-2* (GPI-Anchored protein), *cho-1* (choline transporter), and *unc-17* (synaptic vesicle acetycholine transporter), were also abolished in *lim-4* mutants and LIM-4 maintains its own expression by autoregulation in the SMB neurons. To investigate the molecular mechanism of *lim-4*, we did promoter analyses and bioinformatics searches with the SMB marker genes and identified several *cis*-regulatory motifs including putative LIM-4 binding sites. We confirmed that these regulatory elements were sufficient to drive the expression of a non-SMB marker gene in the SMB neurons. In addition, we expressed *lim-4* cDNA under the control of the heat shock promoter which not only fully restored *flp-12* gene expression in *lim-4* mutants, but induced the ectopic expression of *flp-12* in other cell-types. Since *lim-4* is evolutionary well-conserved throughout different species, we also expressed the LIM-4 human orthologue, LHX6 and LHX8 cDNA under the control of heat shock promoter in *lim-4* mutants, and found that *lim-4* mutant phenotypes were fully rescued. Furthermore, we are currently expressing the human LHX6 or *C. elegans* LIM-4 in the human neuroblastoma cells to test if expression of these genes could induce the cholinergic cell fate. Taken together, LIM-4 appears to be necessary and sufficient to promote specification of the cholinergic and peptidergic SMB motor neuron fate.

Contact: yeonj718@dgist.ac.kr

Internal metabolic status modulates pheromone-mediated neural plasticity in *C. elegans*

Leesun Ryu

Department of Brain Science, DGIST (Daegu Gyeongbuk Institute of Science & Technology), Daegu, 711-873, Republic of Korea

The nervous system senses and processes environmental signals in the context of internal and/or external signals to generate proper behaviors. Behavioral plasticity such as habituation, sensitization, and short/long-term memory is essential for survival and evolution of animals and can be mediated by changes at the sensory receptor and/or neuronal circuit levels. However, the molecular and cellular mechanisms underlying these mechanisms of behavioral plasticity are not well understood. *C. elegans* provides an ideal system in which to explore the molecular and neural basis of behavioral flexibility. Previously, we and others found that adult hermaphrodites specifically avoid the ascaroside pheromone C9 (ascr#3) (Srinivasan et al., 2008; Macosko et al. 2009; Jang et al., 2012). The ADL chemosensory neurons detect and drive repulsion to C9 (Jang et al., 2012). *In vivo* Ca^{2+} imaging experiments showed that addition of C9 induces a rapid increase in ADL intracellular Ca^{2+} levels which returns quickly to baseline even in the presence of C9. When animals were exposed to 10-second pulses of C9 at 10-second intervals, ADL exhibited Ca^{2+} transients to the addition of only the first pulse of C9 but not to following pulses indicating that ADL quickly habituates to C9 (Jang et al., 2012). We now found that after animals were removed from the food source for just 5mins, ADL exhibited Ca^{2+} transients to not only the first pulse but also to a second pulse of C9 and animals also repelled to the second C9 exposure. In addition, we found that neurotransmitters such as dopamine and serotonin that have roles in mediating food-related neuronal responses regulated this behavioral plasticity. These observations suggest that C9 habituation of ADL is modulated by internal feeding status. To investigate how internal metabolic conditions influence ADL pheromone responses, we are currently performing rescue experiments and Ca^{2+} imaging to identify neuronal mechanisms in the pheromone mediated neural plasticity.

Contact: leesunryu@dgist.ac.kr

Coordinated regulation of peptidergic SAA interneuron traits through a conserved terminal selector gene

Myeongjin Hong

Department of Brain Science, DGIST, Daegu, Republic of Korea

Neuronal specification and differentiation are orchestrated through signaling molecules and transcription factors. However, the mechanisms underlying how specific transcription factors regulate cell fate are not fully understood. Using *C. elegans* as a model system, we are investigating the molecular and cellular mechanisms for neuronal cell-fate specification. In *C. elegans*, the SAA cholinergic and peptidergic interneurons consist of two dorsal (SAADL/R) and two ventral (SAAVL/R) neurons that have been implicated in head foraging via the circuit comprising the RME and SMB motor neurons (White et al., 1986). To examine how the SAA neurons are specified during development, we conducted genetic screens and candidate gene searches utilizing the expression pattern of the *flp-7* neuropeptide gene, which is expressed in the SAA neurons as well as other neurons (Kim et al., 2004). We identified over 30 mutants that showed altered *flp-7* expression patterns. In *lsk24* mutants *flp-7* expression was completely abolished in the SAA neurons. Through complementation tests, we found that *lsk24* mutants were allelic to previous identified *unc-42* mutant animals, suggesting that *unc-42* is required for the expression of *flp-7* in these neurons. UNC-42 is a paired-like homeodomain protein of the Q50 class homologous to ARX (Jamel et al., 2002). ARX had been shown to have roles in interneuron migration in the developing brain (Stromme et al. 2002). To investigate how UNC-42 regulates *flp-7* expression in SAA, we examined the promoter of *flp-7* gene and identified a *cis*-regulatory motif that is required for *flp-7* expression in SAA. In addition, the promoters of other SAA markers, including *lad-2* (L1CAM adhesion molecule homolog), are being analyzed. We are also testing whether expression of *unc-42* is autoregulated and whether transgenic worms expressing *unc-42* cDNA under the control of *hsp*-16.2 heat shock promoter restores *flp-7* expression in SAA. Finally, we are planning to clone other mutants that control SAA specification.

Contact: mjhong@dgist.ac.kr

C. elegans apl-1 allows practical analysis of Alzheimer's Disease-Related Amyloid Precursor Protein function

Alexander B. Mir

City College of New York, USA

The progressive, neurodegenerative disorder Alzheimer's disease (AD) can be characterized by amyloid plaques in the brain. These plaques consist chiefly of the b-amyloid peptide, a cleavage product of the poorly understood amyloid precursor protein (APP). The study of APP in mammalian systems is complicated by the presence of three separate genes encoding functionally redundant proteins: APP, APLP1 and APLP2. In contrast, the free-living nematode *Caenorhabditis elegans* has only a single APP-related gene: *apl-1*. We utilize two specific alleles of *apl-1*, *apl-1(yn5)* and *apl-1(yn10)*, in our dissection of the apl-1 pathway.

The *apl-1(yn5)* allele has a deletion mutation which removes the coding region for the cytoplasmic and transmembrane segments of the APL-1 protein as well as much of the 3' UTR. *apl-1(yn5)* mutants are viable, demonstrating that the extracellular domain of APL-1 is sufficient for survival. These mutants show a delayed development as well as a partially penetrant temperature-sensitive embryonic lethality with is greatly enhanced by the knockdown of the insulin/IGF-1 receptor ortholog *daf-2*. To further investigate this interaction, we have generated a *daf-2(e1368);apl-1(yn5)* double mutant that experiences a highly penetrant embryonic lethality at 25°C. We are performing a forward mutagenic screen to identify mutations rescuing the *apl-1(yn5);daf-2(e1368)* phenotype, indicating potentially novel agents in the APL-1 pathway.

apl-1(yn10) is a null allele in which a 1,931bp deletion removes the coding region for the extracellular domain of APL-1. These mutants are not viable. We wish to identify the proximal promoter elements critical for APL-1 expression. To identify these elements, we are generating a series of rescue constructs containing the APL-1 coding sequence with partial upstream elements. We will micro-inject these rescue plasmids into *apl-1(yn10) lon-2* heterzygotes and screen for viable long progeny (indicating homozygosity), demonstrating that the given promoter fragment is sufficient for viability. We aim to better understand *C. elegans* APL-1 by characterizing its interaction with the insulin receptor pathway as well as by illuminating its key proximal regulatory elements. These insights will allow us to characterize the normal function of human APP in greater detail, in turn contributing to our overall knowledge of the pathogenesis of AD.

Contact: a.bernier.mir@gmail.com

SVH-5 transcription factor regulates axon regeneration in *C. elegans* by activating the transcription of the *svh-2* gene encoding a receptor tyrosine kinase

Chun Li, Naoki Hisamoto, Kunihiro Matsumoto

Nagoya University, Japan

Axon regeneration is an evolutionarily conserved process from nematodes to humans. Recently, we have identified a growth factor SVH-1 and its receptor tyrosine kinase SVH-2 as positive regulators of axon regeneration in *Caenorhabditis elegans*. Axon cutting by laser microsurgery induces the transcription of the *svh-2* gene specifically in severed neurons. However, the mechanism by which how the *svh-2* gene is induced in response to axon cutting is not well understood. In this study, we identified SVH-5 transcription factor as a regulator involved in the transcriptional induction of the *svh-2* gene. In *svh-5* null mutants, *svh-2* induction by axon cutting did not occur, and frequency of axon regeneration was greatly reduced. In addition, forced expression of the *svh-2* gene in neurons by the constitutive promoter was able to suppress the axon regeneration defect in *svh-5* mutants. The *svh-2* promoter region contains the consensus sequences of the transcription factor-binding site. We introduced mutations in these sites and found that one of them is essential for the transcriptional induction of the *svh-2* gene. These results suggest that SVH-5 acts as a transcription factor, which activates the transcription of the *svh-2* gene in response to axon cutting.

Contact: li_spring2006@hotmail.com

The multidrug resistance protein MRP-7 inhibits methylmercury-associated animal toxicity and dopaminergic neurodegeneration in *Caenorhabditis elegans*

Richard Nass, Natalia VanDuyn

Indiana University School of Medicine, USA

Background: Methylmercury (MeHg) exposure from occupational, environmental, and food sources is a significant threat to public health. Recent epidemiological and vertebrate studies suggest that MeHg exposure may contribute to dopamine (DA) neuron vulnerability and the propensity to develop Parkinson's disease (PD). We have developed a novel *Caenorhabditis elegans* (*C. elegans*) model of MeHg toxicity and have shown that low, chronic exposure confers embryonic defects, developmental delays, and DA neuron degeneration, and that the toxicity is partially dependent on the phase II antioxidant transcription factor SKN-1/Nrf2. Aims/Objectives: In this study we asked what genes and molecular pathways are involved in MeHg-induced whole animal and DA neuron pathology. Methods: We utilized a reverse genetic screen, biochemical assays, immunofluorescence, transgenic *C. elegans*, RT-PCR, ICP-MS, Western analysis, and neuronal morphology analysis to characterize expression, localization and the role that SKN-1, MRP-7 and post-translational modifications play in MeHg-induced whole animal and DA neuronal death. Results: Over 17,000 genes were screened for whole animal sensitivity to MeHg, and 92 genes were identified (90% have strong human homologues) that affect whole animal and/or DA neuron pathology. These genes are strongly biased towards mechanisms that affect the mitochondria, transcription, apoptosis and calcium signaling. Here we report detailed analysis of a putative plasma membrane transporter's role in MeHg-associated DA neuroprotection. Specifically we demonstrate that genetic knockdown of MRP-7 results in a 2-fold increase in Hg levels and a dramatic increase in stress response proteins associates with the endoplasmic reticulum, golgi apparatus, and mitochondria, as well as an increase in MeHg-associated animal death. Chronic exposure to low concentrations of MeHg induces MRP-7 gene expression, while exposures in MRP-7 genetic knockdown animals results in a loss of DA neuron integrity in without affecting whole animal viability. Transgenic animals expressing a fluorescent reporter behind the endogenous MRP-7 promoter indicate that the transporter is expressed in DA neurons. Conclusions: These studies show for the first time that a multidrug resistance protein is expressed in DA neurons, and its expression inhibits MeHg-associated DA neuron pathology.

Contact: ricnass@iupui.edu

Depletion of intermediate filament IFB-1 negatively affects mitochondrial trafficking, development and functionality of *C. elegans* amphid sensory neurons

Muniesh Muthaiyan Shanmugam, Yen Chang, Gong Her Wu, Ahmed F Elshikh, Oliver I Wagner

National Tsing Hua University, Taiwan

Mitochondria are essential cellular organelles for ATP synthesis, calcium buffering and apoptosis. Mitochondria accumulations based on impeded transport mechanisms, as well as neuronal intermediate filaments (IF) accumulations, are both hallmarks in neurodegenerative diseases. Long-range bidirectional transport of mitochondria depends on microtubules and microtubule-based motor proteins, while short-range transport and docking mainly relies on actin and actin-based motors. Unlike actin and microtubules, intermediate filaments (IFs) do not possess associated motor proteins. Further, it is poorly understood whether a link between neuronal IFs and mitochondria mobility exist.

In *C. elegans*, among the 11 cytoplasmic IF family proteins, IFB-1 is of particular interest as it is expressed in a subset of sensory neurons and forms an obligate heteropolymer with one of the essential IFA proteins crucial for worm viability. Particularly, IFB-1 is expressed in amphidic neurons (necessary for chemosensation) and depletion of this protein leads to significant chemotaxis defects, however, no dye-filling defects. Mitochondria motility in worms carrying different mutations in the IFB-1A gene, as well as in IFB-1 knockdown animals, was largely impeded with reduced velocity, frequency of directional changes, increased pausing frequency and increased moving persistencies. Similarly, when eliminating the mitochondrial membrane potential *in vitro*, we observed that mitochondria motility is similarly impeded as compared to IFB-1A knock-outs. We also revealed significant changes in mitochondrial shapes, sizes, densities as well as in oxygen consumption. Importantly, IFB-1A colocalizes with mitochondria at discrete regions in *C. elegans* neurons. We also demonstrate that axonal growth is reduced in cultured cells from IFB-1A mutants and that the development of dendritic sensory neurons in worms is significantly affected. Taken together, we propose a model in which intermediate filaments may serve as important docking stations for mitochondria during their long transport in neurons as (miniature) molecular motors may not provide full mechanical support for these large organelles.

Contact: shanmugambms@gmail.com

Genetic analysis of a neomorphic tubulin mutation that redirects synaptic vesicle targeting and causes neurite degeneration in *C. elegans*

Jiun-Min Hsu[1], Chun-Hao Chen[1], Yen-Chih Chen[1], Kent McDonald[2], Mark Gurling[3], Albert Lee[4], Gian Garriga[3], Chun-Liang Pan[1]

[1]Institute of Molecular Medicine, College of Medicine, National Taiwan University, Taipei, Taiwan
[2]Electron Microscope Laboratory, University of California, Berkeley, USA
[3]Department of Molecular and Cell Biology and Helen Wills Neuroscience Institute, University of California, Berkeley, USA
[4]Department of Chemistry, National Taiwan University, Taipei, Taiwan

The interaction between microtubules and the molecular motors is critical for the polarized distribution of synaptic vesicles to the axon. The anterograde motor KIF1A/Kinesin 3 and the retrograde motor dynein are responsible for synaptic vesicle transport and both interact with a shared C-terminus region of alpha-tubulins enriched in acidic residues. Here we report a neomorphic mutation in the touch neuron-specific α-tubulin, *mec-12*, that altered an absolutely conserved glycine to glutamic acid. The mutant, *mec-12* (*gm379*), showed mislocalization of synaptic vesicles to the short posterior process of the PLM touch neuron and also extensive swellings of the touch neuron processes. Both phenotypes were not seen in the *mec-12* null mutants and could be eliminated by *mec-12* RNAi or removing *mec-7*, a touch neuron-specific β-tubulin. Mutations in *unc-104*/Kif1a enhanced synaptic vesicle mislocalization in the *mec-12* (*gm379*). Strikingly, mutations or RNAi of *dhc-1*, the cytoplasmic dynein heavy chain, suppressed synaptic vesicle mislocalization. In microtubule sedimentation assay, DHC-1 was much more likely to co-sediment with the mutant microtubules compared to the wild-type microtubules. By comprehensive site-directed mutagenesis we showed that the density of the acidic residues in the H12 helix of MEC-12 governed synaptic vesicle targeting in the PLM. Our study provides an *in vivo* validation of the electrostatic interaction between α-tubulin and dynein, and contributes to the mechanistic understanding of human neurological syndromes caused by missense tubulin mutations.

Contact: jimmyhsu0419@gmail.com

A CaMK cascade and neuropeptide signaling modulate pheromone-mediated developmental plasticity

JiSoo Park[1], Zhi Fang[1], Piali Sengupta[2], Kyuhyung Kim[1]

[1]Department of Brain Science, DGIST, Daegu, Republic of Korea
[2]Department of Biology, Brandeis University, Waltham, MA 02454, United States

Animals maintain homeostasis in response to ever-changing environmental conditions and metabolic demands. These regulatory processes are known to be mediated partly by changes in gene expression. However, the exact mechanisms by which environmental signals are transduced and integrated with internal metabolic status are still unknown. Ascaroside pheromone exposure down-regulates expression of a *daf-7* TGF-β and a subset of putative G-protein coupled chemosensory receptor genes such as *str-3* in the ASI neurons; this downregulation influences dauer formation (Nolan et al., 2002; Peckol et al., 1999; Kim et al., 2009). To dissect out the signaling pathways underlying this pheromone-mediated developmental plasticity, we performed a genetic screen and also isolated candidate genes (Kim et al., 2009). We found that the *cmk-1* (Ca^{2+}/calmodulin-dependent protein kinase I: CaM kinase I) is required to downregulate *str-3* GPCR expression upon crude or synthetic pheromone exposure. As expected, *cmk-1* mutants were also defective in the pheromone-mediated dauer decision. We also found that *crh-1* (cyclic AMP response element binding protein: CREB) is required for *str-3* expression even in the absence of pheromone. Surprisingly, CMK-1 and CRH-1 act non-autonomously in the ASE/AWC chemosensory neurons to regulate *str-3* expression in the ASI neurons, suggesting that *cmk-1* and/or *crh-1* may act in these neurons to transmit environmental signals to ASI. We next investigated if neuropeptides function in pheromone-mediated *str-3* gene expression and observed that *flp-18* FLP neuropeptides and *npr-5* neuropeptide receptor mutants (Cohen et al., 2009) exhibited defects in down-regulation of *str-3* by pheromone exposure, suggesting that FLP-18 relays the signals from AWC/ASE to ASI.

Contact: jissu85@gmail.com

Expression of an expanded CGG-repeat RNA impairs the olfactory response in a *C. elegans* model of Fragile X-associated tremor/ataxia syndrome

Bi-Tzen Juang[1], Noelle L'Etoile[2], Paul Hagerman[3]

[1]National Chiao Tung University, Taiwan
[2]University of California, San Francisco, USA
[3]University of California, Davis, USA

Fragile X-associated tremor/ataxia syndrome (FXTAS) is a severe neurodegenerative disorder caused by the expansion of CGG trinucleotide repeats in the 5' untranslated region (5'UTR) of the fragile X mental retardation 1 (*FMR1*) gene. Men with CGG repeats of 60-200 are at risk for developing FXTAS, a disease that progresses to death. Although current evidence supports an important role of the expanded-CGG-repeat-containing mRNA leading to cellular toxicity in FXTAS, the mechanisms are unclear. One symptom is a decrease on the plasticity of the startle response to environmental stimulus. To gain genetic and cell biological insight into FXTAS, we investigate the effect of 99 CGG repeats on the plasticity of the olfactory behavior in *C. elegans*. Of the nematode primary sensory neurons, the AWC (amphid wing cells) neurons conduct odor-attractive behavior (chemotaxis), while prolonged odor stimulation in the AWC neurons attenuates the response (adaptation). We found that expression of the *FMR1* 5'UTR with 99 CGGs interfered with the ability to adapt to the AWC-sensed odor. We performed a candidate genetic screen and showed that the microRNA-specific Argonuate, ALG-2, is required to cause the olfactory adaptation defects induced by the expanded-CGG-repeats. This observation suggests that microRNA may play a role in the development of the cellular toxicity to produce FXTAS.

Contact: btjuang@nctu.edu.tw

Modulation of Axon Growth on *C. elegans* with TiO2 Nanoparticles

Chun-Chih Hu

National Tsing Hua University, Taiwan

Due to the widespread applications of titanium dioxide (TiO2) nanoparticles (NPs) in food, cosmetics and medical devices, it is receiving increasing attention to evaluate its toxicities to the environment. Many toxicity studies using different organisms have concluded that the toxicity of anatase TiO2 NPs is based on its photocatalytic effect. However, the toxic effect of anatase TiO2 in the dark condition as not been evaluated. Recently, it has been shown that rutile TiO2 nanotubes are able to induce the differentiation of neuronal rat cells and provide decent biocompatibility. The most elusive question has been whether TiO2 NPs exert direct toxic effects to organisms. Here, we used anatase and rutile TiO2 NPs to treat the neurons of *C. elegans* and see how this particle affects neuronal growth. Here we show that TiO2 NPs with concentration 50μg/ml promote the outgrowth of neurites and inhibit the outgrowth of axons at high concentration from 100μg/ml to 300μg/ml, suggesting TiO2 NPs might be beneficial to axon extension at low concentration but toxic to axons at high concentrations. However, for cell body area no significant difference can be found.

Contact: a1087038@yahoo.com.tw

IFE-1, a *C. elegans* isoform of eIF4E, regulates GSP-3/4, a family of sperm-enriched protein phosphatases for sperm activation

Mohammad Al-Amin[1], Yhong-Hee Shim[1,2], Ichiro Kawasaki[1]

[1]Department of Bioscience and Biotechnology, Konkuk University, Seoul, 143-701, Republic of Korea
[2]Institute of KU Biotechnology, Konkuk University, Seoul, 143-701, Republic of Korea

IFE-1 is one of the five *C. elegans* isoforms of eIF4E, the mRNA 5' cap-binding component of the translation initiation complex. *ife-1* mutants are temperature-sensitive sterile due to a defect in sperm function (Amiri et al., 2001; Henderson et al., 2009). To further analyze the molecular function of IFE-1, we previously performed a proteomic analysis to search for proteins that are down regulated in *ife-1* mutants (Kawasaki et al., 2011). Through the analysis, GSP-3/4 (Glc Seven like Phosphatases) and Major Sperm Proteins (MSPs) were identified as two major groups of down-regulated proteins in *ife-1* mutants. Among them, RNAi of *gsp-3/4* caused an *ife-1*-like phenotype. That is, *gsp-3/4(RNAi)* hermaphrodites produced unfertilized oocytes that were rescued by mating with wild-type males. It was also reported that *gsp-4 gsp-3* double mutant males show defects in sperm activation (Wu et al., 2012).

We therefore analyzed the function of IFE-1 during sperm development; we examined their sperm activity *in vivo* and *in vitro*. By using transgenic strains carrying *Pspe-11::mCherry::H2B*, in which sperm were marked with *mCherry*, we observed that, although *ife-1* mutants produced spermatids, they were immediately depleted from the spermatheca after ovulation started in hermaphrodites. Further, the proportions of *in vitro* activated sperm were significantly reduced in *ife-1* mutant males compared to wild-type males, especially at 25°C. These results support the view that IFE-1 has an essential function for sperm activation or spermiogenesis possibly through the regulation of GSP-3/4. Interestingly, through qPCR analysis, we found that *gsp-3/4* and *msp* mRNA levels were significantly reduced in *ife-1* mutants at high temperature, suggesting that *gsp-3/4* and *msp* gene expressions are controlled at a pre-translational level rather than at translational level by IFE-1. In mammalian cells, it is known that eIF4E has an essential function for nuclear export of subset of mRNAs. We are currently testing whether IFE-1 regulates the nuclear export of *gsp-3/4* and *msp* mRNAs rather than their translation initiation. This study was supported by grants NRF-2013R1A1A2009090, NRF-2013R1A1A2009820, and 2014 KU Brain Pool Program of Konkuk University.

Contact: mohammad.alamin@gmail.com

cdc-25.4 is required for successful male reproduction in *Caenorhabditis elegans*

Sangmi Oh[1], Jae-Hyung Park[1], Ichiro Kawasaki[1], Yhong-Hee Shim[1,2]

[1]Department of Bioscience and Biotechnology, Konkuk University, Seoul 143-701, Republic of Korea
[2]Institute of KU Biotechnology, Konkuk University, Seoul 143-701, Republic of Korea

Cell division cycle 25 (CDC25) phosphatases progress cell cycle by activating cyclin-dependent kinases (CDKs), which are inactivated by WEE1/MYT1 kinases. *C. elegans* has four *cdc-25* family members, *cdc-25.1*, *cdc-25.2*, *cdc-25.3*, and *cdc-25.4*. Comparison of amino acid sequences of CDC25 phosphatase domains between *C. elegans* and *Homo Sapience* shows a high score of similarity. CDC-25 family members appear to have distinct function during *C. elegans* development. Among them, functions of *cdc-25.4* have not been elucidated, yet. We examined *cdc-25.4* expression levels during postembryonic developmental stages and in different genetic backgrounds including *fem-1(hc17lf)* that produces only oocytes, *fem-3(q20gf)* that produces only sperm, and *glp-1(q231lf)* that is germline proliferation defective. We found that *cdc-25.4* mRNA level was highly abundant in wild-type adult males, in L4-stage wild-type hermaphrodites, and in *fem-3gf* adult hermaphrodites. On the other hand, *cdc-25.4* mRNA level was very low in *glp-1* mutant that contains few germ cells. These results suggest that *cdc-25.4* functions during male germline development. To examine whether *cdc-25.4* mutants have a defect in male reproduction, we tested the male fertility of *cdc-25.4(tm4088)* deletion mutant by mating the *cdc-25.4(tm4088)* males with *fog-2(q71)* hermaphrodites which are sterile due to the absence of sperm. We found that *fog-2* hermaphrodites did not produce offspring after mating with *cdc-25.4* mutant males while *cdc-25.4* hermaphrodites produced self-progeny normally. Furthermore, we observed that *cdc-25.4* mutant males and hermaphrodites produced spermatids of normal appearance in their gonads. These results suggest that although *cdc-25.4* mutants can produce spermatids, only the males cannot utilize the spermatids successfully for fertilization. We are currently investigating whether there is any functional difference between male sperm and hermaphrodite sperm in *cdc-25.4* mutants, or whether the sperm produced in *cdc-25.4* mutant males are successfully delivered to hermaphrodites by mating. This study was supported by a grant NRF-2013R1A1A2009090, NRF-2013R1A1A2009820, and 2014 KU Brain Pool Program of Konkuk University.

Contact: cheristal@hanmail.net

Sperm-derived TRP-3 channel mediates the onset of the calcium wave in the fertilized egg of *Caenorhabditis elegans*

Jun Takayama, Shuichi Onami

RIKEN QBiC, Japan

Calcium waves during fertilization trigger embryonic development. How the fertilizing sperm induces the fertilization calcium wave is not well understood. Here we show that sperm-derived TRP-3 channel mediates the onset of the calcium wave in the fertilized egg of *Caenorhabditis elegans*. We monitored the fertilization calcium response by using a chemical calcium indicator and spinning disk confocal microscopy. By computational image analysis of the microscopy movies, we found that the sperm entry induces a rapid (~ 1 s) local calcium rise near the sperm entry point and a following slow (~40 s) global wave traveling from the sperm entry point to the opposite pole of the egg. No calcium responses were observed in *spe-9* or *spe-42* mutant, whose sperm cannot enter the oocyte. The rapid local calcium rise was not observed in the fertilized egg of *trp-3* mutant, which lacks a sperm-specific transient receptor potential (TRP) channel. In the *trp-3* mutant, the traveling wave was observed but its onset was significantly delayed by ~ 26 s. In addition, the success rate of the embryogenesis was decreased in the embryos fertilized by *trp-3* mutant sperm. To examine the localization dynamics of the TRP-3 protein, we expressed a TagRFP-T-fused *trp-3* transgene in sperm. The TRP-3::TagRFP-T fluorescence was associated with the plasma membrane of the mature sperm. By observing the fertilization between the TRP-3::TagRFP-T-expressing sperm and GFP::PH-expressing oocytes, in which the plasma membrane was labeled, we found that TRP-3 proteins were transferred from sperm to the plasma membrane of the fertilized egg. Moreover, high-speed (49 fps) imaging revealed that the calcium concentration in the fertilizing sperm cytoplasm increased after sperm-egg fusion. These results demonstrate that TRP-3 is transferred from the plasma membrane of the fertilizing sperm to that of the fertilized egg upon plasma membrane fusion and induces the rapid local calcium rise and mediates the timely onset of the traveling calcium wave in the fertilized egg. The sperm-derived TRP-3 channel may act as a calcium conduit that allows calcium entry from the extracellular space to the cytoplasm of the fertilized egg.

Contact: jtakayama@riken.jp

Using next-generation sequencing to determine gene identity in temperature-sensitive, embryonic lethal mutants with adult germline development defects

Josh B Lowry, Bruce Bowerman

Institute of Molecular Biology, University of Oregon, USA

Conditional alleles of essential genes are uniquely valuable genetic tools, as many if not most essential genes have multiple requirements during the life of an organism, and many essential genes are widely conserved. *C. elegans* is unique as an animal model in which one can feasibly screen through very large mutagenized populations for rare conditional (heat-sensitive) mutations in essential genes. However, this virtue remains greatly under-exploited. Genome wide RNAi screens have identified ~2500 essential *C. elegans* genes, yet there are conditional alleles for only about 100 of them. We are using next generation DNA sequencing-based approaches in an effort to identify conditional mutations in more essential *C. elegans* genes. We have applied these methods to 25 recessive TS-EL mutants that are both (i) eggshell-defective (and hence osmotically sensitive) after late larval (L4) upshifts to the restrictive temperature, and also (ii) adult sterile after early larval (L1) upshifts to the restrictive temperature (Osm/Ste mutants). Because eggshell production (mediated by proteins secreted by the zygote after fertilization), and development of the complex membrane architecture of the *C. elegans* gonad both depend on membrane trafficking, we hypothesized that the affected genes might be important for gonad membrane morphogenesis and dynamics. To identify the causal mutations, we use a combined genome-wide SNP mapping and whole genome sequencing strategy. We now have WGS data for 19 of the TS-EL mutants. We have confirmed the identity of the causal mutations in 8 of these mutants based on non-complementation results from genetic crosses with deletion alleles that are available for candidate genes identified in the WGS data. Consistent with our initial hypothesis, some of the identified genes are known to be involved in membrane biology (*abtm-1*, *drp-1*, and *vps-15*). Other conserved identified loci include *atx-2*, *crn-3*, and Y17G7B.13. We have narrowed the candidate genes to a single locus for an additional 5 mutants, and to two loci for 3 others: the TS-EL mutations complement deletion alleles in all but one or two candidate genes, for which no deletion alleles are currently available. We will describe the WGS approach and present images of the gonad defects in sterile adult mutants.

Contact: jlowry@uoregon.edu

The *C. elegans* gene *spe-45*, expressed in the male germline, is essentially required for gamete fusion like mouse *Izumo1*

Tatsuya Tajima[1], Jun Takayama[2], Skye Comstra[3], Shuichi Onami[2], Steven W. L'Hernault[3], Hitoshi Nishimura[1,3]

[1]Department of Life Science, Setsunan University, Japan
[2]Laboratory for Developmental Dynamics, RIKEN Quantitative Biology Center, Japan
[3]Department of Biology, Emory University, USA

Mouse spermatozoa contain the immunoglobulin (Ig)-like protein IZUMO1, which is indispensable for sperm-oocyte fusion. However, the molecular basis of gamete fusion is still largely unknown. For example, IZUMO1 is unlikely to be the sperm fusogen that directly mediates membrane fusion, since this protein has no hydrophobic motif besides its transmembrane domain. It implies that IZUMO1 may carry a real fusogen to comprise the sperm fusogenic machinery.

By reverse genetics, we have recently identified the *C. elegans* gene F28D1.8. This gene encoded an Ig-like, single-pass transmembrane protein and seemed specifically expressed in the male germline. Moreover, the deletion allele tm3715 of F28D1.8 caused the self-sterility of hermaphrodites, so that F28D1.8 was named *spe-45*. In this study, we examined the functional role(s) of *spe-45* in the *C. elegans* male germline functions; spermatogenesis, spermiogenesis and fertilization.

First of all, in *him-5(e1490)* males, we expressed a *gfp* gene that is flanked by native 5'- and 3'-UTRs of *spe-45*. The resulting GFP signals were detected in the proximal male gonads, confirming that *spe-45* is expressed in male germ cells. Next, spermatids released from *spe-45(tm3715)* males were similar to those from wild-type worms in cell number and cytology, suggesting that spermatogenesis normally occurs in mutant males. As we out-crossed *spe-45(tm3715)* males to *fem-1(hc17)* hermaphrodites (almost no self-sperm), followed by DAPI staining of the females, numerous sperm nuclei were detected in her spermatheca. Therefore, mutant male spermatids seemed to normally undergo spermiogenesis *in vivo*. Indeed, spermatids from *spe-45* mutant males were activated into normal-looking spermatozoa by Pronase treatment *in vitro*.

Our data on live imaging analysis, however, showed that *spe-45(tm3715)* male spermatozoa may fail to fuse with the oocyte plasma membrane. These combined findings suggest that *spe-45(tm3715)* worms produce otherwise normal spermatozoa that are defective presumably in gamete fusion, like male mice deficient for *Izumo1*. Intriguingly, *spe-45* mutant hermaphrodites were partially rescued by expressing a transgene coding for mutant SPE-45 protein where the Ig-like domain is swapped to that of mouse IZUMO1. Therefore, functional role(s) of the Ig-like domains in sperm-oocyte fusion might be conserved between *C. elegans* and mouse.

Contact: 14m908tt@edu.setsunan.ac.jp

A forward genetic screen for new gamete function mutants

Amber R Krauchunas, Gunasekaran Singaravelu, Andrew Singson

Rutgers University, USA

A detailed understanding of fertilization and egg activation will require knowing the full complement of molecules that act in these processes. While the basic steps of fertilization are well described, the specific molecules that are required for sperm-egg interactions and fusion are not well understood or still unidentified. Likewise, the initial trigger of egg activation has been worked out in a number of species but the molecules and signaling pathways that regulate the events of egg activation remain largely unknown. Our lab has developed a forward genetic screen for new temperature-sensitive gamete function mutants based on a strategy previously used by others to identify embryonic and maternal effect lethal mutations[1-2]. The screen utilizes a strain of worms that is homozygous for the *sem-2(n1343)* mutation and carries an integrated *elt-7:gfp* reporter. Worms that carry the *sem-2(n1343)* mutation are unable to lay eggs, though fertilization still occurs normally, leading to a “bag of worms” phenotype[2]. This phenotype can be completely suppressed by sterility or maternal effect embryonic lethality. The *elt-7:gfp* reporter, which is expressed in embryos and the adult gut, allows us to quickly determine if surviving animals are producing zygotes, distinguishing fertilization/egg activation mutants from mutations that effect later developmental processes. The screen is carried out at 25^{o}C; once sterile candidates are identified they are shifted to 16^{o}C to test if fertility can be restored. Our lab has previously used this screening strategy to identify 14 conditional gamete function mutants. In one of two pilot screens wild-type males were added to sterile candidates, which recovered an additional 15 new non-conditional sperm function mutants. Our goal now is to focus the screen on identifying new egg function (*egg*) mutants. To our knowledge no previous genetic screens have been conducted to specifically identify egg mutants in *C. elegans*. We discuss the results of this screen and newly identified gamete function mutants.

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Contact: ark@waksman.rutgers.edu

The mechanism for enrichment of a chromodomain protein MRG-1 into the primordial germ cells in *C. elegans*

Takashi Miwa, Kunio Inoue, Hiroshi Sakamoto, Teruaki Takasaki

Kobe University, Japan

Germ cells are the only cells that are capable of giving rise to the next generation. In the nematode *C. elegans*, germ granules, which have been thought to contain the germline determinants, are asymmetrically segregated to the germline blastomeres from the first cleavage. On the other hand, a chromodomain protein MRG-1, which is essential for germline development, is present uniformly in all nuclei in early embryos and then gradually enriched in the primordial germ cells (PGCs) in late embryos. To understand the mechanism underling the unique expression pattern of MRG-1, we first determined whether MRG-1 in the PGCs is maternal or zygotic product. GFP-fusion reporter analysis revealed that *mrg-1* promoter is not active in PGCs during embryogenesis, suggesting that MRG-1 in PGCs is mostly maternal and accumulated through post-transcriptional regulation. Indeed, a maternally supplied GFP reporter carrying the 3'-UTR of *mrg-1* mRNA recapitulated the enrichment of GFP into PGCs. We next searched for the factor(s) that regulates the MRG-1 expression via 3'-UTR. Germ granules are not involved in the MRG-1 expression because inhibition of PPTR-1, which is required for the proper asymmetric segregation of germ granules, did not disturb the MRG-1 enrichment into PGCs. Intriguingly, when RNA polymerase II was depleted by RNAi to inhibit entire zygotic gene expression, the MRG-1 enrichment in PGCs was disappeared, suggesting that zygotic factor(s) regulates the expression of maternal *mrg-1* mRNA via its 3'-UTR.

Contact: takasaki@port.kobe-u.ac.jp

Evolution of sex and reproductive strategies: hermaphroditism and sex determination in the fungal feeding nematode *Bursaphelenchus okinawaensis*

Ryoji Shinya[1,2], Koichi Hasegawa[2], Anthony Chen[1], Natsumi Kanzaki[3], Paul W. Sternberg[1]

[1]HHMI and California Institute of Technology, USA
[2]Chubu University, Japan
[3]Forestry and Forest Products Research Institute, Japan

Nematodes have many different reproductive strategies along with their divergent life-histories; the ability of hermaphrodite to self- and cross-fertilize is useful for genetic manipulation. Here we demonstrate the hermaphroditism of the fungal feeding nematode *Bursaphelenchus okinawaensis*, which was formerly described as a parthenogenetic nematode, and we show its other unique sexual characteristics. To determine the precise reproductive mode (*i.e.* parthenogenesis or hermaphroditism) in *B. okinawaensis*, we performed the following experiments: 1) observation of the pronuclear and chromosome behavior during oogenesis and early embryogenesis; 2) observation of the spermatogenesis during the L4 stage; 3) investigation of sperm utilization; 4) investigation of the phenotypic segregation after cross-mating using an EMS-induced visible mutant. The results of these experiments clearly showed the hermaphroditism of *B. okinawaensis*. We then investigated the mating preference and spermatid size between males and hermaphrodites. *B. okinawaensis* males successfully mated only with sperm-depleted old hermaphrodites, and the spermatid sizes of males were almost the same as those of hermaphrodites. In addition, the sex ratio of cross-fertilized progeny in *B. okinawaensis* was highly skewed toward hermaphrodites. To determine the sex distortion and determination mechanism, we then evaluated the activity of sperm from males and investigated the impact of various environmental factors, autosomal sex determination genes, and endosymbiotic microbes. The exact mechanism of the sex distortion and determination in *B. okinawaensis* is still unclear but seems to involve other unknown factors. Our study demonstrates that this close relative of *B. xylophilus*, the pathogen causing pine wilt disease, is amenable to genetic analysis. Furthermore, there are many differences in the sex allocation and sex determination mechanisms between *B. okinawaensis* and *Caenorhabditis elegans*. *B. okinawaensis* will be useful for future studies in the evolution of sex and reproductive strategies.

Contact: shinya@caltech.edu

Characterizing loss-of-function suppressors of conditional centrosome-defective mutants

Chien-Hui Chuang, Sean O'Rourke, Bruce Bowerman

University of Oregon, USA

Centrosomes are microtubule organizing centers that mediate mitotic spindle assembly and function and can influence cell polarity. To investigate centrosome function, we performed RNAi modifier screens to identify genes that when reduced in function can restore viability to one or more of 3 temperature-sensitive (TS) centrosome-defective mutants: *spd-2*(or183ts), *spd-5*(or213ts) and *zyg-1*(or278ts). We identified 9 non-essential genes and one essential gene that specifically when knocked down by feeding RNAi suppress the embryonic lethality associated with one or more of these three mutants when grown at semi-permissive temperatures. Surprisingly, the strength of the suppression can change over time, and this temporal effect varies in different TS mutants. This variability has complicated analyzing the significance of the interactions. Nevertheless, we have focused further effort on two genes, *hpo-11* and *inx-14*, that can suppress lethality in all three centrosome-defective TS mutants. When mutant embryos were upshifted to the semi-permissive temperature used for the screen, we observed abnormally short P0 spindles and and failures in P0 cell division. In embryos that did undergo P0 cell division, progression through the cell cycle was abnormal compared to wild-type. When we knocked down *hpo-11* or *inx-14* in the *spd-2*, *spd-5* or *zyg-1* mutant embryos, P0 spindle length was more normal. We also used feeding RNAi to knock down *hpo-11* or *inx-14* in wild type embryos and observed a loss of P0 anaphase spindle oscillations and some delays in cell cycle progression. We will present these data and further analysis of these suppressor loci.

Contact: cchuang@uoregon.edu

Analyzing the effect of neopeltolide in cell division using *C. elegans* embryos

Akira Shibukawa, Eisuke Sumiyoshi, Haruhiko Fuwa, Makoto Sasaki, Asako Sugimoto

Grad. Sch. of Life Sci., Tohoku Univ, Japan

Natural compounds have been an attractive source for pharmaceutical agents. There are still many natural compounds that are not yet fully utilized because their mode of action is unknown. *C. elegans* has been highly successful as a model system in cell and developmental biology because of its transparent cells/body and stereotypic development, thus it can offer a unique platform to dissect the mode of action of less-characterized natural compounds and to search for their target molecules.

In this study, we used *C. elegans* early embryos to characterize the *in vivo* effect of neopeltolide, a natural compound with a potent antiproliferative activity isolated from a deep-water sponge of the family *Neopeltidae*. Recently total chemical synthesis of neopeotolide has been accomplished, and genetic and biochemical analysis implicated that its molecular target may be the cytochrome bc_1 complex in the electron transport chain in mitochondria. However, it has been uncertain whether the inhibition of the cytochrome bc_1 fully accounts for the potent antiproliferative activity of neopeltolide. We analyzed the cell cycle-dependent phenotypes caused by neopeltolide treatment in early *C. elegans* embryos by live imaging of the GFP/mCherry-labeled markers for chromosomes, centrosomes and microtubules. We found that embryos treated with neopeltolide showed a significant delay in cell cycle progression and arrested at various cell cycle phases. The neopeltolide-treated embryos arrested at metaphase formed abnormal mitotic spindles, in which microtubule arrays emanating from centrosomes to chromosomes continued to elongate without attaching kinetochores. To examine whether the inhibition of the cytochrome bc_1 complex accounts for these phenotypes, we are currently analyzing the RNAi phenotypes for the genes encoding components of the cytochrome bc_1 complex, which will lead to the understanding of the mode of action of neopeltolide *in vivo*.

Contact: s-akira@life.biology.tohoku.ac.jp

A novel centrosomal protein GTAP-3 is involved in centriole duplication and the recruitment of γ-tubulin to centrosomes

Shinsuke Uchiya[1], Eisuke Sumiyoshi[1], Nami Haruta[1], Masahiro Terasawa[2], Nobuhito Oyama[1], Yukihiko Kubota[1], Asako Sugimoto[1]

[1]Graduate School of Life Sciences, Tohoku University, Sendai, Japan
[2]RIKEN Center for Developmental Biology, Kobe, Japan

The centrosome is the major microtubule-organizing center (MTOC) in animal cells, playing a key role to form and maintain mitotic spindles. The γ-tubulin complex, a major microtubule nucleator, is recruited to centrosomes and increases its microtubule nucleation activity during mitosis. However, the factor(s) that regulate localization and activity of the γ-tubulin complex are not known in *C. elegans*. To elucidate the regulation of the γ-tubulin complex, we analyzed proteins that co-immunoprecipitated with γ-tubulin from *C. elegans* egg extract using mass spectrometry, and identified a novel centrosomal protein, GTAP-3 (Gamma-Tubulin Associated Protein 3). In *C. elegans* embryos, GFP-GTAP-3 was localized at the centrioles throughout the cell cycle. Depletion of GTAP-3 by RNAi resulted in embryonic lethality at a high penetrance. In *gtap-3(RNAi)* embryos, some cell showed defects in the centriole assembly or separation. The defects of centriole duplication resulted in monopolar spindles, which later produced multinucleate cells. In addition, γ-tubulin and microtubules at the centrosomes were significantly reduced during interphase. Previously, an interactome analysis (Boxem M *et al.,* 2008) suggested that GTAP-3 binds to DLC-1 (Dynein Light Chain 1), a component of the dynein complex. We determined the specific site of GTAP-3 that interacted with DLC-1 by yeast two-hybrid assay. Depletion of DLC-1 by RNAi increased the amount of GTAP-3 at centrioles at metaphase in 1-cell embryos. These data suggest that GTAP-3 contributes to centriole duplication and γ-tubulin recruitment to centrosomes in interphase, and that DLC-1 negatively regulates the localization of GTAP-3 to centrioles.

Contact: sin5099@gmail.com

The *C. elegans* MOZART1 ortholog is essential for the recruitment of the γ-tubulin complex to centrosomes

Nami Haruta, Asako Sugimoto

Graduate School of Life Sciences, Tohoku University, Japan

The gamma-tubulin complex (γTuC) is a major microtubule nucleator at MTOC, such as centrosome and SPB in yeast. Many organisms have two types of γTuCs, known as the γ-tubulin small complex (γTuSC) and the γ-tubulin ring complex (γTuRC). The γTuSC is composed of γ-tubulin, GCP2 and GCP3. The γTuRC consists of six γTuSCs with several γTuRC-specific components, which forms a ring-like structure with a higher nucleating activity than the γTuSC. MOZART1 (Mitotic-spindle organizing protein associated with a ring of γ-tubulin 1) is a conserved small protein recently identified as a γTuC-binding protein, and it was shown to bind to the N-terminal region of GCP3. We previously found that the composition of the *C. elegans* γTuCs is unusual and identified two novel components, GTAP-1 and -2, but it has not been known whether the MOZART1 ortholog is present in the complexes. Here, we identified and analyzed the function of the *C. elegans* ortholog of MOZART1 (Ce-MOZART1). Yeast two-hybrid analysis showed that Ce-MOZART1 strongly interacted with the N-terminal of GIP-1 (GCP3 ortholog), but not with GIP-2 (GCP2 ortholog). Live imaging of the GFP-tagged Ce-MOZART1 in *C. elegans* embryos revealed that Ce-MOZART1 colocalized with γ-tubulin (TBG-1) at centrosomes throughout cell cycle, and this centrosomal localization was dependent on γ-tubulin and GIP-1. RNAi of *Ce-MOZART1* resulted in embryonic lethality with cytokinesis defects. In these embryos, the centrosomal localization of γ-tubulin and GIP-1 are significantly reduced, but that of SPD-5 was unaffected. These data suggest that Ce-MOZART1 is a component of the γTuC and essential for its recruitment to centrosomes.

Contact: nharuta@m.tohoku.ac.jp

ZYG-9 contributes to multiple aspects of cell cycle-dependent microtubule behaviors

Satoshi Namai, Eisuke Sumiyoshi, Asako Sugimoto

Graduate School of Life Sciences, Tohoku University, Japan

Microtubules (MTs) are dynamic cellular components and change their behaviors temporally and spatially. Upon mitotic entry, MTs in the cytoplasm are rapidly depolymerized, and mitotic spindles are organized mainly from MTs assembled at centrosomes. It is also known that condensed mitotic chromosomes can stimulate MT assembly independently of centrosomes. In *C. elegans*, mitotic chromosome-stimulated MTs and meiotic spindle MTs are organized independently of the γ-tubulin complex, a conserved major MT nucleator. Although spatial and temporal control of MT regulators is regarded to be responsible for these cell cycle-dependent MT behaviors, its details are still not well understood.

XMAP215 is an evolutionarily conserved processive MT polymerase. It has also been shown to have a MT depolymerization activity, and γ-tubulin-dependent and -independent MT nucleation activities *in vitro*. The *C. elegans* XMAP215 orthologue ZYG-9 was shown to have MT polymerization activity at centrosomes. To investigate whether ZYG-9 has other regulatory roles of cell cycle-dependent MT behaviors, we analyzed the contribution of ZYG-9 to various types of MT behaviors throughout cell cycle.

We first investigated the contribution of ZYG-9 to non-centrosomal, γ-tubulin-independent MTs. In *zyg-9(RNAi)* zygotes, female meiotic spindles were initially formed, but MTs were decreased at meiotic anaphase when spindle MTs are reorganized. By inhibiting pronuclear migration, the female pronucleus stays separated from sperm-derived centrosomes attached to the male pronucleus, thus non-centrosomal, γ-tubulin-independent MTs assembled around oocyte-derived chromosomes can be readily observed. Knock-down of *zyg-9* under this condition decreased the MTs around oocyte-derived chromosomes. Thus, ZYG-9 contributes to polymerization or maintenance of non-centrosomal, γ-tubulin-independent MTs during mitosis and meiotic anaphase. Additionally we noticed that depletion of ZYG-9 significantly increased the cytoplasmic MTs during mitosis and meiosis, suggesting that ZYG-9 might be actively involved in depolymerization of cytoplasmic MTs upon mitotic entry and during meiosis. Collectively, ZYG-9 contributes to multiple aspects of cell cycle-dependent MT behaviors not only at centrosomes but also around chromosomes and cytoplasm.

Contact: s-namai@life.biology.tohoku.ac.jp

Role of CDC-48 and UBXN-2 in spindle orientation in *C. elegans*

Ainoa Figuerola-Conchas[1,2], Karolina Niewola[1,2], Elsa Kress[1], Francoise Schwager[1], Veit Goder[3], Robbie Loewith[1,2], Gerardo Turcatti[2,4], Monica Gotta[1,2]

[1]Université de Genève, Switzerland
[2]NCCR Chemical-Biology, Switzerland
[3]University of Seville, Spain
[4]École polytechnique fédérale de Lausanne, Switzerland

Asymmetric cell division is important for the generation of cell diversity. Since the mitotic spindle determines the plane of cell cleavage, its orientation will determine the fate of the daughter cells. Recently, our group has found that UBXN-2, a CDC-48 adaptor, regulates spindle orientation in the *C. elegans* embryo. CDC-48 was first identified in a screen for mutants affecting cell division (cdc, cell division cycle) in yeast. It is a conserved protein that regulates several cellular processes including Endoplasmic Reticulum Associated Degradation (ERAD), spindle-chromosome attachment, spindle disassembly and organelle membrane fusion at the end of mitosis. Its association with several adaptors confers the specificity to CDC-48. To study the implication of CDC-48 in cell division in the embryo, we propose a screen for chemical compounds that will inhibit CDC-48 function. To identify inhibitors, we will make use of yeast and the well characterized function of CDC-48 in ERAD. The rationale of the screen consists in using an ERAD degradable substrate fused to a green luciferase which allows identifying ERAD blockage by high through-put readout of bioluminescence. In parallel, we have carried out a yeast two-hybrid screen to find UBXN-2 interactors and identified a transmembrane coiled-coil protein called TCC-1. Interestingly, TCC-1 is involved in spindle movements in the one-cell *C. elegans* embryo (Berends et al, 2013). Our aim is to elucidate how TCC-1 and UBXN-2 function together in spindle positioning.

Contact: ainoa.figuerola@unige.ch

Syndecan/SDN-1 regulates Wnt-dependent spindle orientation in *C. elegans*

Katsufumi Dejima[1,2], Sukryool Kang[3], Shohei Mitani[2], Pamela C Cosman[3], Andrew D Chisholm[1]

[1]Division of Biological Sciences, Section of Cell and Developmental Biology, University of California San Diego, USA
[2]Department of Physiology, Tokyo Women's Medical University, School of Medicine, Japan
[3]Department of Electrical and Computer Engineering, University of California San Diego, USA

Orientation of the mitotic spindle defines the axis of cell division. Wnt signals orient mitotic spindles in development, but little is known about how such cues are spatially restricted during mitosis. We show that the *C. elegans* heparan sulfate proteoglycan (HSPG), syndecan/SDN-1 is required for precise orientation of the mitotic spindle in response to an external Wnt signal. We find that SDN-1 is the predominant HSPG expressed in the early embryo, and that loss of HS biosynthesis or of the SDN-1 core protein results in misorientation of the spindle of the ABar blastomere. Quantitative analysis indicates that SDN-1 is required for spatial regulation of the external cue during mitosis. Spindle orientation of the ABar and EMS blastomeres involves parallel Wnt and Src dependent pathways; we find that HS/SDN-1 acts in Wnt signaling specifically in ABar, but not in EMS. The ABar spindle orients in response to Wnt cues from the C blastomere; we find that SDN-1 transiently accumulates on the ABar surface as it contacts C. SDN-1 accumulation in ABar is followed by local concentration of MIG-5/Dishevelled at the ABar cortex. Subsequently, SDN-1 is bi-directionally internalized into ABar or C, possibly by endocytosis. Our data suggest SDN-1 transiently forms a special junction that defines the MIG-5/Dishevelled accumulation site, and reveal a new role for syndecan in Wnt dependent-spindle orientation in the context of a nascent cell contact site.

Contact: kdejima@research.twmu.ac.jp

ppk-1/PIP5K regulates *cwn-2*/Wnt function to orient asymmetric cell divisions in *C. elegans*

Noaki Yoshida, Hitoshi Sawa

SOKENDAI, Japan
NIG, Japan

During development, the asymmetric cell division is an important mechanism to generate cell diversity. In *C. elegans*, each epithelial stem cell (V cell) is polarized and divide asymmetrically, giving rise to an anterior daughter that terminally differentiates and a posterior one that retains the V cell fate. Polarity of these cells is redundantly regulated by three Wnt genes (*cwn-1, cwn-2, egl-20*). Since ectopically expressed Wnts rescued polarity defects of Wnt mutants, these Wnts do not appear to function as positional cues, even though they are necessary to properly orient polarity. Therefore the mechanism for these Wnts to orient the polarity remains a mystery.

From RNAi screening, we identified the *ppk-1* gene, encoding a phosphoinositide-4, 5-bisphosphates synthesis kinase (PIP5K), as a polarity modulator of the V2.pa cell which is one of the V cells. A *ppk-1* null mutant showed reversed but not loss-of polarity of this asymmetric division, suggesting that *ppk-1* functions to promote the correct orientation of the cell polarity, but is dispensable for polarity generation. Since the *ppk-1* phenotype was suppressed and enhanced by loss-of-funtion and the overexpression of *cwn-2*/Wnt, respectively. The reversed polarity in *ppk-1* mutant is caused by the *cwn-2* function that promote reversed orientation of the polarity. In contrast, in wild type, *cwn-2* promotes the normal orientation of the polarity. These results suggest that *cwn-2* has two functions promoting both normal and reversed polarity orientation.

How does *cwn-2* have two opposite functions? We hypothesized that distinct receptors of CWN-2 mediate each *cwn-2* function. To evaluate this hypothesis, we are analyzing the genetic interactions between *ppk-1* and Wnt receptors. We found that a *lin-18* mutation suppresses the *ppk-1* phenotype. This result suggests that *lin-18* promotes the reversed polarity in *ppk-1* mutant. We will report our progress on the relationship between *ppk-1, lin-18* and *cwn-2* in asymmetric cell divisions of V cells.

Contact: nyoshida@nig.ac.jp

Measurement-based mathematical modeling of PAR-2 protein localization in *C. elegans* embryo

Yukinobu Arata[1], Michio Hiroshima[2], Chan-Gi Pack[1], Kenichi Nakazato[3], Tetsuya J. Kobayashi[4], Tatsuo Shibata[5], Yasushi Sako[1]

[1]Cellular Informatics Laboratory, RIKEN, Japan
[2]Laboratory for Cell Signaling Dynamics, Quantitative Biology Center, (QBiC), RIKEN, Japan
[3]Theoretical Biology Laboratory, RIKEN, Japan
[4]Institute of Industrial Science, the University of Tokyo, Japan
[5]Laboratory for Physical Biology. RIKEN Center for Developmental Biology, Japan

The PAR/aPKC system maintains cell polarity through the mutual inhibition mechanism. However, quantitative aspects how polarity protein dynamics are balanced for stable polarization remain largely unexplored. In living *C. elegans* embryos, we comprehensively measured intracellular dynamics of a RING protein PAR-2 using single-molecule imaging and fluorescent correlation spectrometry. On the cortex, PAR-2 formed oligomers. The size distribution of PAR-2 particles varied along the a-p axis of embryos. By analyzing trajectories of PAR-2 particles, we found that PAR-2 dissociation from the cortex was promoted by PKC-3-dependent phosphorylation, while that was suppressed by PAR-2's oligomerization. Therefore, asymmetric control of PAR-2's dissociation rate along the a-p axis was achieved by PKC-3 dependent phosphorylation and PAR-2's oligomerization. In addition, we found that the association rate from the cytoplasm was also asymmetric along the a-p axis, which was at least regulated by PKC-3-dependent phosphorylation. By measuring diffusion coefficient on the cortex, we concluded that there were novel PAR-2 components, in which the diffusion distance was much shorter than embryos size. Using a reaction-diffusion model, we reproduced asymmetric localization of PAR-2 by a bi-stable mechanism *in silico*, under the condition that all the parameters of PAR-2 were determined by kinetic values obtained from measurements *in vivo*. Interestingly, our model suggested that cortical asymmetry of PAR-2 was stably maintained against the density fluctuation through a mechanism due to the short diffusion distance. The short diffusion distance facilitate to adapt cortical asymmetry to smaller-sized cells generated by successive cleavages in early *C. elegans* development. These results raised a possibility that a unique balance of polarity protein dynamics provides cellular pattern with stability and adaptability *in vivo*.

Contact: arata@riken.jp

Mirror asymmetric contraction of actomyosin drives left-right symmetry breaking in *C. elegans*

Yuko Kamkikawa, Michael Tiongson, Pavak Shah, Zhirong Bao

Sloan-Kettering Institute, USA

The breaking of Left/Right (L/R) symmetry during embryogenesis is indispensable for proper development in bilateral organisms. In vertebrates, such as the mouse and zebrafish, directional beating of cilia breaks the L/R symmetry. However, in many organisms cilia are not present at the developmental stage when L/R symmetry is broken, suggesting that other mechanisms must exist.

We show that mirror asymmetric actin cortex dynamics during cytokinesis drive L/R symmetry breaking in *C. elegans*. Actin forms tilted filaments relative to the ingressing contractile ring, revealing mirror asymmetry of the cortex. Mutations in *act-2*, an actin isoform, as well as RNAi against *pfn-1*/Profilin, disrupt the actin filaments and randomize the L/R outcome. Laser ablation experiments further suggest that myosin contraction along the mirror asymmetric actin filaments drives the directional twist of ABa/p cells that breaks L/R symmetry. These results suggest a novel form of actomyosin-based cellular asymmetry in directional tissue morphogenesis.

To further elucidate the underlying mechanisms of the mirror asymmetry, we have conducted genetic and RNAi screens for *situs inversus totalis*. Current results suggest that mirror asymmetry of actomyosin requires cell contact-based inside-outside polarity, linking the two forms of cellular asymmetry in ABa/p cells. We propose that this link couples the L/R and dorsoventral axes.

Contact: kamikawy@mskcc.org

PLST-1 is essential for cortical contractility during early *C. elegans* embryogenesis.

Wei Yung Ding, Ronen Zaidel-Bar

Mechanobiology Institute, Singapore

Cortical contractility, driven by an actomyosin network underlying the plasma membrane, is responsible for the control of cell shape and the generation of contractile forces that drive polarization and cell division. Contractility depends on myosin activity as well as the organization of filamentous actin, which is determined by the concerted action of a multitude of actin binding proteins. Plastin/fimbrin is an evolutionary-conserved actin-bundling protein with two tandem repeats of calponin homology (CH) domains. To examine the role of *C. elegans* plastin, PLST-1, in early embryogenesis, we characterized the *plst-1(tm4255)* allele, a deletion that abrogates the third and fourth CH domains.

We found in early *plst-1(tm4255)* embryos a severe attenuation of cortical ruffling and complete absence of pseudocleavage. During polarization, the anterior cortical flow speed was more than two-fold slower in the mutant, resulting in only partial establishment of polarity. Subsequent maintenance of polarity was also defective, as manifested by a distinctively slanted anterior cap. We also found that PLST-1 is required for two highly asymmetric cell division events: the second polar body extrusion and the first cytokinesis. 85% of mutant embryos exhibited a delay in cytokinesis initiation, while 15% failed to initiate or failed to complete cytokinesis. Interestingly, following the delay in initiation, the subsequent furrowing speed was similar to that of wild-type. A careful evaluation of cortical NMY-2 at anaphase onset revealed that PLST-1 is essential for the proper cortical arrangement of NMY-2 into a furrowing band. On the other hand, partially compromising NMY-2 function in a *plst-1(tm4255);nmy-2(ne3409)* double mutant (at 20°C) lead to complete failure in initiation of furrowing, implying that PLST-1 and NMY-2 function in parallel to modulate the cortical cytoskeleton to initiate cytokinesis. Current work, employing high-resolution imaging along with genetic and optical perturbations, is aimed at elucidating the molecular mechanism by which PLST-1 modulates the actomyosin network.

PLST-1 is unique among eukaryotes as it contains only three repeats of CH domain, challenging the notion that tandem CH domains are required to form a fully functional F-actin binding domain. We will employ biochemical assays as well as electron microscopy in order to characterize the interaction between PLST-1 and actin and elucidate its capability to bundle F-actin.

Contact: dwy@nus.edu.sg

BAR-1/β-catenin is involved in asymmetric cell divisions of seam cell

Yinhua Jin, Masako Yokoo, Hitoshi Sawa

National Institute of Genetics, Japan

Wnt signaling pathways are important for various biological phenomenon including cell fate decision and morphogenesis. In canonical Wnt signaling pathway, the stability of β-catenin, a key player in the pathway, is regulated through phosphorylation by the GSK3β/Axin/APC complex. It was recently reported that β-catenin can be asymmetrically localized in ES cells to regulate distinct fates of the daughter cells.

In *C. elegans*, asymmetric cell division (ACD) is controlled by the Wnt/β-catenin asymmetry pathway that involves asymmetric localization of WRM-1/β-catenin and APC. WRM-1 is divergent β-catenin that is not phosphorylated by GSK3β. *C. elegans* has another β-catenin, BAR-1 that is regulated by GSK3 and PRY-1/Axin. However, the involvement of BAR-1 in ACD has not been reported. We found that polarity of asymmetric division of specific seam cells (Vn.pa) is frequently reversed in *pry-1* mutants at L2 stage. This phenotype is suppressed by a *bar-1* mutation. Furthermore, ACD is disrupted by the expression of dominant active *bar-1*. These results support that the canonical Wnt/β-catenin pathway operates in ACD in *C. elegans*.

Similar to *pry-1* mutants, we found that *vang-1* mutants show polarity reversal specifically in the Vn.pa cells. Since VANG-1 is component of the planar cell polarity (PCP) pathway, our results suggest cooperation of canonical and PCP pathways in the regulation of ACD. We will further study the genetic interactions between *vang-1* and *bar-1*.

Contact: misun@nig.ac.jp

Role of egg shell geometry in *C. elegans* embryonic development

Pei Yi Tan[1], Ronen Zaidel-Bar[1,2]

[1]Mechanobiology Institute, National University of Singapore, Singapore
[2]Department of Biomedical Engineering, National University of Singapore, Singapore

In egg-laying animals, the formation of an egg shell not only serves as a protective envelope, but also defines a constrained volume in which the embryo will develop. In *C. elegans*, egg shell formation takes place in the spermatheca immediately after fertilization. Deposition of chitin, which provides structural integrity to the egg shell, contours the surface of the one-celled embryo, thus outlining a geometric space in which the embryo will undergo further development.

The stereotypical shape of the *C. elegans* egg shell is a prolate ellipsoid of revolution with semi principal axes of 30 µm and 50 µm. However, knockdown of SPV-1, a negative regulator of spermatheca contractility, gives rise to round or elongated embryos by physically squeezing the embryos prior to their passage into the uterus. Interestingly, although SPV-1 is solely expressed in the spermatheca, loss of SPV-1 is associated with 50% embryonic lethality.

We found that 56% of round embryos with an aspect ratio (embryo length/breadth) below 1.4 arrested prior to or during epidermal morphogenesis, while 83% of elongated embryos with aspect ratio above 2.0 are unaffected. Using HMR-1::GFP as an epidermal marker, we observed misarrangement of intercalating dorsal cells and the failure of ventral cells to fully enclose in round embryos. Furthermore, KAL-1::GFP, which marks ventral neuroblasts, revealed mislocalization of neuroblast cells. Strikingly, embryos encased in elongated egg shells were able to compact along the anteroposterior axis and revert to the more favorable aspect ratio.

We hypothesize that egg shell geometry provides global spatial information determining the cellular division planes. Thus, we are testing the possibility that embryos with an aspect ratio below 1.4 fail to accurately position their cell division plane, affecting relative cell positioning and cell-cell contacts of daughter cells, which in turn perturb cell signaling and cell fate determination.

Our results highlight the importance egg shell geometry in *C. elegans* development. However, we cannot rule out the possibility that embryos with low aspect ratio, which resulted from being severed by the spermatheca, may lack some cytoplasmic contents required for embryogenesis. Therefore, we will proceed with physical manipulation of shape in wild-type embryos *in-vitro* to corroborate the finding that normal development requires an ellipsoid geometry with aspect ratio of 1.4.

Contact: a0038688@nus.edu.sg

C. elegans chaperonin CCT/TRiC is required for actin biogenesis and microvillus formation in intestinal epithelial cells

Keiko Saegusa[1], Miyuki Sato[1], Katsuya Sato[1], Junko Shimada[2], Akihiro Harada[3], Ken Sato[1]

[1]Laboratory of Molecular Traffic, Institute for Molecular and Cellular Regulation, Gunma University, Japan
[2]Department of Laboratory Sciences, Gunma University School of Health Sciences, Japan
[3]Department of Cell Biology, Graduate School of Medicine, Osaka University, Japan

Intestinal epithelial cells have unique apical membrane structures, known as microvilli, which contain bundles of actin microfilaments. However, the mechanism of microvillus formation is largely unknown. Here, we report that the *Caenorhabditis elegans* cytosolic chaperonin containing TCP-1 (CCT) is essential for proper formation of microvilli in intestinal cells. In intestinal cells of CCT-deficient animals, a significant amount of actin is lost from the apical area, forming large aggregates in the cytoplasm, and the apical membrane is deformed into bubble-like structures. The length and number of intestinal microvilli are decreased in those animals. However, overall actin protein levels remain relatively unchanged when CCT is depleted. We also found that CCT depletion causes a reduction in tubulin levels, and disorganization of the microtubule network. In contrast, the stability and localization of the intermediate filament, IFB-2, which forms a dense filamentous network underneath the apical surface, appears to be superficially normal in CCT-deficient cells, suggesting substrate specificity of CCT in the folding of filamentous cytoskeletons *in vivo*. Our findings indicate that CCT is required for the biogenesis of actin and tubulin, and is thereby essential for microvillus formation in intestinal cells.

Contact: m13702012@gunma-u.ac.jp

PKG-1 affects OSM-3 clustering at the distal tip of cilia and negatively affects IFT particle transport in the distal segment

Prerana Mahendra, Hsinyi Huang, Muniesh M Shanmugam, Ron Hsieh, Helly Punjabi

National Tsing Hua University, Taiwan

Cilia are specialized subcellular organelles which exist in various cell types in most eukaryotic organisms. Non-motile cilia, or primary cilia, are mainly responsible for sensory function and are recognized to inherit essential roles in physiology and development. Ciliary diseases such as polycystic kidney disease (PKD) are one of the most common and life threatening genetic diseases world-wide. However, little is known about the underlying molecular mechanism and pathways that control cilia development and intraflagellar transport (IFT). Based on previous studies revealing critical roles of ciliary kinases such as DYF-5 and DYF-18 in IFT, we expected more kinases and phosphatases to regulate ciliogenesis and IFT. We therefore used data mining tools and data from the literature to identify putative candidates. For our candidate screen, we adopted a broad range of methods, including dye-filling, chemotaxis assays, IFT component expression pattern, and motility analysis of IFT particles in cilia, to investigate the effect of mutations in genes encoding for ciliary kinases and phosphatases. Here, we report the effect of six kinases and phosphatases on dye-filling and chemotaxis assay, and identified three potential kinases (PKG-1, GCK-2 and DYF-18) that significantly affect ciliogenesis and IFT particle transport. In detail, PKG-1 and DYF-18 both affect the distribution of molecular motors kinesin-II and OSM-3. The strongest effect was observed in PKG-1 mutants with obvious clustering of OSM-3 motors at the distal tip of cilia. Also abnormal cilia morphology was observed in PKG-1 mutants while in GCK-2 mutants cilia morphology was similar to wild type animals. On the other hand, GCK-2 affected transport characteristics of IFT particle A (CHE-11) in both the middle and distal cilia segment (similar as observed in DYF-18 mutants), however, PKG-1 affects the transport only in the distal segment. PKG-1 rescue experiments lead to partial recovery of the observed phenotypes concluding that PKG-1 plays a pivotal role in ciliogenesis and intraflagellar transport.

Contact: prerana.pcbiotech@gmail.com

A systematic *in vitro* and *in vivo* screen in axonal motors reveals synaptic vesicle transport and neuron generation direct and indirect regulating by Dynein/Dynactin

Chih-Wei Wayne Chen[1], Yu-Fei Peng[1], Ying-Cheng Yen[1], Victor D. Lee[1], Geng Ming Hu[2], Shang-Yu Wu[3]

[1]National Tsing Hua University, Taiwan
[2]National Taiwan Normal University, Taiwan
[3]National Chiao Tung University, Taiwan

Various neurodegenerative diseases display accumulation of proteins (cargo) in the nervous system and one hypothesis is that cargo accumulation (for example APP or tau) is based on defective axonal transport powered by molecular motors. Kinesins and their opposing dynein/dynactin motor complex are the major motor proteins for transport along axonal microtubules in neurons. Frequently observed directional changes of motors and synaptic vesicles along axons in living worms might be explained by regulated cooperative interactions between kinesin and dynein or by passive tug-of-war model. The bidirectional movement is from unbalanced opposing forces produced by kinesin and dynein/dynactin motors.

The functional interactions between more than a dozen subunits of kinesin-3 and dynein/dynactin motors were tested employing yeast two-hybrid and BiFC (bimolecular fluorescence complementation) assays as well as RNAi knockdown in living worms. We identified crucial regulatory interaction sites between specific kinesin-3 and dynein /dynactin domains. A mathematical model by fitting our recorded motility data (using kymograph analysis) supported the development of a thorough model how these domains interact. Besides understanding how these interactions affect axonal transport in the living worm, we also use neuron cell cultures to investigate the effect on axonal growth and degeneration.

Contact: d9580810@oz.nthu.edu.tw

Fertilization-induced K63-linked ubiquitination mediates clearance of maternal membrane proteins

Miyuki Sato, Ryosuke Konuma, Katsuya Sato, Kotone Tomura, Ken Sato

Laboratory of Molecular Traffic, IMCR, Gunma University, Japan

In *Caenorhabditis elegans*, fertilization triggers endocytosis and rapid turnover of maternal surface membrane proteins in lysosomes, although the precise mechanism of this inducible endocytosis is unknown. We found that high levels of K63-linked ubiquitin chains transiently accumulated on endosomes upon fertilization. Endocytosis and the endosomal accumulation of ubiquitin were both regulated downstream of the anaphase-promoting complex, which drives the oocyte's meiotic cell cycle after fertilization. The clearance of maternal membrane proteins and the accumulation of K63-linked ubiquitin on endosomes depended on UBC-13 and UEV-1, which function as an E2 complex that specifically mediates chain elongation of K63-linked polyubiquitin. CAV-1-GFP, an endocytic cargo protein, was modified with K63-linked polyubiquitin in a UBC-13/UEV-1-dependent manner. In *ubc-13* or *uev-1* mutants, CAV-1-GFP and other membrane proteins were internalized from the plasma membrane normally after fertilization. However, they were not efficiently targeted to the multivesicular body (MVB) pathway but recycled to the cell surface. Our results suggest that UBC-13-dependent K63-linked ubiquitination is required for proper MVB sorting rather than for internalization. These results also demonstrate a developmentally controlled function of K63-linked ubiquitination.

Contact: m-sato@gunma-u.ac.jp

Nuclear membrane proteins act in transport of the Netrin receptor UNC-5 in cell migration in *C. elegans*

Hon-Song Kim, Kiyoji Nishiwaki

Department of Bioscience, Kwansei-Gakuin University, Japan

The KASH protein interacts with the SUN protein in the nuclear membrane and recruits kinesin-1 to regulate nuclear migration along microtubules. In *C. elegans*, UNC-83/KASH, UNC-84/SUN and kinesin-1(UNC-116 and KLC-2) act in nuclear migration of the distal tip cells (DTCs), the leader cells of gonadal development. We found that *unc-83(e1408)* and *unc-84(e1410)* mutants exhibited abnormal gonad formation due to the precocious dorsal turn of DTCs, in addition to the nuclear migration defect. The dorsal migration of DTCs is regulated by the repulsive action of the UNC-5 receptor in response to the guidance molecule UNC-6/Netrin. We previously reported that precocious distribution of UNC-5 in the plasma membrane could cause the precocious dorsal turn of DTCs. We analyzed the localization and behavior of the UNC-5::GFP(a functional fusion) vesicle in intracellular trafficking pathway. The UNC-5::GFP was first detected as vesicles at the perinuclear region and the cytoplasm, where they colocalized with Rab-1, at the beginning of dorsal turn of DTCs. The UNC-5::GFP vesicles were gradually colocalized with the Golgi and spread to the plasma membrane, suggesting that they could be transported to plasma membrane through the general secretory pathway. During dorsal turn of DTCs, the UNC-5::GFP vesicles showed strong colocalization with both Rab-1 and Rab-11.1-positive vesicles at perinuclear region, suggesting that the UNC-5 could be transported and tethered to perinuclear region through these Rab-containing vesicles. In the *unc-83(e1408)* mutant or in kinesin-1 depleted DTCs, the UNC-5::GFP vesicles were first detected near the plasma membrane and were never accumulated to the perinuclear region until the end of dorsal turn, indicating tethering defects of UNC-5 to the perinuclear region. Surprisingly, the Rab-1 and Rab-11.1-positive vesicles also mislocalized to the region near the plasma membrane in the *unc-83(e1408)* mutant and kinesin-1 depleted DTCs. These results raise the possibility that UNC-83, UNC-84 and kinesin-1 may function in tethering UNC-5 around the nucleus through Rab-1 and Rab-11.1-positive vesicles, thereby restricting UNC-5 transport in proper timing and amount during the dorsal turn.

Contact: hskim@kwansei.ac.jp

Analysis of the molecular mechanisms involved in the cellular uptake of double-stranded RNA

Rieko Imae[1,2], Hiroyuki Arai[2], Shohei Mitani[1,3]

[1]Department of Physiology, Tokyo Women's Medical University School of Medicine, Japan
[2]Graduate School of Pharmaceutical Science, University of Tokyo, Japan
[3]Tokyo Women's Medical University Institute for Integrated Medical Sciences, Japan

Introduction of double-stranded RNA (dsRNA) into cells induces potent and specific gene silencing, a phenomenon called RNA interference (RNAi). In some organisms, including *C. elegans*, RNAi has a potential to spread between cells, leading to non-cell autonomous RNAi in cells distant from the cells where dsRNA is initially introduced. Intercellular RNAi signal, which is assumed to dsRNA, is imported into the cell via a conserved transmembrane protein SID-1, which is considered as a dsRNA channel or transporter in *C. elegans*. On the other hand, it has been reported that endocytic pathway plays an important role in cell entry of dsRNA in drosophila S2 cells and mammalian cells, and it is suggested that the same mechanism is conserved in *C. elegans*. Recently, SID-3 was reported to be involved in the import of mobile dsRNA into *C. elegans* cells, and the mammalian homolog of SID-3, activated *cdc-42*-associated kinase (ACK), is implicated in the early endocytic pathway. These reports suggest the involvement of membrane traffic in systemic spread of silencing RNA, however, little is known about the entire trafficking pathway regulating the phenomenon. To elucidate the role of membrane traffic in the intercellular transport of dsRNA, we screened the mutants of membrane traffic related genes exhibiting defects in systemic RNAi spreading. Through this screening, we identified a gene required for the uptake of dsRNA into both the somatic and germ cells. The gene product was localized to endosome and trans-Golgi network (TGN), indicating that endomembrane trafficking plays an important role in cellular uptake of silencing RNA.

Contact: rimae@mol.f.u-tokyo.ac.jp

Genetic screen to identify genes involved in maintaining lysosome morphology, function and dynamics in *C. elegans*

Yuan Li, Yubing Liu, Yanan Sun, Wei Zou, Baohui Chen

National Institute of Biological Sciences, Xiaochen Wang's Lab, China

Lysosomes are membrane-bound acidic organelles that degrade cargoes derived from endocytosis, phagocytosis or autophagy through the action of a variety of lysosomal hydrolases. The degradation products are exported from lysosomes by membrane transporters for the re-utilization in cellular metabolism. Defects in either lysosomal hydrolytic enzymes or exports of hydrolysis products lead to inherited lysosomal storage disorders, indicating the importance of lysosomes in cellular homeostasis.

We observed that lysosomes, which are labeled by either the lysosomal enzyme NUC-1 or the lysosomal membrane protein LAAT-1 or are stained by lysotracker, appear as small puncta or thin tubules, and display a highly dynamic pattern during development. To understand how lysosome morphology and dynamics are regulated, we performed genetic screens to search for mutants with altered lysosomalmorphology. From a forward genetic screen that covers 10,000 haploid genomes, we have isolated 50 mutants which contain abnormal lysosomes. Here we report our characterizationof three recessive mutants, qx348, qx245 and qx268, which affect3 different genes. We found that tubular lysosomal structures are disrupted in all three mutants and lysosomes appear as big puncta.We examined degradative activity of lysosomes and found that these mutants affect degradation of several kinds of cargoes, with more severe defects observed in the degradation ofautophagic cargo. Moreover, all three mutants display severe developmental defects includinghigh percentage of embryonic lethality and larval arrest. These datasuggestthat lysosome-mediated processes regulated by the affected genes are essential for *C. elegans* development. We are now in the process of cloning the affected genes and further characterizing their functions in regulating lysosome morphology, function and animal development.

Contact: liyuan@nibs.ac.cn

Autolysosome formation requires clearance of phosphatidylinositol-3-phosphate by themyotubularin phosphatase MTM-3 in *C. elegans*

Yanwei Wu

National Institute of Biological Sciences, China

Autophagy is a lysosome-mediated degradation process that removes portions of the cytoplasm or selectively clears mis-folded or aggregate-prone proteins and damaged organelles. The core machinery that controls autophagosome biogenesis was identified by yeast genetics, and is well conserved in higher organisms. Nevertheless, autophagy in higher eukaryotes displays fundamental differences from that in yeast. Genetic screens performed in the nematode *C. elegans* identified both conserved Atg homologs and metazoan-specific autophagy regulators, establishing *C. elegans* as a useful genetic model to study autophagy. The hierarchical order of autophagy proteins in the aggrephagy pathway (which degrades protein aggregates) has also been established in worms, providing a genetic platform for mechanistic studies of autophagosome formation and maturation in higher eukaryotes.

Here, we study functions of mytobularin phosphatases in autophagy regulation. It is reported that initiation of autophagosome formation is promoted by the PI3-kinase complex, and negatively regulated by myotubularin phosphatases including MTMR14 and MTMR3, indicating a tight control of local phosphatidylinositol-3-phosphate (PtdIns3P) levels at this step. However, Ymr1, the only myotubularin phosphatase in yeast, is recently reported to be essential for normal progression of autophagy. It is unclear whether the distinct effect of human and yeast myotubularin phosphatases on autophagy is due to the intrinsic differences between mammalian and yeast autophagy, or because PtdIns3P turnover has opposing effects if it occurs at different stages of autophagy. We found that loss of MTM-3, but not other myotubularin phosphatases in C.elegans causes defects in degradation of various autophagic substrates. Our epistasis analyses indicate that MTM-3 acts at the step downstream of the ATG-2/EPG-6 complex and upstream of EPG-5 in the aggrephagy pathway to promote autophagosome maturation into autolysosomes. MTM-3 can be recruited to autophagosomes by PtdIns3P and loss of MTM-3 causes increased autophagic association of ATG-18 in a PtdIns3P-dependent manner. Our data reveal critical roles of PtdIns3P turnover in autophagosome maturation and/or autolysosome formation.

Contact: wuyanwei@nibs.ac.cn

Screening genes that regulate the nucleolar size in *C. elegans*

Li-Wei Lee[1], Hsuan Liu[2], Bertrand Chin-Ming Tan[1], Szecheng J Lo[1]

[1]Department of Biomedical Sciences, Chang Gung University, Taiwan
[2]Department of Biochemistry, Chang Gung University, Taiwan

The nucleolus is a non-membrane bound compartment in the nucleus of eukaryotes. The major biological function of nucleolus is in ribosome biogenesis. Although the presence of nucleolus is dynamic during cell cycle and growth, the increase of nucleolar size and number is a hallmark of many human cancer cells due to the high demand of ribosome for rapid cell dividing, which can be used as a marker for pathological diagnosis. *C. elegans* has around 1000 cells, which provides a good model for searching genes that regulate the nucleolar size and these genes are conserved from worms to humans. Using the EMS mutagenesis method, two mutants (*ncl-1* and *ncl-2*) which appear enlarged nucleoli have been reported previously. To seek more genes that regulate the nucleolus size, we established a FIB-1::GFP reporter strain as a starting material for EMS mutagenesis. Three mutants were obtained by a low-power fluorescence microscopic screening. After having performed the whole genome deep sequencing, results showed that these worms are mutated at the *ncl-1* gene. Two are mutated at the nucleotide position 316, converting a glutamine codon (CAA) to a stop codon (TAA), which is the same as a mutation site of known *ncl-1(e1942)* strain. The third one, a new allele, is mutated at the nucleotide position 1690, also a conversion of CAA to a stop codon of TAA. It raised an interesting question why mutations of *ncl-1* are easier than other genes to be obtained. It could be that *ncl-1* is the most important nucleolus-size suppressor in worms, which affects all types of cells.

Contact: losj@mail.cgu.edu.tw

Use of laser nano-ablation to study mechanical properties of epidermal acto-myosin cortex during *C. elegans* embryo elongation

Thanh Thi Kim Vuong, Michel Labouesse

Institute of Genetics and of Molecular and Cellular Biology, France

Elongation - the morphogenesis step of *C. elegans* embryogenesis, occurs in part through the activity of the mechanical forces produced by non-muscle myosin II in the epidermis, and at a later embryonic stage through the activity of muscles. Acto-myosin forces are thought to squeeze the embryo circumferentially (dorso-ventrally) to drive its elongation in the anterior-posterior direction. We used a laser nano-ablation technique to dissect quantitatively acto-myosin-dependent tension during elongation, and to understand the coupling between acto-myosin forces with muscle contractions.

The technique consists of cutting a thin line in the labeled actin cortex with an infrared femto-second laser. The shape of the cut and its recoil velocity give a direct assessment of the mechanical tension and cortex stiffness. First, we found that before muscle start contracting, elongation is not driven by anisotropy of tension in all lateral epidermal cells, as previously thought, but more likely by the ones in the middle and tail regions. Moreover, there is little tension in the acto-myosin network in dorso-ventral cells in the head at this stage. Second, we observed an increase of tension magnitude and anisotropy in the head after the onset of muscle contractions but still present in a muscle mutant, indicating that it is independent of muscle contractions. The finding of a significant lower tension in a *let-502/rho-kinase* mutant compared to the wild type embryo supports the validity of the method. We will present a preliminary model which features the embryo as a sphere (head) attached to a cylinder (body/tail) with an internal hydrostatic pressure. Acto-myosin network in the body/tail part induces stress preferentially in the dorso-ventral direction in order to satisfy Laplace's law (constraint between stress, hydrostatic pressure and the shape of the embryo) and drive elongation in this region.

Contact: vuong@igbmc.fr

Study of epidermal morphogenesis during early elongation at a single cell level

Emmanuel Martin, Marie-Hélène Ouellette, Karim Hamiche, Sarah Jenna

UQAM, Canada

Elongation of *Caenorhabditis elegans* embryo into a long, thin worm occurs during late embryogenesis. These morphogenic events are divided into two phases: the early and late elongation. Early elongation is currently believed to be driven by contractions of circumferential actin-filament bundles (CFBs) at the apical pole of hypodermal cells. These contraction events are thought to be regulated by two parallel pathways: the *let-502/mel-11* and the *pix-1/pak-1* pathways. These pathways are thought to control the phosphorylation level of the myosin-light chain MLC-4. According to the currently accepted model, while MLC-4 phosphorylation and consequently myosin contraction is thought to be high in lateral hypodermal cells, ventral and dorsal hypodermal cells are thought to be mainly in a relaxed form. We recently showed that the *pix-1/pak-1* pathway controls the head to tail morphology of elongating embryos in parallel to *let-502/mel-11*. These data suggest the existence of a planar polarity control of morphogenic events driving elongation.

To better understand this planar polarity control of early elongation, we developed a novel approach to study morphogenic events occurring at that stage at a single cell level. To do so, we characterized the membrane deformation of hypodermal cells along the antero-posterior axis of the embryo using the AJM-1::GFP cell junction marker and a 3D-image analysis tool. Using this approach, we showed that that the shrinking of transversal membranes of ventral hypodermal cells is not negligible compared to lateral cells and consequently that dorsal and ventral hypodermal cells are not equally in a relaxed state during early elongation. Surprisingly, our data show that shrinking of transversal membranes of lateral cells cannot explain on their own the elongation speed of longitudinal membranes of these cells. We also observed distinct membrane deformations of cells located at the anterior versus posterior part of the embryo. Considering the significant deviation brought by our data from the currently accepted model we are currently using this novel approach and mutants of signalling pathways controlling early elongation to readdress the molecular basis of hypodermal cell morphogenesis at that stage.

Contact: martin.emmanuel.2@courrier.uqam.ca

Analysis of expression pattern, localization and function of the PAF1 complex, which is essential for epidermal morphogenesis in *C. elegans*

Kenji Tsuyama[1], Yukihiko Kubota[1], Yusuke Takabayashi[1], Nami Haruta[1], Rika Maruyama[2], Naoko Iida[2], Asako Sugimoto[1]

[1]Grad. Sch. of Life Sci., Tohoku Univ., Japan
[2]RIKEN CDB, Japan

During morphogenesis, cells undergo dynamic changes including polarization, migration and rearrangement of cell groups. Through an RNAi screen, we identified the ortholog of Ctr9 (CTR-9) whose depletion caused a body elongation defect at a high penetrance. Ctr9 is a component of the Polymerase-Associated Factor 1 complex (PAF1C). The PAF1C consists of five conserved proteins and is implicated in diverse transcription related processes. We found by RNAi that, in addition to the CTR-9, orthologs of all other four components of the PAF1C (PAFO-1, LEO-1, CDC-73 and RTFO-1) were required for epidermal morphogenesis. The loss of the PAF1C component did not affect cell specification for neurons, muscles and epithelia, but is required for cell shape change and positioning of epidermal cells. PAFO-1::mCherry, mCherry::LEO-1 and GFP::RTFO-1 driven by their authentic promoters were localized to the nuclei of virtually all embryonic cells. Although LEO-1 was universally expressed, mutant rescue experiments revealed that its epidermal expression was crucial for the viability of this animal. The vast majority of PAF1C was not tightly associated with chromatins, because it was diffused to the cytoplasm after NEBD. The nuclear localization of mCherry::LEO-1 and PAFO-1::mCherry were lost by RNAi knockdown of other components except RTFO-1. In contrast, the nuclear localization of GFP::RTFO-1 was unaffected by the knockdown of other components. Thus, the nuclear localization of RTFO-1 was independent from other components of the PAF1C, but that of other four components was interdependent. Because cytoskeletons are involved in epidermal cell shape change and migration, we analyzed the organization of F-actin and microtubules in epidermal cells using VAB-10(ABD)::GFP and mCherry::TBB-2, respectively. Depletion of CTR-9 affected the alignment of microtubules more severely than actin filaments in epidermal cells during the morphogenesis stage. Take together, we speculate that the PAF1C regulates transcription of specific genes in epithelial cells during morphogenesis stage, and that some of the targets might affect the organization of microtubules.

Contact: t-kenji@life.biology.tohoku.ac.jp

A genome-wide RNAi screen to identify new players of a muscle-to-epidermis mechanotransduction pathway essential for embryonic elongation

Christelle Gally, Agnés Aubry, Sarah Yoessle, Michel Labouesse

IGBMC, France

Loss of muscle activity in the *C. elegans* embryo results in embryonic elongation arrest at the 2-fold stage (Pat phenotype) for a reason that has long remained unclear. We recently unraveled a mechanotransduction pathway between muscles and epidermis that accounts to a large extent for the Pat phenotype (Zhang et al., 2011). Specifically, during the second phase of embryonic elongation, muscle contractions activate the serine/threonine p21-activated kinase PAK-1 in the epidermis and trigger the remodeling of hemidesmosomes (HDs) in the epidermis through intermediate filament phosphorylation. The most upstream known component of this pathway is the adaptor protein GIT-1, whose localization to HDs requires muscle activity. However, since *git-1* mutants do not induce a two-fold arrest, we predict that muscle contractions also trigger a second parallel pathway, probably to activate actomyosin remodelling. In order to identify this putative parallel pathway, we have undertaken a genome-wide RNAi screen in the *git-1(tm1962)* mutant background, looking for enhancers that lead to an elongation arrest.

In the primary screen, we have identified 78 candidates that induce body-morphology defects as well as an elongation arrest in the *git-1* mutant but much less so in the wild-type N2 background. We have confirmed this *git-1* enhancement for 39 candidates in a secondary screen. Among them, four genes encode HD components and six genes are involved in muscle differentiation/function or attachment to the extracellular matrix, hence validating our approach.

Now, we are focusing on candidates that were not previously linked to embryonic elongation. One of them is FARL-11, which is orthologous to human FAM40A and FAM40B whose functions remains unclear. The progeny of *farl-11(tm6233)* homozygote mutants (provided by the National Bioresource Project) displays 50% early dead embryos. When combined with the *git-1* mutant, the embryonic lethality reaches 80%, with half of the dead embryos being arrested at the 1.5 fold stage. We generated a CRISPR *GFP-farl-11* knock-in (Dickinson et al., 2013) and observed that the protein is present at the level of HDs during elongation. These results confirm that HDs represent a central platform for the elongation process in response to muscle contractions. First it allows IFs remodeling through PAK-1 activation; second it recruits FARL-11, triggering the activation of a parallel pathway that we are now unraveling.

Contact: gally@igbmc.fr

Tissue mechanics regulate epidermal stem cell dynamics coupled to the molting cycle

Sophie Katz, Alison Frand

Department of Biological Chemistry, School of Medicine, University of California Los Angeles, USA

Mechanical properties of the extracellular matrix (ECM) are known to affect the behavior of stem cells, but the underlying mechanisms have not been well defined in any organism. We study this question using the molting cycle of *C. elegans* as a model. The worm cuticle is a collagen-rich ECM that is remade by underlying epidermal cells and syncytia during each of the four molts. The seam cells, which are pluripotent epidermal blast cells, repeatedly switch between non-proliferative and proliferative states in phase with the molting cycle. During the molts, the seam cells secrete new ECM, behaving like differentiated, polarized epithelia. Immediately after the first three molts, the seam cells undergo asymmetric divisions characteristic of stem cells. In preliminary research, we found that mutations affecting certain fibril-forming extracellular proteins such as MLT-10 and FBN-1 lead to defects in epidermal morphogenesis. These results suggested that tension-dependent cell-ECM signaling pathways influence seam cell behavior. By extension, we hypothesized that cycles in the mechanical stress on certain cell-ECM or cell-cell attachment complexes license seam cell division as animals exit the molts. To identify components of these anticipated pathways, we selected 59 candidate genes similar in sequence to known components of mechanotransduction pathways in other systems. Gene-specific knockdowns were then screened for defects in seam cell development, including hyperplasia, hypoplasia and abnormal cell morphology. Based on the results of our screen and further genetic and molecular analyses, we propose that the actin cytoskeleton is critical for the conversion of mechanical forces into chemical cues that regulate seam cell division and proliferation. Our findings may apply to human cancer biology, because the deregulation of mechanical signaling between epidermal cells and matrices has been implicated in both tumorigenesis and metastasis.

Contact: sophiekatz@ucla.edu

The role of PIGV-1 in maintaining epithelial integrity during *C. elegans* morphogenesis

Thang Dinh Doan, Yemima Budirahardja, Ronen Zaidel Bar

Mechanobiology Institute, Singapore

Glycosylphosphatidylinositol (GPI) is a post-translational modification resulting in the attachment of modified proteins to the plasma membrane outer leaflet. GPI anchored proteins (GPI-AP) have diverse functions, but common to all is their targeting apical membranes of epithelial cells and association with lipid rafts. GPI biosynthesis is essential for viability of all eukaryotes. However, due to early embryonic lethality of null mutants, the role of GPI-AP in development is poorly understood.

The highly conserved GPI biosynthesis pathway involves more than 20 phosphatidylinositol glycan (PIG) enzymes, among them PIGV. Recently, missense mutations in human pigv were associated with a multiple congenital malformation syndrome with a high frequency of Hirschsprung disease and renal abnomalies [1]. However, little is known on how such phenotypes are linked to PIGV function.

We took advantage of a hypomorphic allele of PIGV in *C. elegans*, *pigv-1(qm34)*, to study the role of GPI-AP in development. Originally isolated in a genetic screen by Hekimi *et. al*. [2] we characterized the temperature-sensitive allele *qm34* as a missense mutation (G361E) in *pigv-1*. At the restrictive temperature (above 20°C) we found an 85% reduction in GPI-AP at the surface of embryonic cells. Consequently, 65% of *pigv-1(qm39)* embryos arrested during elongation, while exhibiting internal cysts and/or surface ruptures. Closer examination of the defects revealed that all epithelial tissues are affected in *pigv-1(qm34)*. Cysts are formed in the intestine, pharynx and excretory canal, and ruptures occur through epidermal cells, suggesting epithelial membrane weakening. Epithelial-specific expression of PIGV-1 was sufficient to rescue these phenotypes and embryonic lethality. Importantly, strengthening the actin cortex or the links between the apical membrane and cortex by overexpression of ACT-5 or ERM-1, respectively, significantly rescued cyst formation, ruptures and elongation arrest.

In conclusion, we propose GPI-APs play a critical role in maintaining the integrity of the apical membranes of epithelial cells, allowing them to withstand the stresses of morphogenesis.

1. Horn, D., *et. al.* Delineation of PIGV mutation spectrum and associated phenotypes in hyperphosphatasia with mental retardation syndrome. Eur J Hum Genet, 1-6 (2013).

2. Hekimi, S., *et. al.* Viable maternal-effect mutations that affect the development of the nematode *Caenorhabditis elegans*. Genetics 141, 1351-1364 (1995).

Coment: doandinhthang@gmail.com

Sequential apical and lateral cell constrictions initiate vulval lumen morphogenesis in *C. elegans*

Qiutan Yang, Juan Miguel Escobar, Alex Hajnal

MLS University of Zurich, Switzerland

During epithelial morphogenesis, linear arrays of cells are converted into three dimensional tubular structures that are the building blocks of most epithelial organs. In this process, the contraction of the actomyosin network generates intracellular forces that cause the bending of the tissue and induce tube formation. We have investigated the actomyosin-induced cell shape changes that regulate the morphogenesis of the *C. elegans* hermaphrodite vulva, a tubular organ formed by 22 epithelial cells. The first discernible event that initiates vulval invagination and lumen formation is the constriction of the apical membranes in the inner-most pair of primary vulval cells (VulF). After completing apical constriction, the VulF cells constrict their lateral membranes along the apico-basal axis to extend and connect the lumen to the anchor cell (AC), which is located in the overlaying uterus. This sequence of apical and lateral cell constrictions correlates with the proper subcellular distribution of the cortical actomyosin network and the divisions of the adjacent VulE cells. Lateral but not apical VulF constriction requires the prior invasion of the AC into the vulval epithelium, which permits the formation of direct AC-VulF cell contacts. Moreover, luminal pressure created by the migration of the secondary cells towards the vulval midline assists in the dorsal extension of the vulval lumen. Taken together, a regulated sequence of cell shape changes coordinated by the invading AC and interspersed with the last round of cell divisions initiates vulval lumen morphogenesis.

Contact: qiutan.yang@imls.uzh.ch

Dopamine regulates body size independently of TGFβ pathway in *C. elegans*

Takashi Nagashima[1], Eitaro Oami[2], Satoshi Suo[2], Shoichi Ishiura[1,2]

[1]Dept. of Biological Sciences, Graduate School of Science, The Univ. of Tokyo, Japan
[2]Dept. of Life and Sciences, Graduate School of Arts and Sciences, The Univ. of Tokyo, Japan

Body size is a fundamental feature of animals and its regulation is important for adaptation to their environment. In *C. elegans*, many genes that regulate body size have been identified. Some of these genes function in neurons, indicating that the nervous system plays a role in regulating body size. However, the mechanisms for regulating the body size by nervous system are not fully understood. Dopamine, an amine neurotransmitter, is required for food sensing and food-dependent behaviors in *C. elegans*. The role of dopamine in the development of *C. elegans* is not well-understood. We have shown that *cat-2* mutants (defective in dopamine synthesis) are larger than the wild type animals. We also found that *cat-2* mutants lay eggs that are slightly more developed than that of the wild type animals. The larger body size of *cat-2* mutants was not due to the delayed egg-laying since *cat-2* mutants were larger than the wild type animals even when animals were synchronized at the L4 stage. These results suggest that dopamine negatively regulates the body size independently of the developmental rate or egg-laying. It is reported that *cat-2* mutants exhibit increased locomotion compared to the wild type animals. We found that the large body size of *cat-2* is not caused by this increased locomotor activity. However, *unc-52* and *unc-54*, which function in muscles, suppressed the increase in the body size of *cat-2*, suggesting that the dopamine-regulation of body size does require functional muscles. The TGFβ ligand DBL-1 has been shown to increase the body size of *C. elegans* by suppressing LON-1. To examine whether the dopamine and TGFβ pathways are related, we conducted epistasis analyses and found that the dopamine-dependent pathway regulates the body size independently of the TGFβ pathway and these pathways converge on the LON-1.

Contact: tnagashima22@gmail.com

Dissection of the facilitating role of *lon-1* on the DBL/SMA pathway based on a novel hypodermal marker

Ho Tsan Wong, King Lau Chow

Division of Life Science, Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong

DBL/SMA pathway controls *C. elegans* body length. A reduced pathway signal de-represses the transcription of a downstream target gene, *lon-1*, which inhibits hypodermal endoreduplication. In *lon-1*(e185) mutant, the worms display a long phenotype (Lon). Double mutant of *lon-1* with *dbl-1* or *sma-6* failed to conclude *lon-1* acting solely as a downstream target gene (Morita et al., 2002; Maduzia et al., 2002). We aim to directly measure the pathway activity using an intracellular signal readout in the hypodermis, *lon-1p::gfp*. The reporter responds inversely to the DBL/SMA signal, similar to the intestinal BMP reporter *pspp-9::gfp*. The stronger reporter signal in *lon-1* mutant indicates that *lon-1* is a positive regulator of *dbl-1*. In *lon-1* mutant, weakened DBL/SMA signal should show a Sma phenotype, not the displayed Lon phenotype. We hypothesize that *lon-1* may regulate body length through two counter-acting pathways: (1) through direct repressing of the endoreduplication, and (2) through enhancing DBL/SMA signal to inhibit endoreduplication. In *lon-1* mutant, (1) is epistatic to (2). Since LON-1 is a membrane-associated protein, we expect its extracellular regulation of BMP pathway might be DBL-1 dosage dependent. We will quantify *lon-1p::gfp* reporter signal in double mutants of *lon-1* and *dbl-1* as well as mutants of pathway components to prove this hypothesis.

We have also confirmed that the N-terminus and the conserved SCP-CRISP domain of LON-1 can physically interact with CRM-1 CR-motif by yeast-2-hybrid assays (Wong K.H. unpublished). Either N-terminus or SCP-CRISP domain alone rescued *lon-1* mutant body length. The result implies that each of these domains can act alone to execute LON-1 function in (1) or/and (2). Focusing on (2), truncated LON-1 with different domains will be produced and displayed on the cell surface in *lon-1* mutant background to see how these proteins alter the DBL/SMA pathway responsive *lon-1::gfp* reporter signal to demonstrate the activity of each domain. (This study is supported by Research Grants Council, Hong Kong)

Contact: Frankiewong2012@yahoo.com.hk

Analysis of *flp-10* that controls migration of the gonadal leader cells in *C. elegans*

Keisuke Morita, Hon-song Kim, Kiyoji Nishiwaki

Sch. of Sci. and Tech., Kwansei Gakuin Univ., Japan

The anterior and posterior gonadal leader cells called distal tip cells (DTCs) migrate in a U-shaped pattern to form the U-shaped gonad arms during *C. elegans* larval development. The *flp-10(tk28)* mutation results in abnormal gonad formation due to the misdirected migration of DTCs. *flp-10* encodes an FMRFamide like peptide (FLP). FMRFamide has Phe-Met-Arg-Phe-NH2 in its carboxyl terminus. These peptides are known to be involved in regulation of neuronal activities. FLP-10 is supposed to be proteolytically processed into four peptides, secretion signal peptide, A, B and C peptides having 16, 11 and 16 amino acids, respectively. Although the B peptide corresponding to FMRFamide was expected to act in cell migration, we found that the C peptide having the secretion signal had this activity by injection rescue experiments. The expression of the C peptide in in body wall muscle cells, DTCs or touch neurons rescued the mutant phenotypes, suggesting that the C peptide is secreted and acts on a certain receptor. We identified a protein in the worm lysate specifically co-precipitated with the C peptide in a Pull-down assay using the biotinylated C peptide. LC/MS/MS analysis revealed that the specific protein corresponded to the product of *H28O16.1*. *H28O16.1* encodes the alpha subunit of mitochondrial ATP synthase. Interestingly, there are some reports that the mitochondrial ATP synthase is also expressed in the plasma membrane. We are trying to uncover the possibility that H28O16.1 could be the receptor for the FLP-10 C peptide.

Contact: morimorike@gmail.com

Requirement of an ADAM (a disintegrin and metalloprotease) protease in promoting a BMP-like signaling pathway in *Caenorhabditis elegans*

Lin Wang

Cornell University, USA

Bone morphogenetic proteins (BMPs) belong to the transforming growth factor β (TGFβ) superfamily of ligands, and the BMP signaling pathway plays important roles in multiple developmental and physiological processes. Malfunction of the pathway in humans can cause skeletal abnormalities, cardiovascular diseases and cancer. Studies of the BMP-like Sma/Mab signaling pathway in *C. elegans* has provided insights into the understanding of BMP signal transduction. Our lab is interested in identifying and characterizing factors involved in modulating the BMP pathway. We have previously shown that an evolutionarily conserved transmembrane protein UNC-40 positively promotes BMP signaling at the ligand-receptor level (Tian et al., 2013). Interestingly, the extracellular domain of UNC-40 is sufficient to promote BMP signaling *in vivo*, suggesting that the UNC-40 protein might be processed to release its extracellular domain. ADAM (a disintegrin and metalloprotease) proteases are known to be critical for cleaving transmembrane domain proteins in a process called ectodomain shedding. We have found that mutations in one of the ADAM proteases in *C. elegans*, SUP-17, caused defects in Sma/Mab signaling and that *sup-17* appears to function at the ligand-receptor level to positively promote Sma/Mab signaling. Using a functional SUP-17::GFP translational fusion and various cell-type specific expression constructs, we showed that like *unc-40*, *sup-17* is also expressed and functions in the signal-receiving cells. Our results provide *in vivo* evidence for the involvement of ADAM proteases in modulating BMP signaling. We are currently testing the hypotheses that SUP-17 promotes Sma/Mab signaling mainly by shedding the ectodomain of UNC-40.

Contact: lw494@cornell.edu

Genetic interactions among ADAMTS metalloproteases and basement membrane molecules in cell migration in *C. elegans*

Ayaka Imanishi, Tomomi Takano, Kiyoji Nishiwaki

Sch. of Sci. and Tech., Kwansei Gakuin Univ., Japan

During development of *C. elegans* gonad, the gonadal leader cells called distal tip cells (DTCs) migrate in a U shaped pattern to form the U shaped gonad arms. The ADAMTS (a disintegrin and metalloprotease with thrombospondin motifs) family metalloproteases, MIG-17 and GON-1 are required for DTC migration. Mutations in *mig-17* result in misshapen gonads, and mutations in *gon-1* result in shorten and swollen gonads due to the abnormal DTC migration. We have isolated mutations in basement membrane proteins, *emb-9*, *let-2* (both encode collagen IV) and *fbl-1/fibulin-1* as genetic suppressors of *mig-17* and *gon-1* mutations. To understand the role-sharing between these two proteases, we examined whether the suppressors of *mig-17* can suppress *gon-1* and those for *gon-1* can suppress *mig-17*. We found that some of the *emb-9*, *let-2* and *fbl-1* mutations suppress both *mig-17* and *gon-1*, while others result in opposite outcomes. Although the reason for these allele specific responses in molecular levels is not clear at present, these results suggest that *mig-17* and *gon-1* have their specific functions as well as the functions commonly shared between them. Probably the loss of the gene specific functions should be the cause for the very different phenotypes shown by *mig-17* and *gon-1* mutations.

Contact: ayaka_nw@outlook.com

Possible regulation of endocytosis by semaphorin during ray morphogenesis

Ayana Kobayashi, Hiroki Tanaka, Shin Takagi

Nagoya University, Japan

The semaphorin family of intercellular signaling proteins acts via the plexin family transmembrane proteins to govern cellular morphogenesis in a variety of developmental events. Since the semaphorin signaling system is now known to regulate a wide range of cellular events including cytoskeletal reorganization, mRNA translation and mTOR partnering, much remain to be elucidated about its downstream pathways.

In the *C. elegans* male tail, two transmembrane-type semaphorins, SMP-1 and SMP-2, and their cognate receptor, PLX-1, are required for proper positioning of rays, which are adult sensory organs derived from larval epidermal cells. In *plx-1* single or *smp-1*, *smp-2* double mutants, the anterior-most ray1, which is usually closely associated with ray2 in the wild type animal, is displaced anteriorly. This defect is caused by the positioning defects of ray forming larval epidermal cells. Taking advantage of the high penetrance of the ray phenotype, we have been carrying out a genetic suppressor screening of *plx-1* mutants.

We have identified mutations in *unc-26* and *unc-57* as novel suppressors of *plx-1* mutants. These mutations by themselves do not affect ray positioning in the wild type genetic background. As UNC-26/synaptojanin and UNC-57/endophilin are both well-known endocytotic factors, our results suggest that the semaphorin signal represses the endocytosis in ray forming epidermal cells. To further examine this possibility, we are trying to visualize the localization of UNC-26 and UNC-57 in wild type and *plx-1* mutants.

Contact: kobayashi.ayana@c.mbox.nagoya-u.ac.jp

Analysis of morphological changes of epidermal cells producing sensory rays in the *C. elegans* male tail

Shin Takagi, Hiroki Tanaka, Ayana Kobayashi

Nagoya University, Japan

Some organs generated during the larval development of *C. elegans* are interesting experimental systems for studying epidermal morphogenesis. In the tail of the adult male, there are a set of nine male-specific genital sense organs known as rays. Development of rays starts at the 3rd larval stage, when nine ray precursor cells, R(n) cells, are generated from specialized epidermal cells on each side of the posterior body of the larval male. Each ray precursor cell, after 3 divisions, gives rise to a ray precursor cluster comprising three cells, which later differentiate into two neurons and one support cell of a mature ray. Each ray precursor cell also produces Rn.p, which fuses later with other Rn.ps to form a multi-nucleated cell called the tail seam.

Examination with the ajm-1::gfp apical junction marker showed that descendants of R(n) cells change their shape and position dynamically but in a stereotyped fashion during development. The marker, however, does not allow complete visualization of cell-shape. To examine the cell-shape in details, we have generated a lin-17p::gfp::PH transgene marking the plasma membrane of larval male tail cells.

Preliminary observations revealed that the descendants of R(n) cells change overall shapes during the development. In particular, ray precursor clusters change their shapes drastically to form a process by thinning the apical part during the 3rd larval stage. The horizontal position of the apical surface of the cells is also displaced from that of their nuclei, suggesting that cell bodies shift anteriorly.

Contact: i45116a@nucc.cc.nagoya-u.ac.jp

Identification of proteins that interact with the AMPK-related protein kinase UNC-82/ARK5/SNARK/NUAK in *C. elegans* striated muscle.

Pamela E Hoppe[1], Christopher D Duchesneau[1], Latrisha S Lane[1], April R Reedy[1,2], Hiroshi Qadota[2], Guy M Benian[2]

[1]Western Michigan University, USA
[2]Emory University, USA

The UNC-82 serine/threonine kinase is required for the organization of the myosin filaments of body-wall muscle and is located at or near the M-line (Hoppe et al., 2010), which is the site where myosin filaments attach. The mechanism of UNC-82 kinase activation, the signal that promotes activation and the identities of UNC-82 targets have not been established. To better understand the role of UNC-82, we analyzed the distribution of components representing different “complexes” at the M-line, which contains many structural and probably signaling components, in *unc-82* mutants. Thick-filament and thick-filament-associated proteins are severely affected, whereas membrane-proximal M-line components show modest disorganization. We tested for physical interaction between UNC-82 and other M-line or thick-filament proteins by using strains in which myosin, paramyosin or UNC-82 is ectopically localized within muscle cells, and determining which proteins are recruited to the ectopic structures. Our results suggest that UNC-82 physically interacts, either directly or indirectly, with one or more of these three proteins: myosin, paramyosin, and UNC-98, which is a potential chaperone required for the incorporation of paramyosin into thick filaments (Miller et al., 2008). Antibody staining of *unc-82* mutant alleles revealed that ectopic accumulations of paramyosin occur in three different kinase domain missense mutants (including two from the Million Mutation Project), but not in the presumptive null that contains a premature stop codon. An UNC-82::GFP fusion containing a missense mutation in the kinase domain (*e1220*) expressed from an extrachromosomal array shows wild-type localization in an N2 background, but localization to ectopic structures that contain paramyosin in the *unc-82* null background. The kinase-dead UNC-82 protein appears to drive formation of these distinctive paramyosin accumulations, which are absent in the *unc-82* null mutant alone, suggesting that paramyosin is an UNC-82 substrate. Further, normal catalytic activity is required for proper localization of UNC-82, but the presence of wild-type UNC-82 allows proper localization of kinase-dead UNC-82 and paramyosin. Paramyosin accumulations present in the paramyosin missense allele *unc-15(e73)* also recruit UNC-82::GFP, as well as UNC-98. A genetic interaction between *unc-82* and UNC-98::GFP transgenes further suggests functional interaction of these protein during thick filament assembly or maintenance.

Contact: pamela.hoppe@wmich.edu

Mutational analyses of protein-protein interaction between UNC-112 (kindlin) and PAT-4 (ILK)

Hiroshi Qadota, Rachel E. Corbitt, Annie McPherson, Yohei Matsunaga, Kim M. Gernert, Guy M. Benian

Department of Pathology, Emory University, USA

Kindlins are highly conserved integrin-associated proteins implicated in several human diseases. The single kindlin ortholog in *C. elegans*, UNC-112, is required for muscle sarcomere assembly, and is part of a conserved four-protein complex that associates with the cytoplasmic tail of integrin at the base of muscle integrin adhesion sites. The proper localization of UNC-112 to muscle adhesion sites depends on PAT-4 (integrin linked kinase; ILK). In addition, PAT-4 binds to the N-terminal half of UNC-112 (Mackinnon et al. 2002). UNC-112, but not PAT-4, directly interacts with the cytoplasmic tail of integrin. Our combined biochemical, genetic and cell biological data (Qadota et al. 2012) suggests a molecular model for this requirement: UNC-112 exists in a closed form that interacts weakly with integrin, and an open form that interacts more strongly with integrin, and conversion to the open active form is promoted by binding of PAT-4 to UNC-112.

To learn more about the interaction between UNC-112 and PAT-4, we isolated several PAT-4 mutants that can bind to PAT-6 (α-parvin), but cannot bind to UNC-112. Of 16 clones isolated, 4/16 contained single amino acid changes, and 3/4 are located in the kinase domain. Placement of these three residues on a homology model of PAT-4 kinase, reveals that they reside on a similar surface of PAT-4 kinase that also contains several residues that when mutated permit binding of PAT-4 to UNC-112 D382V (Qadota et al., 2014). We created transgenic lines that express from heat shock promoters HA tagged versions of either wild type, Q308H or I432F PAT-4. Although wild type PAT-4 localizes to integrin adhesion sites (dense bodies and M-lines), the two PAT-4 mutants (Q308H and I432F) fail to localize to these structures, suggesting that PAT-4 localization to integrin adhesion sites requires interaction with UNC-112 consistent with our model. Beginning with mutant PAT-4s (Q308H and I432F) that cannot bind to UNC-112, we used random mutagenesis of UNC-112 and yeast 2-hybrid assays to identify mutations in UNC-112 that allow it to bind to mutant PAT-4. We have determined the sequence alterations in 6 UNC-112 mutant clones that restore binding to PAT-4 I432F, and 16 UNC-112 mutant clones that restore binding to PAT-4 Q308H. Two single and two double mutants have been identified. Four of the six map within or very close to the FERM_N domain of UNC-112. Functional activity will be assessed by transgenic worms experiments.

Contact: hkadota@emory.edu

Simple genome editing of essential genes by the CRISPR/Cas9 system using temperature sensitive lethal mutant strains

Yu Honda, Nami Haruta, Yukihiko Kubota, Asako Sugimoto

Laboratory of Developmental Dynamics, Graduate School of Life Sciences, Tohoku University, Sendai, Japan

CRISPR/Cas9-based DNA cleavage provides an efficient tool for genome editing and is applied to many model organisms including *C. elegans*. In the CRISPR/Cas9-based genome editing in *C. elegans*, various selection maker genes (e.g., *unc-119*$^{+}$, GFP/mCherry visible markers, and antibiotics-resistance genes) have been used to screen for recombinants. Here, we report a simple genome editing of essential genes by the CRISPR/Cas9 system, using temperature sensitive (*ts*) lethal mutants, which eliminates the requirement of a selection marker. In this method, a homologous repair template with the desired modification (for example, insertion of the *gfp*-coding sequence) is designed to contain a truncated gene fragment, so that the fragment cannot rescue the *ts* phenotype when present as an extrachromosomal array; only when it replaces the *ts* allele region, the *ts* phenotype will be rescued. The purified recombinant Cas9 protein, *in vitro* transcribed sgRNA and the repair template are co-injected into the *ts* worms, then viable progeny are screened at a restrictive temperature 25°C. Using the *tbb-2(gk129)* worms that show *ts* lethality as the host strain, we successfully applied this method to construct a *gfp::tbb-2* (β-tubulin) strain, in which the *gfp*-coding sequence is inserted into the N-terminus of the *tbb-2* coding region. Because this method does not use a selection marker, alteration of the neighboring genome sequence can be kept to the minimum, which will reduce the risk to affect the expression of the gene of interest. The GFP signal of the obtained *gfp::tbb-2* strain was almost twice as bright as that of the *pie-1* promoter driven *gfp::tbb-2* strain constructed by the microparticle bombardment method. Multiple worm laboratories have isolated a large collection of *ts* lethal mutants, which can be used as the starting strain for this simple CRISPR/Cas9-based genome editing method.

Contact: y-honda@biology.tohoku.ac.jp

A method for integrating 4D images of *C. elegans* embryos expressing different fluorescent markers

Yusuke Azuma, Shuichi Onami

RIKEN, Japan

Advances in microscopy and molecular labeling enabled us to understand embryogenesis in terms of cellular dynamics. In *C. elegans*, fluorescently labeled nuclei can be semi-automatically tracked up to the 550-cell stage. To further understand cellular dynamics, we need to track multiple proteins and visualize their relationships since biological processes such as cell division are composed of dynamics of many kinds of proteins. However, currently there is no effective method to monitor the dynamics of all those proteins because multi-color imaging is limited to display only three to four proteins at one time.

We solved this problem by developing a method to integrate 4D images of *C. elegans* embryos expressing different fluorescent markers. The integration is enabled by the invariant embryogenesis of *C. elegans*. This method allows integrating unlimited number of strains expressing different marker genes. It first detects the positions of nuclei in each image. The images are then registered to match the positions of corresponding nuclei. These process are calculated automatically and overlapped images are created.

We evaluated performance of the method by applying it to images of pairs of embryos whose nucleus and cellular membrane were labeled by different fluorescent proteins. Consistent overlapped cellular dynamics were observed in the integrated embryos. Additionally, the cellular membranes were segmented by image processing methods and overlap volume ratios were calculated. The averaged ratio was about 85% for early stage embryos. By subtracting the overlapped images in each pair, differences in cellular shapes can be located. In this way, we found differences in lamellipodial dynamics in one of the pairs. Now we are further analyzing whether the differences are intrinsic nature in the *C. elegans* embryogenesis or artifacts generated by the integration method. Finally, we integrated embryos labeled with membrane and F-actin markers and found their colocalization. Thus, the method succeeded in integrating embryos labeled with different proteins. We plan to introduce this method to many kinds of proteins.

Contact: yazuma@riken.jp

AMPK and HIF-1 regulates ROS levels by a feedback loop to promote longevity and immunity in *C. elegans*

Ara B. Hwang[1], Eun-A Ryu[1], Murat Artan[1], Hsin-Wen Chang[2], Mohammad Humayun Kabir[3], Hyun-Jun Nam[1], Dongyeop Lee[1], Jae-Seong Yang[1], Sanguk Kim[1], William B. Mair[4], Cheolju Lee[3], Siu Sylvia Lee[2], Seung-Jae Lee[1]

[1]Department of Life Sciences/IBIO/ and WCU ITCE, Pohang University of Science and Technology, Pohang, Kyeongbuk, 790-784, South Korea
[2]Department of Molecular Biology and Genetics, Cornell University, New York, USA
[3]Theragnosis Research Center, Korea Institute of Science and Technology, Seoul and Department of Biological Chemistry, University of Science and Technology, Daejeon, South Korea
[4]Department of Genetics and Complex Diseases, Harvard School of Public Health, Boston, Massachusetts 02115, USA

Mild inhibition of mitochondrial respiration extends the lifespan of many species. We previously reported that increased reactive oxygen species (ROS) caused by reduced mitochondrial respiration promote longevity by activating hypoxia-inducible factor 1 (HIF-1) in *C. elegans*. Here we further elucidated a feedback-regulatory mechanism and functional significance of the ROS-induced longevity. We initially found that mutations in *aak-2* (catalytic α subunit of AMP-activated protein kinase [AMPK]) suppressed the longevity induced by an ROS-generating chemical, paraquat (0.25 mM). In addition, we showed that mitochondrial ROS activated AMPK, and that 0.25 mM paraquat did not further increase the longevity of gain-of-function AAK-2 transgenic animals. These data suggest that AMPK mediates the longevity induced by mitochondrial ROS. We then investigated the mechanisms by which AMPK contributes to the ROS-induced longevity. We demonstrated that AMPK acts as a negative feedback regulator of internal ROS levels, because *aak-2* mutants were hyper-sensitive to ROS and contained increased ROS levels. In contrast, HIF-1 was necessary and sufficient for increasing internal ROS levels, suggesting that HIF-1 acts as a positive regulator of ROS. Next, we sought to identify genes downstream of HIF-1 that affect lifespan and/or ROS levels. Thus, we used whole-genome microarrays to compare the transcriptomes of the mitochondrial *isp-1* mutants, which display high levels of ROS, and *isp-1; hif-1* mutants. We found that *smf-3* and *ftn-1*, two genes that are implicated in the regulation of cellular levels of iron and ROS, act downstream of HIF-1 to mediate the ROS-induced longevity. Our microarray analysis also indicated that *isp-1* mutants exhibited an up-regulation of pathogen-responsive genes compared to that of the *isp-1; hif-1* mutants. Thus, we directly examined whether the longevity caused by *isp-1* mutation is linked to the pathogen resistance of *C. elegans*. We found that *isp-1* mutants were resistant to several pathogens, and both *aak-2* and *hif-1* were required for the enhanced pathogen resistance. Conversely, we found that feeding non-pathogenic bacteria increased the lifespan of wild type but not that of the long-lived *isp-1* mutants. Taken together, we propose that the feedback mechanism involving AMPK and HIF-1 regulates ROS levels to maintain an optimal immunity and longevity.
Contact: seungjaelee@postech.ac.kr

Myc and Mondo like transcription factor *mml-1* regulates fat metabolism and lifespan in germline deficient worms

Shuhei Nakamura[1], Özlem Karalay[1], Christian Latza[1], Arno Meiler[2], Ben Becker[1], Kayo Nakamura[1], Bianca Habermann[2], Adam Antebi[1,3]

[1]Max Planck Institute for Biology of Ageing, Germany
[2]Max-Planck Institute of Biochemistry, Germany
[3]Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, Germany

The reproductive system of an animal is important for procreation but also has an impact on longevity. In *C. elegans*, removal of the germline stem cells during early development results in lifespan extension (termed 'gonadal longevity'). This longevity depends partly on fatty acid desaturation, lipolysis and autophagy. However, little is known about the molecular links connecting these different pathways involved in lifespan extension.

Through RNAi based genetic screening, we have found that Myc and Mondo-like transcription factor MML-1 and its heterodimer partner MXL-2 are both required for longevity conferred by germline ablation. Gene expression studies indicate that genes involved in fatty acid desaturation as well as many DAF-16/FOXO target genes important for fat metabolism are affected when *mml-1* or *mxl-2* function is abolished in germline deficient animals. This suggests that MML-1/MXL-2 might link fat metabolism and lifespan in germline deficient worms. In concordance with this, we found that total stored fat and Mono Unsaturated Fatty Acid levels were decreased in germline deficient *mml-1* mutants. Interestingly MML-1::GFP is upregulated in germline deficient animals and this upregulation partly depends on NHR-80/HNF-4 activity. Mammalian homologs of MML-1, MondoA and ChREBP, positively regulate lipogenesis and glycolysis, and the reduction of these activities improves obesity and insulin resistance. Our results imply that within gonadal longevity, presence of *mml-1* has a beneficial effect on animal lifespan by linking reproduction and fat metabolism. Thus, detailed characterization of MML-1 functioning using *C. elegans* will potentially contribute to understanding the molecular relationships between reproduction, fat metabolism and lifespan in the higher organisms.

Contact: shuhei.nakamura@age.mpg.de

Identification and characterization of PDZ domain-containing proteins that regulate lifespan via insulin/IGF-1 signaling pathway

Seon Woo An[1], Eunseok Choi[1], Jae-Seong Yang[2], Keunhee Seo[1], Wooseon Hwang[1], Heehwa Son[1], Hyun-Jun Nam[1], Sanguk Kim[1,3], Seung-Jae Lee[1,2,3]

[1]Department of Life Sciences, Pohang University of Science and Technology, Pohang, Gyeongbuk, 790-784, South Korea
[2]IBIO, Pohang University of Science and Technology, Pohang, Gyeongbuk, 790-784, South Korea
[3]ITCE, Pohang University of Science and Technology, Pohang, Gyeongbuk, 790-784, South Korea

C. elegans aging is regulated by a variety of cellular signaling pathways, including insulin/IGF-1 signaling (IIS). Many individual components that regulate aging in IIS have been identified, including DAF-2/insulin/IGF-1 receptor, DAF-18/PTEN phosphatase and DAF-16/FOXO. However, how protein-protein interactions among IIS components contribute to aging regulation is incompletely understood. Here, we focused on the roles of proteins containing PDZ domains, which act as scaffolds to mediate protein-protein interactions. To identify PDZ proteins that mediate lifespan regulation via IIS, we performed an RNAi lifespan screen by knocking down 49 genes that encode PDZ domain-containing proteins. Among them, genetic inhibition of *kin-4*, a homolog of a mammalian MAST family kinase, significantly decreased the longevity of *daf-2*/insulin/IGF-1 receptor mutants while having a small effect on that of wild type. To identify interaction partners of KIN-4, we performed a yeast two-hybrid screen using the PDZ domain of KIN-4 as a bait. Among 21 prey proteins we recovered so far, we found that DAF-18/PTEN bound to KIN-4. We will confirm the physical and genetic interaction between DAF-18 and KIN-4, and will further determine the functional significance of this interaction. This study may yield insights into understanding how protein-protein interactions via PDZ domains contribute to aging regulation by IIS.

Contact: swa.alice@gmail.com

A microRNA hormone in insulin/IGF signaling regulates lifespan across tissues

Yidong Shen, Renate Einrich, Adam Antebi

Max Planck Institute for Biology of Ageing, Germany

Ageing is modulated by conserved longevity signaling pathways, including insulin/IGF signaling (IIS). A significant feature of ageing is that various tissues exhibit coordinated decline in structure and function with ageing, which must be achieved by communication of the longevity signals across tissues. However, how these pathways communicate amongst the various tissues and what the molecular messenger is remain poorly studied. In *C. elegans*, we discovered a conserved microRNA under the regulation of IIS. In addition to the function in its origin tissue, this microRNA also functions in a tissue non-autonomous manner, getting secreted into intestine and modulating intestinal DAF-16/FOXO by directly binding to its 3'UTR. The microRNA mutation increases *C. elegans* lifespan and enhances age-related phenotypes, including mid-life mobility, by activating DAF-16/FOXO. In mammals, this microRNA is also able to modulate oxidative stress cell non-autonomously, with its targeting against FOXO well conserved. Our results therefore discovered a microRNA hormone in IIS, coordinating the ageing process across tissues.

Contact: Yidong.Shen@age.mpg.de

Deficiency of uracil exclusion genes and 5-fluoro-2'-deoxyuridine treatment increase lifespan of the short-lived AP endonuclease mutant worm

Yuichi Kato, Qiu-Mei Zhang-Akiyama

Kyoto University, Japan

Apurinic/apyrimidinic (AP) sites are the major DNA lesions generated even under normal conditions. Because they inhibit DNA replication and transcription, repair of AP sites is essential for all organisms. AP endonucleases are ubiquitous enzymes that play an important role in the repair of AP sites. They initiate the repair process based on DNA resynthesis by recognizing AP sites and cleaving the DNA strand at the sites. Function of AP endonucleases has been studied well by *in vitro* experiments. However, it remains to be elucidated how AP endonucleases function in multicellular organisms.

EXO-3 is an AP endonuclease in *C. elegans*. In order to determine whether AP endonucleases contribute to longevity, we analyzed phenotypes of the *exo-3* mutant worm. We found that deletion of the *exo-3* gene caused shortened lifespan in a *ung-1*-dependent manner. UNG-1 is a uracil DNA glycosylase that eliminates uracil in DNA to generate AP site. This result suggested that main cause of AP sites affecting lifespan was removal of uracil by UNG-1. Knockdown of the *dut-1* gene and 5-fluoro-2'-deoxyuridine (FUdR) treatment also rescued shortened lifespan of the *exo-3* mutant. DUT-1 is an enzyme that hydrolyzes dUTP to prevent from its misincorporation into DNA. This result suggested that uracil metabolism and/or regulation of cell division also affected longevity of the *exo-3* mutant. In addition, it is possible that FUdR has both positive and negative effects on lifespan in *C. elegans*.

C. elegans possesses another AP endonuclease, APN-1, but no *apn-1* mutant strain suitable for our experiment has been isolated yet. To elucidate the role of APN-1, we are currently trying to generate an *apn-1* deletion mutant using TALENs and the CRISPR/Cas9 system. This study would be valuable for understanding how AP endonucleases function in multicellular organisms.

Contact: yuichi@kingyo.zool.kyoto-u.ac.jp

Lifespan-extending effect of the rare sugar D-psicose on *Caenorhabditis elegans*

Masashi Sato[1], Hirofumi Sakoguchi[1], Tomoya Shintani[2], Kazuhiro Okuma[2], Ken Izumori[3]

[1]Department of Applied Biological Science, Kagawa University, Japan
[2]Matsutani Chemical Industry Co. LTD, Japan
[3]Rare Sugar Research Center, Kagawa University, Japan

Calorie-restricted diets are known to extend lifespans of model animals, including nematodes, flies, mice and monkeys. It is believed that calorie-restricted diets delay the onset of aging-associated diseases such as diabetes, cancer and Alzheimer's disease, and also prolong human longevity. However, in reality, it is difficult to continue calorie-restricted diets for a long period of time. Therefore, the development of calorie restriction mimetics (CRMs) that have similar effects as calorie-restricted diets is desired. Our preliminary study showed that the rare sugar D-psicose extended the lifespan of *Caenorhabditis elegans*. The findings suggest that D-psicose, a stereoisomer of D-fructose, serves as a CRM in *C. elegans*. The extension of *C. elegans* lifespan under calorie-restricted diet conditions is thought to be dependent on the induction of oxidative stress-related proteins, including Cu/Zn-superoxide dismutase (SOD), Mn-SOD and catalase regulated by the insulin/IGF signaling pathway. We aimed to elucidate the molecular mechanism of the lifespan extension by D-psicose. Wild-type *C. elegans* N2 was used in this study. Treatment of worms with 28 mM D-psicose increased mean lifespan of N2 by 20% (control: 20.9 days; D-psicose: 25.1 days). Using Quantitative real-time RT-PCR, we found that the mRNA expression levels of mitochondrial Mn-SOD (*sod-3*), cytosolic catalase (*ctl-1*) and peroxisomal catalase (*ctl-2*) were enhanced by 1.4, 1.5 and 1.6-fold, respectively, after treatment of D-psicose (Fig. 2). In contrast, expression of cytosolic Cu/Zn-SOD (*sod-5*) was unaffected by 28mM D-psicose treatment. Total SOD and catalase enzyme activities in N2 with 28 mM D-psicose treatment were increased by 1.4 and 2.1-fold, respectively. D-psicose was thought to extend the lifespan of *C. elegans* by increasing the oxidative-stress resistance of the nematode. These results indicate that D-psicose is a candidate calorie restriction mimetic.

Contact: sato@ag.kagawa-u.ac.jp

Aging in Space

Yoko Honda[1], Akira Higashibata[2], Yohei Matsunaga[3], Yukiko Yonezawa[3], Tsuyoshi Kawano[3], Atsushi Higashitani[4], Kana Kuriyama[5], Toru Shimazu[5], Masashi Tanaka[1], Nathaniel J Szewczyk[6,7], Noriaki Ishioka[2], Shuji Honda[1]

[1]Tokyo Metropolitan Institute of Gerontology, Japan
[2]Japan Aerospace Exploration Agency, Japan
[3]Department of Bioresource Sciences, Faculty of Agriculture, Japan
[4]Graduate School of Life Science, Tohoku University, Japan
[5]Japan Space Forum, Japan
[6]MRC, UK
[7]Arthritis Research UK Centre for Musculoskeletal Ageing Research, University of Nottingham, UK

Lifespan and aging rate in metazoans are implicated to be determined in response to environmental conditions. In fact, they are influenced by temperature, oxygen, pheromone, and food intake. Moreover, perturbation of sensory perception or signaling of mechanical, chemical, osmotic, thermal, or cold stimuli changes the lifespan of *Caenorhabditis elegans*. Microgravity has been shown to induce several physiological or pathological changes including disturbance of the sense of equilibrium and loss of muscle and bone mass, mainly from the observations in spaceflight. However, how microgravitational space environments affect aging is not well understood. We examined an aging marker in space-flown *C. elegans* and explored the involvement of the genes whose expression was changed during spaceflight, in the control of lifespan. We observed that, in *C. elegans*, spaceflight suppressed the formation of transgenically expressed polyglutamine aggregates, which normally accumulate with increasing age. Furthermore, the inactivation of each of eight genes that were down-regulated in space extended lifespan on the ground. These genes encode proteins that are likely related to neuronal or endocrine signaling. We also found that hypergravity shortened the lifespan in wild type. These results suggest that aging in *C. elegans* is controlled through neuronal and endocrine response to gravitational conditions.

Contact: hondas@center.tmig.or.jp

Systematic regulation for temperature experience-dependent cold habituation

Akane Ohta, Tomoyo Ujisawa, Misato Uda, Tomohiro Ishiwari, Makoto Ioroi, Natsune Takagaki, Mai Kimura, Satoru Sonoda, Atsushi Kuhara

Konan Univ, Japan

Temperature is essential information for survival and proliferation of animal. We are using cold tolerance as a model for studying temperature sensation and memory. 15°C-cultivated animals can survive at 2°C, however, 20°C- or 25°C-cultivated animals can not survive after cold shock. Here we show that a light and pheromone-sensing neuron (ASJ) regulates cold tolerance through insulin signaling. Mutants impaired with ASJ phototransduction, including trimeric G protein and cGMP-gated channel, showed abnormal cold tolerance. Ca^{2+} imaging demonstrated that ASJ responds to temperature. Temperature-responsiveness of ASJ was also detected in *snb-1* mutant impairing synaptic transmission, suggesting that ASJ itself plays a role as thermosensory neuron. Previous study for dauer formation indicated that insulin DAF-28 and INS-6 are secreted by ASJ. Genetic epistasis analysis suggested that DAF-28 and INS-6 regulate cold tolerance signaling redundantly. Tissue specific rescue experiments indicated that insulin receptor/DAF-2 receives insulin at intestine and neurons in cold tolerance. Altogether, our study exhibited a systematic regulation for controlling cold habituation in living animal.

Recently, we are studying temperature memory by using temperature shift assay in cold habituation. To understand the time required for animals to establish cold tolerance, we shifted the temperature of wild-type adult animals. We found that only three hours after temperature shift from 25 to 15°C cold tolerance was newly established. *crh-1*/CREB mutant delayed reconfiguration of cold tolerance in temperature shift assay. So far, this abnormality was rescued by expressing *crh-1*cDNA in ASJ sensoryneuron.

Contact: aohta@center.konan-u.ac.jp

Natural variation modifies temperature responses and memory

Misaki Okahata, Shoko Furukawa, Akane Ohta, Atsushi Kuhara

Konan Univ, Japan

Temperature is one of the critical information for living and proliferation of organisms on the earth. Animals therefore have adapted to environment temperature changes by accumulation of natural mutations. We are studying about natural variation of genes involved in temperature habituation of *C. elegans*. Most of 20 degree-cultivated wild-type N2 animals isolated from Bristol can not survive at 2 degree, while 15 or 17 degree-cultivated N2 animals can survive at 2 degree. We found that variety of wild-type strains isolated from various area showed different cold habituation phenotypes. CB4854, CB4855, CB4858, RC301 and KR314 can not survive at 2 degree after cultivation at 15 or 17 degree, whereas N2, CB4853, CB4857 and CB4932 can survive at 2 degree after cultivation at 17 degree. Since CB4854 showed the strongest decrement of cold tolerance, we focused on CB4854 strains. To identify the genes involved in the natural variations, we utilized deep DNA sequencer and classical genetic mapping. By the classical linkage mapping and three factor crosses, responsible gene polymorphism for the cold habituation was mapped on chromosome X. Deep DNA sequencer analysis showed that there are eighty thousand DNA polymorphisms including SNPs between CB4854 and N2. By the SNPs analysis, we have mapped responsible polymorphism onto -1.6~ -1.5cM region on chromosome X, in which six genes are predicted. We therefore measured cold habituation phenotype of the mutants impairing these genes, and we found that *vps-52* mutant showed abnormal cold habituation, which is similar phenotype as CB4854. *vps-52* gene encodes Golgi-associated retrograde protein (GARP) that is mainly expressed in neurons. CB4854 has a nucleotide insertion in intron near splicing site. Now, we are going to do rescue experiment and quantification of expression levels of *vps-52* in natural variation strains. Recently, we are studying temperature memory by using temperature shift assay in cold habituation. In N2 animals, only three hours after temperature shift from 25 to 15°C, cold tolerance was newly established. We found that natural variation CB4857 rapidly memorizes new temperature. We are planning to identify the responsible genes for the memory variations by using deep DNA sequencer.

Contact: ss162024@center.konan-u.ac.jp

Artificial evolution and screening for mutants defective in temperature habituation

Yukari Kinoshita, Misato Uda, Akane Ohta, Atsushi Kuhara

Konan Univ, Japan

Temperature response is important for animal, and animals have habituation mechanisms to environmental temperature changes. We are utilizing temperature experience-dependent cold tolerance of nematode, *C. elegans*, as a model for studying temperature sensation and memory. After cultivation at 20°C, wild-type were destroyed by cold stimuli. In contrast, after cultivation at 15°C, most of animals can survive. To isolate genes involved in the cold tolerance, we are using two approaches, (1) Artificial evolution and (2) EMS-mutagenesis.

(1) *C. elegans* has strong advantage for artificial evolution analysis, since life cycle is short and strains can be preserved at -80°C. *C. elegans* has strong advantage for artificial evolution analysis. We are maintaining *C. elegans* at 15 or 23 degrees for gradually accumulating spontaneous mutations, and frozen-stocks of animals were made at every generation. We so far obtained about 110 generations animals, and cold tolerance phenotypes have been gradually changed. We are planning to decode the genomes of frozen-animals by using deep DNA sequencer.

(2) We isolated several mutants defective in cold tolerance from two thousands genomes screening by EMS mutagenesis. KHR1 showed significant decrement of cold habituation. To isolate responsible genes for KHR1, we utilized single nucleotide polymorphism (SNP) in wild-type strains isolated from different areas, Bristol and Hawaii. So far, Responsible gene of cold tolerance in KHR1 strain was mapped on chromosome X -7.48 cM~-0.83 cM. We are now analyzing detailed-mapping position by using classical genetics and deep DNA sequencer.

Contact: m1323002@center.konan-u.ac.jp

Molecular and tissue networks underlying cold tolerance

Satoru Sonoda, Saki Tanaka, Akane Ohta, Atsushi Kuhara

Konan Univ, Japan

Temperature is a critical environmental stimulus and causes biochemical changes. Animals including nematode *C. elegans* have habituation mechanisms to environmental temperature changes. For example, 20 degree-cultivated *C. elegans* were destroyed by cold stimuli, while 15 degree-cultivated animals can survive under cold condition. We recently found that trimeric G-protein-coupled temperature signaling in ASJ releases insulin, which regulates cold habituation by expressing genes through FOXO transcriptional factor /DAF-16 in intestine (Ohta, Ujisawa, et al., under revision). To isolate downstream molecules of insulin signaling for cold habituation, we performed DNA microarray analysis comparing between wild-type and insulin signaling mutant. We found that 15 genes, such as protein phosphatase (PP1)/ *gsp-3*, *gsp-4*, *fem-3*, laminin/*lam-3*, were required for cold habituation. GSP-3 encoding protein phosphatase1 (PP1) is involved in sperm development and motility, and GSP-4 and FEM-3 are also involved in sperm or male development. Mutants defective in these genes showed abnormal cold habituation, suggesting that sperm is involved in cold habituation.In order to investigate how sperm affects cold habituation, and whether sperm is interact with the known-signaling in cold habituation, such as ASJ temperature signaling and insulin pathway in intestine, we introduced genetic epistasis analysis. Abnormal cold habituation of sperm mutant was not suppressed by the mutation in insulin signaling. Additionally, expression levels of sperm genes are significantly altered in daf-2/insulin receptor mutant. These suggest that sperm affects cold habituation in downstream of insulin signaling of intestine. We unexpectedly found that abnormal cold habituation in *gsp-4* was strongly suppressed by mutations in the temperature signaling in ASJ, hypothesizing that sperm affects temperature signaling in ASJ by using any feedback system such as secretly signaling. To investigate what molecules are reacquired for these systems, we focused on hormonal signaling. Our DNA microarray analysis identified that the expression levels of 58 genes encoding nuclear hormone receptor (nhr) were significantly changed by temperature stimuli. We found that the mutants defective in *nhr-88* and *nhr-114* showed abnormal cold tolerance. We are now using genetic epistasis analysis and calcium imaging of ASJ to investigate whether these mutations affect temperature signaling in ASJ.

Contact: d1422003@center.konan-u.ac.jp

Oxidative stress suppresses locomotion and pumping motion in *Caenorhabditis elegans*

Michiyo Suzuki[1], Tetsuya Sakashita[1], Yuya Hattori[2], Yasuhiko Kobayashi[1]

[1]Microbeam Radiation Biology Group, Japan Atomic Energy Agency, Japan
[2]Research Group for Radiation Effect Analysis, Japan Atomic Energy Agency, Japan

The nematode *Caenorhabditis elegans* is a good *in vivo* model system for the examination of radiobiological effects. Using *C. elegans*, we recently investigated the effects of ionizing radiation on locomotion (crawling) and found that whole body irradiation reduced locomotion, though the mechanisms are not clear [1]. It is well known that free radicals such as OH^- and H^- are produced following exposure of ionizing radiation. The reactions of free radicals cause the production of oxidative stress including hydrogen peroxide (H_2O_2). In *C. elegans*, it has been reported that exposure to H_2O_2 induces a decrease in the tap response [2]. Oxidative stress are known as important factors of aging, and may relate to the decrease of motility observed in the aging process. We therefore explored the potential effectiveness of H_2O_2 in the suppression of *C. elegans*'s locomotion. Wild-type animals were exposed to graded doses of H_2O_2 and the locomotory rate was evaluated. The locomotory rate was significantly decreased in H_2O_2-exposed animals, in which the dose response was similar to that in the irradiated animals [1]. To investigate the radiation effects and the involvement of oxidative stress on other types of movement, we subsequently focused on the pumping motion (chewing and swallowing) which is a rapid periodic motion using the pharyngeal muscles. As a result, the proportion of the pumping-motion arrest significantly increased after whole body irradiation and the pumping-motion arrest was restored within several hours. Furthermore, the response in H_2O_2-exposed animals was similar to that in the irradiated animals. These results support the possibility that radiation-induced suppression of both locomotion and pumping motion in *C. elegans* was caused by radiation-produced H_2O_2. In this presentation, we will give an outline of the radiation effects on *C. elegans* and discuss the involvement of the radiation-induced oxidative stress in the phenomena.

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Contact: suzuki.michiyo@jaea.go.jp

Stress-enhanced spermatogenesis and sperm quality in aged male *C. elegans*

Wan-Yi Chou, Ying-Hue Lee

Institute of Molecular Biology, Academia Sinica, Taiwan

The main task for the living organisms is to survive and reproduce. When encountering adverse environment like famine, to survive, living organisms have evolved numerous mechanisms to re-adjust their physiology, such as diapause. Scientists are highly interested in these stress-induced physiological changes, and great effort has been devoted to uncover the mechanisms underlying these changes. We have used adult male *C. elegans* as a model to investigate how the starvation stress affects the physiology of male worms. We previously found that starvation stress can stimulate ribosome biogenesis and that DAF-2 insulin/IGF-1 receptor signaling is involved in the process. Our subsequent studies reveal that a short-term starvation stress at the young age can significantly improve vitality of the aged male *C. elegans*, when compared to those unstressed male worms of the same age. To further study the effects of starvation stress on the production and quality of sperms in male worms, we examined the spermatogenesis activity and fertility of the aged male worms upon receiving a starvation stress at the earlier age. We found that the stressed aged males have maintained their reproductive activity, while the un-stressed control males lose the activity quickly and no longer reproduce at the same ages examined. Our results show that a short-term starvation stress, particularly at young age, can preserve the number and quality of sperms in the aged male worms, as evidenced by the significantly increased activity of spermatogenesis to produce sperms, enhanced expression of semen factors for sperm activation and the preserved ability of sperm to be activated for fertilization.

Contact: yinghue@gate.sinica.edu.tw

Effect of Repeated Starvation on Fat Content

Shinya Matsumoto[1], Kosuke Kato[2], Yasuki Matsumura[2], Nao Sato[1], Yukari Yamamoto[1], Akari Sawanaga[1], Midori Ogai[1], Haruka Saito[1]

[1]Kyoto Women's University, Japan
[2]Kyoto University, Japan

It is widely considered the regular feeding is important to maintain our health. However, irregularity in feeding rhythm such as late supper and skipping meals are almost impossible to avoid in our busy modern life. If and how such irregular feeding pattern can affect our life is not always clear. The aim of the study is to evaluate the effect of irregular feeding rhythm on the various physiological aspects using *C.elegans* as a model organism.

Feeding pattern of human and worm is different; while human eat intermittently, worms eat continuously. Therefore, the repeated starvation was introduced as the model of irregular feeding rhythm, because the regimen disturbs normal feeding pattern of worms.

A cycle of 6 hr starvation - 18 hr feeding was introduced twice to adult worms, and its effect on fat accumulation, life span, mobility and fecundity was analyzed. Although no obvious effect was observed in fecundity and mobility, fat content, assessed by Nile Red staining, was shown to increase in worms that experienced starvation compared to that of fed worms.

The increment of fat content in starvation-experienced worms may have resulted by the fat metabolic shift toward accumulation rather than consumption to prepare for the food shortage.

Contact: matumots@kyoto-wu.ac.jp

Age- and nutrition-associated change in the morphology and population of intestinal granules in *Caenorhabditis elegans*

Hirohisa Shiraishi[1], Maya Kamikawara[1], Seijun Suzuki[1], Eri Matsuura[2], Katsutoshi Ogasawara[2], Kinji Ishida[2], Koujiro Tohyama[2], Kenji Nishikori[1], Takahiro Tanji[1], Ayako Ohashi-Kobayashi[1]

[1]School of Pharmacy, Iwate Medical University, Japan
[2]The Center for Electron Microscopy and Bio-Imaging Research, Iwate Medical University, Japan

We have reported that a major subset of *C. elegans* intestinal granules, which is observed from late larval to early adult stage, vanishes in an age- and starvation-dependent manner (Nishikori *et al.*, *C. elegans* Topic Meeting 2012, Madison, WI). Such a granule is a non-acidic organelle to which HAF-4 and HAF-9, ABC transporters homologous to a mammalian lysosomal peptide transporter TAP-like (ABCB9), co-localize. The deletion mutants for *haf-4* and *haf-9* exhibit the loss of the HAF-4/HAF-9-positive granules and some physiological defects such as slow growth and reduced brood size, suggesting that the granules play important roles in the storage and supply of nutritional components during reproductive period. Hence we named the organelle HEBE (HAF-4/HAF-9-enriched body evanescent with age) after the goddess of youth and the cupbearer in Greek mythology. Besides HEBE granules, several types of granules including autofluorescent acidic ones, birefringent ones and lipid droplets exist in the intestinal cells. We also found that lipid droplets are increasing with age instead of the reduction of HEBE granules.

Here we performed the transmission electron microscopic (TEM) observation of the ultrastructure of adult intestinal granules upon starvation and ageing. Characteristic granules were observed by 3-hr starvation, which seem to exhibit the breakup process of HEBE granules, and then HEBE granules almost disappeared by 5-hr starvation. Overnight starvation gave rise to semilunar-shaped membrane structures. Age-dependent alterations of morphology and population of intestinal granules were also observed: the emergence of putative collapsing HEBE granules and another double structures with sunny-side up egg-like appearance (day-3 to day-5 adult), and the increase in white granules containing some electron-dense material (day-5 to day-10 adult). These TEM images will propose the visual clue on the intracellular membrane dynamics in dietary condition and aging.

Contact: hshirais@iwate-med.ac.jp

Burkholderia pseudomallei suppresses *Caenorhabditis elegans* immunity by specific degradation of a GATA transcription factor

Song-Hua Lee[1], Rui-Rui Wong[1], Chui-Yoke Chin[1], Tian-Yeh Lim[1], Su- Anne Eng[1], Cin Kong[1], Nur Afifah Ijap[1], Ming-Seong Lau[1], Mei-Perng Lim[1], Yunn-Hwen Gan[2], Fang-Lian He[3], Man-Wah Tan[3], Sheila Nathan[1,4]

[1]School of Biosciences and Biotechnology, Faculty of Science and Technology, University Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia
[2]Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117597, Singapore
[3]Departments of Genetics and Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA 94305-5120, USA
[4]Malaysia Genome Institute, Jalan Bangi, 43000 Kajang, Selangor, Malaysia

The gram-negative soil bacteria *Burkholderia pseudomallei* is the causative agent of human and animal melioidosis. However, very little is known about the molecular mechanisms that underlie *B. pseudomallei* virulence and how the organism is able to interact with its host to elicit the clinical symptoms of melioidosis. To gain insights into the host defense responses to *B. pseudomallei* infection within an intact host, we employed a genome-wide transcriptome analysis on age-matched adult worms infected for 2, 4, 8 and 12 hours. Our analyses revealed that approximately 6% of the *C. elegans* genes were transcriptionally regulated when compared to uninfected animals. The genes modulated were both defence-specific genes as well as non-immune-related genes. Changes in expression of metabolic, longevity and stress response genes following *B. pseudomallei* infection have not been previously reported. An unexpected feature of the transcriptional response to *B. pseudomallei* was a progressive increase in the proportion of down-regulated genes, of which ELT-2 transcriptional targets were significantly enriched. ELT-2 is an intestinal GATA transcription factor with a conserved role in immune responses. We demonstrate that *B. pseudomallei* down-regulation of ELT-2 targets is associated with degradation of ELT-2 protein by the host ubiquitin-proteasome system. Degradation of ELT-2 requires the *B. pseudomallei* type III secretion system. Together, our studies using an intact host provide evidence for pathogen-mediated host immune suppression through the destruction of a host transcription factor.

Contact: songhua82@yahoo.com

Is mutation rate fitness-dependent? II. Fitness decay and mtDNA mutation rate in mutation accumulation lines of the Nematode worm *Caenorhabditis elegans*

Chikako Matsuba[1,2], Matthew Salomon[1,3], Ayush Saxena[1], Dejerianne G. Ostrow[1,4], Charles Baer[1,5]

[1]Department of Biology, University of Florida, USA
[2]Unidad Mixta de Investigacion en Biodiversidad, University of Oviedo, Spain
[3]Department of Molecular and Computational Biology, University of Southern California, USA
[4]Molecular Pathology, Children's Hospital Los Angeles, USA
[5]University of Florida Genetics Institute, USA

Disparate lines of evidence suggest that physiological condition may affect mutation rate, which in turn suggests that the mutation rate may depend on the underlying fitness of the organism. To investigate that possibility we chose five high-fitness and five low-fitness "first-order mutation accumulation" (1° MA) lines that had previously evolved under extremely relaxed selection (Ne≈1) for 250 generations subsequent to divergence from a common ancestor. 48 replicate "second order MA (2° MA) lines were initiated from each 1° MA progenitor and allowed to evolve under relaxed selection for an additional 150 generations. On average, the rate of decay of fitness of the 2° MA lines did not differ between high-fitness and low-fitness lines, but several non-significant trends are consistent with low-fitness lines having greater mutation rate. Re-sequencing of the genomes of 4-5 2° MA lines and their 1° MA progenitors revealed that large deletions in the mtDNA genome of low fitness line may partially explain the observed fitness patterns. The 1kbp and 3kbp deletions presented respectively in the low fitness 1° MA l progenitors 504 and 508 with the most steep fitness decay in 2° MA process. The degree of deletion heteroplasmy was 0.78 and 0.71 for each line. All the 2° MA lines of 504 and 508 shared the large deletion from their progenitor except for 504.10. Additionally, three independent novel large deletions were found in their 2° MA lines (4kbp in 504.10, 3kbp in 508.34 and 1kbp in 508.35), however there are no evidence of large deletions in the rest of the 2° MA lines. The degree of deletion heteroplasmy was varied from 0.57 to 0.87 in 2° MA lines.

Contact: chikako.matsuba@live.com

Characterization of a *Caenorhabditis elegans* mutant confers selective bacterial resistance

Yi-Wei Chen[1], Po-Lin Chen[2], Wen-Chien Ko[1,2], Chang-Shi Chen[1,3]

[1]Institute of Basic Medical Sciences, College of Medicine, National Cheng Kung University, Tainan, Taiwan
[2]Department of Internal Medicine, National Cheng Kung University Hospital, Tainan, Taiwan
[3]Department of Biochemistry and Molecular Biology, College of Medicine, National Cheng Kung University, Tainan, Taiwan

Aeromonas dhakensis is a bacterial pathogen that causes gastrointestinal symptoms, bacteremia and soft tissue-necrotizing fasciitis in human. Studies have shown that several virulence factors of *A. dhakensis* are responsible for developing these diseases. However, the host factors required for the pathogenicity of *A. dhakensis* are still largely unknown. To discover such host factors, we applied a forward genetic screen in *C. elegans* for isolating mutants that confer resistance to *A. dhakensis* infection. A *C. elegans* mutant strain with significant resistance to *A. dhakensis* was isolated and named as YQ247. By standard genetic methods, we have determined that the mutation in YQ247 is recessive and is located in the region between -9.57 to -4.93 cM on chromosome I. Interestingly, YQ247 is as sensitive as the wild-type N2 to the general stresses, the heavy metal cupper and the oxidative stressor hydrogen peroxide. These data suggested that this mutation allele in YQ247 is specific to bacterial infection. To confirm the resistant specificity of YQ247 to *A. dhakensis*, we also tested YQ247 animals to other bacterial pathogens, including Enterohemorrhagic *Escherichia coli*, *Salmonella Typhimurium*, *Enterococcus faecalis*, and *Staphylococcus aureus*. Of note, YQ247 animals are only resistant to *S. aureus*, besides *A. dhakensis*. Taken all together, our current data suggested that the mutation allele of YQ247 may play roles in the pathogenesis of a common virulence factor of *A. dhakensis* and *S. aureus*.

Contact: yiweichen515@gmail.com

Genome-Wide Identification of Enterohemorrhagic *E. coli* Virulence-Related Genes in *Caenorhabditis elegans*

Cheng-Ju Kuo[1], Hao-Chieh Chiu[2], Chang-Shi Chen[1]

[1]Department of Biochemistry and Molecular Biology, and the Institute of Basic Medical Sciences, National Cheng Kung University, Tainan, Taiwan
[2]Department of Clinical Laboratory Sciences and Medical Biotechnology, National Taiwan University, Taipei, Taiwan

Escherichia coli O157:H7, a member of pathogenic *E. coli*, known as enterohemorrhaghic *E. coli* (EHEC), colonizes the gastrointestinal tract and causes life-threatening infections worldwide. Nowadays, there is no appropriate treatment for EHEC infection due to adverse effects in antibiotic therapy. Therefore, highly effective treatments for prevention and control of EHEC infection are essential. Several virulence factors expressed by *E. coli* O157:H7 contributes to its pathogenicity. *E. coli* O157:H7 produces the Verocytoxins also known as the Shiga-like toxins which are responsible for hemolytic-uremic syndrome (HUS) and hemorrhagic colitis (HC). One of the characteristics of EHEC infection is forming attaching and effacing (A/E) lesions on the mucosal epithelium at the host intestinal cells. The bacterial genes required for formation of A/E lesions are encoded by the locus of enterocyte effacement (LEE), which is a chromosomally encoded pathogenicity islasnd (PAI). Although several virulence factors and mechanism of EHEC had been reported, the pathogenesis of EHEC *in vivo* is still unclear. To these ends, we established a genome-wide transposon mutagenesis library to screen for the bacterial factors that are required for the pathogenesis of EHEC infection in *C. elegans*. From our genetic screen, we discovered the genes involved in the lipopolysaccharide (LPS) and cell exterior constituents biosynthesis pathway that play roles in the pathogenesis of EHEC in *C. elegans*. Moreover, we also identified several unknown genes that are required for EHEC infection. These genes may encode potential uncharacterized EHEC virulence factors. We envision that through investigating the pathogenic mechanisms of these uncharacterized EHEC virulence factors may shed the light onto the development of the novel therapeutics to EHEC infection.

Contact: noturtlekuo@gmail.com

The bacterial metabolite violacein affects *C. elegans* development, fertility and behavior

Kyoung-hye Yoon[1,2], Jin I Lee[2]

[1]UNIST, Republic of Korea
[2]Yonsei University, Republic of Korea

Bacteriovorous nematodes and bacteria likely exist in a constant evolutionary tug of war, defending themselves against the other. Bacteria often produce secondary metabolites that can discourage predation, against which nematodes fight back through their innate immune system. Violacein is a purple bis-indole derivative produced by various genera of soil and water bacteria, and has garnered interest due to its many therapeutic properties such as its effect against cancer. We sought to utilize the well-studied genetics of *C. elegans* to study the mechanism of violacein. We found that purified violacein as well as violacein-expressing OP50 causes developmental delay and decreased fecundity in *C. elegans*. We found several mutants that synthetically exacerbate the developmental delay phenotype, which may point to a molecular mechanism of violacein. *C. elegans* exposed to violacein also showed bacterial avoidance behavior, which may imply the activation of their innate immune system. Currently, we are testing various candidate mutants to further elucidate the molecular target of violacein. In addition, we hope to gain insight into the ecological perspective of how bacteria and bacteriovorous predators compete in their natural environment.

Contact: kyounghyeyoon@yahoo.com

C. elegans has two F_oF_1-ATPase inhibitors that are localized in different cellular compartments

Laura Paulette Fernández-Cárdenas[1], Rosa Estela Navarro-González[1], Marietta Tuena de Gómez Puyou[2]

[1]Cell and Developmental Biology, IFC, UNAM, Mexico, D.F.
[2]Biochemistry and Structural Biology, IFC, UNAM, Mexico, D.F.

The ATPase inhibitor protein (IF1) regulates the hydrolysis activity of mitochondrial F_oF_1-ATPase to avoid ATP depletion. In mammals, IF1 silencing affects multiple mitochondrial parameters such as mitochondrial morphology, cristae density, F_oF_1-ATP synthase dimerization, cell proliferation and death. We are studying the expression and function of the two *C. elegans* IF1 homologs, *mai-1* and *mai-2*. Both proteins inhibit yeast F_oF_1-ATPase *in vitro* but differ in yeast cell localization. MAI-2 is transported to yeast mitochondria while MAI-1 remains diffused in the cytoplasm, suggesting that MAI-2 is *C. elegans* IF1. To study their expression in *C. elegans*, we generated transgenic animals that carry the constructs *pmai-2::mai-2::gfp-mai-2-3'utr* (*mai-2::gfp*) or *pmai-1::mCherry::mai-1-3´utr* (*mCherry-mai-1*). We found that *mai-2::gfp* is expressed in all tissues during all embryonic and larval stages; where it appears to be associated to the mitochondrial network. Unexpectedly, *mCherry::mai-1* expression is not associated to the mitochondrial network and instead appears diffused in the cytoplasm. While *mai-2::gfp* is expressed ubiquitously, *mCherry::mai-1* was only observed in some cells of the nervous system, hypodermis and intestine. We found that *mai-2* (RNAi) animals had normal growth and fertility while their F_oF_1-ATP synthase dimerization, oxygen consumption and mitochondrial morphology do not seem to be affected. We have generated a loss of function *mai-2* mutant by CRISPR/Cas9 to continue the study of *mai-2* function in this nematode.

Contact: paulette_fernandez@hotmail.com

C. elegans W02B12.15b, a homologous gene of human *cisd1*, is involved in oxygen consumption and mitochondria fission

Kuei-Ching Hsiung[1], Kuan-yu Liu[1], Ting-Fen Tsai[2], Sze-Cheng John Lo[1],

[1]Graduate Institute of Biomedical Sciences, Chang Gung University, Taiwan
[2]National Yang-Ming University, Taiwan

Genes containing CDGSH iron-sulfur domains (CISDs) are evolutionally conserved. There are three *Cisd* genes in mammalian, *Cisd1*, *Cisd2* and *Cisd3*. CISD1 is localized to the mitochondrial outer membrane and has the ability to bind redox-active [2Fe-2S] cluster and plays roles in oxidative regulation. *Cisd2* is the causative gene for Wolfram syndrome 2, while the *cisd3* function has not been characterized yet. Based on amino acid sequence comparison and the phylogenetic tree analysis, W02B12.15b of *C. elegans* is the only CDGSH-containing gene and closely related to the mice *Cisd1*. All homologous genes share a common feature of a unique 39 amino acid CDGSH domain, thus W02B12.15b is designated as a *Cecisd* gene. A deletion mutant of W02B12.15b (*tm4993*) showed no obvious phenotypes such as life span variation or hypersensitive to stress. The expression of *Cecisd* was found primarily in the mitochondrial fraction by Western blotting and the fusion protein of Cecisd::GFP showed a mitochondria distribution pattern. The oxygen consumption rate (OCR) of *tm4993* was higher than that of wild type. The OCR represents the mitochondrial activity, which can be influenced by ATP synthesis or reactive oxygen species stress. We also found when the *Cecisd* expression was knockdown, the mitochondrial morphology was preferentially in a fusion state. However, how the *Cecisd* gene plays roles in mitochondria function is required further investigations.

Contact: ecurb224@hotmail.com

Reduction of lipid droplets in the W02B12.15 mutant is AMPK dependent

Kuan-Yu Liu[1], Kuei-Ching Hsiung[1], Ting-Fen Tsai[2], Sze-Cheng John Lo[1]

[1]Graduate Institute of Biomedical Sciences, Chang Gung University, Taiwan
[2]Department of Life Sciences and Institute of Genome Sciences, National Yang-Ming University, Taiwan

Thiazolidinedione (TZD) is a traditional drug used to treat type II diabetes. Drug in this class is thought function on peroxisome proliferator-activated receptor γ (PPAR γ). However, TZD has also been demonstrated to bind to a mitochondria outer membrane protein called mitoNEET. Although the function of mitoNEET is suggested to regulate mitochondrial iron homeostasis, the mechanism is unknown. Amino acid sequence alignment revealed that *C. elegans* W02B12.15 is the mice mitoNEET homolog gene. A deletion mutant of W02B12.15(tm4993) exhibited reduction of lipid droplets. Biochemical analyses showed a less amount of adenosine triphosphate (ATP) production in tm4993 than in N2 worms. Western blot analysis showed the activity of AMP-activated protein kinase (AMPK) was higher in tm4993 mutants than in wild type. Since the phenotype of lipid droplets reduction in the tm4993 mutant could be rescued by function loss of *akk-2* (a subunit of AMPK), it suggested that the mutation of mitoNEET in worms results in the lower ratio of ATP/AMP production and following by the activation of AMPK which in turn increased lipid hydrolysis.

Contact: kevin1990713@hotmail.com

Dietary methionine restriction in axenic medium decreases both methionine and *S*-adenosyl-L-methionine levels.

Rieko Yamauchi[1], Keiko Hirota[2], Zhao Xu[1], Kei Iizuka[2], Hiroaki Daitoku[2], Koichiro Kato[2], Akiyoshi Fukamizu[2]

[1]College of Agrobiological Resource Sciences, University of Tsukuba, Japan
[2]Life Science Center, Tsukuba Advanced Research Alliance, University of Tsukuba, Japan

Methionine is an essential amino acid. It is supplied through four distinct pathways: the absorption of dietary methionine; the methylation of homocysteine, catalyzed by 5-methyltetrahydrofolate-homocysteine methyltransferase-1; the methionine salvage pathway; and the degradation/recycling of cellular proteins. Methionine is converted to *S*-adenosyl-L-methionine (SAM), which serves as a methyl donor for many biological methylation reactions by SAM synthetases. In this study, we investigate the effect of dietary methionine on both methionine and SAM levels in *Caenorhabditis elegans*. To determine the effect of dietary supplementation of methionine on methionine and SAM levels in worms, we culture them under the absence or presence of methionine in a chemically defined liquid medium. The L1 larvae were grown to adulthood on agar plates containing *Escherichia coli*, and then the adult worms were maintained in a methionine-restricted liquid medium. Methionine and SAM levels in the worms were subsequently measured using HPLC and LC-MS/MS, respectively. Methionine restriction led to decrease in both methionine and SAM levels. These data suggest that dietary supplementation with methionine is essential for the maintenance of both methionine and SAM levels in adult nematode worms.

Contact: s1110735@u.tsukuba.ac.jp

Characterization of intestinal amino acid transporter AAT-6 in *C. elegans*

Daisuke Murata[1], Ryuichi Ohgaki[1], Kohei Hagiwara[1], Kanokporn Phetdee[1,2], Shushi Nagamori[1], Ling Wei[1], Taku Hirata[2], Kazuya Nomura[3], Shohei Mitani[4], Yoshikatsu Kanai[1,2]

[1]Division of Bio-system Pharmacology, Osaka University Graduate School of Medicine, Japan
[2]Department of Pharmacology and Toxicology, Kyorin University School of Medicine, Japan
[3]Department of Biological Sciences, Kyushu University, Japan
[4]Department of Physiology, Tokyo Women''s Medical University School of Medicine, Japan

Light subunits of heteromeric amino acid transporters, the members of mammalian SLC7 family, form heterodimers with type II transmembrane glycoproteins (heavy subunits) of the SLC3 family to localize at the plasma membrane and transport amino acids into cells. It has been known that the nematode *C. elegans* has nine light homologs (AAT-1 to AAT-9) and two heavy homologs (ATGP-1 and ATGP-2). We here characterize the amino acid transporter AAT-6 and show that AAT-6 plays an important role in *C. elegans* physiology. AAT-6 transported neutral and basic amino acids when co-expressed with ATGP-1 in *Xenopus* oocytes. In support of this, AAT-6 was specifically expressed in the intestine and co-localized with ATGP-1, but not ATGP-2, at the apical membrane in *C. elegans*. Co-immunoprecipitation analysis revealed that AAT-6 physically interacted with ATGP-1.

Similarly to AAT-6, a *C. elegans* peptide transporter PEPT-1 is also known to be exclusively expressed in the intestinal apical membrane, and absorbs amino acids in the form of di- and tri-peptides from the intestinal lumen. To examine the impact of simultaneous loss of AAT-6 and PEPT-1 on *C. elegans* physiology, we carried out *pept-1* knockdown by RNAi in the *aat-6* mutants. Interestingly, knockdown of *pept-1* caused more than 95% reduction in the number of progeny in *aat-6* mutants. Furthermore, the double mutants of *aat-6* and *pept-1* arrested development at the L1 stage. These results indicate that amino acid absorption mediated by AAT-6 is crucial for post-embryonic development and progeny production in the absence of PEPT-1.

We next examined whether ATGP-1 is involved in the membrane sorting of AAT-6 in *C. elegans*. Unexpectedly, ATGP-1 seems not to be essential for membrane targeting of AAT-6 because AAT-6 still localized to the apical membrane even in the absence of ATGP-1. We previously identified NRFL-1, a *C. elegans* homolog of NHERF (Na^+/H^+ exchanger regulatory factor) family PDZ scaffold proteins, as a novel binding partner of AAT-6. Absence of NRFL-1 also did not affect the membrane sorting of AAT-6, although NRFL-1, but not ATGP-1, is involved in stabilization of AAT-6 on the membrane in aged worms. These results suggest that there may be unknown factors involved in the membrane targeting of AAT-6 or fundamental differences in the mechanism for membrane trafficking of AAT-6 between vertebrates and *C. elegans*.

Contact: murata@pharma1.med.osaka-u.ac.jp

Natural compounds as probes to dissect lysosome homeostasis and functions based on a *Caenorhabditis elegans* screen

Yang Li[1], Meng Xu[1], Guihua Tang[2], Xiaojiang Hao[2], Chonglin Yang[1]

[1]Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, China
[2]Kunming Institute of Botany, Chinese Academy of Sciences, China

Lysosomes are acidic single-membrane organelles that serve as the major degradative sites for deconstructing cargoes resulting from endocytosis, phagocytosis and autophagy. Additionally, recent studies reveal that lysosomes are important for a variety of cellular processes including metabolism, signaling transduction and cell death. Dysfunction of lysosomes is involved in many human disorders such as lysosomal storage diseases (LSD) and neurodegenerative diseases. However, it is still not well understood how lysosome function and dynamics are regulated. In *Caenorhabditis elegans*, there are 6 macrophage-like coelomocytes, where endocytosis and phagocytosis are very active. To this point, we used the nematode *Caenorhabditis elegans* as a model to screen natural chemical compounds isolated from plants for drugs that can interfere with lysosome dynamics or functions. In our system, worms were kept in liquid culture with different dosage of compounds. 48hrs later, specific marker labelled lysosomes were observed in coelomocytes. Several classes of natural compounds were obtained and used as probes for the further mechanism investigation. A new compound called HEC-23 could specifically enlarge LMP-1 and ASP-1 positive lysosomes in coelomocytes, but not affect 2XFYVE positive early endosomes. Several mutants of HOPS complex blocked the HEC-23-induced enlargement of lysosomes, such as *vps-18*, *vps-33* and *vps-16* mutants. Also HEC-23 elevated the No. of cell corpse in germ line. Consistently, HEC-23 specifically enlarged the LAMP-1 positive lysosomes in both Hela and NIH3T3 cells. After HEC-23 administration, enlarged lysosomes are not acidified normally and Cathepsins could not localize in lysosomes properly. CFP-LC3 formed puncta and could not be degradated by enlarged lysosomes due to the impaired lysosomal acidification. Furthermore, HEC-23 promoted cell death in several cell lines and that effect was block by necrosis and Cathepsin inhibitors. Those findings suggest us that HEC-23 promoted necrosis through a Cathepsin-dependent way. Taken together, we found out natural compounds interfering with lysosome homeostasis and functions based on a *Caenorhabditis elegans* model. And compound-induced phenotypes are conserved in mammalian cells.

Contact: oceanyangli@genetics.ac.cn

qx222 affects apoptotic cell clearance in *C. elegans*

Jinchao Liu, Xiaochen Wang

National Institute of Biological Sciences, Beijing, China

In *C. elegans*, dying cells generated by apoptosis are quickly recognized and engulfed by phagocytes during embryonic development and oogenesis. The internalized apoptotic cells are enclosed within phagosomes which undergo a series of maturation process. The two phosphoinositol-3 kinases, VPS-34 and PIKI-1, which convert PtdIns to PtdIns(3)P, function in parallel to regulate phagosome maturation. PtdIns(3)P are also generated on endosomes and autophagosomes, which is regulated by VPS-15/VPS-34 complex but not PIKI-1. From a forward genetic screen, we have isolated a recessive mutation *qx222* which contains increased cell corpses in embryos. We found that *qx222* significantly enhanced persistent cell corpse phenotype in *piki-1* but not *vps-34* mutants and similar embryonic cell corpses were observed in *piki-1*; *qx222* as in *vps-34;piki-1* double mutants. We examined cell corpse engulfment and degradation processes and found that cell corpse recognition is not obviously affected but phagosome maturation is defective in *qx222* worms. We observed significantly reduced 2xFYVE labeling on phagosomes in *qx222* worms, which was greatly enhanced by *piki-1* but not *vps-34*. These data suggest that the gene affected in *qx222* acts together with *vps-34* to regulate PtdIns3P generation on phagosomes. We are now in the process of cloning the gene affected in *qx222* and further characterizing its role in phagosome maturation. We will report our progress in the meeting.

Contact: liujinchao@nibs.ac.cn

The lysosomal cathepsin protease CPL-1 plays a leading role in phagosomal degradation of apoptotic cells in *C. elegans*

Meng Xu

Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, China

During programmed cell death, the clearance of apoptotic cells is achieved by their phagocytosis and delivery to lysosomes for destruction in engulfing cells. The role of lysosomal proteases in cell corpse destruction is, however, not understood. Here we report the identification of the lysosomal cathepsin CPL-1 as an indispensable protease for apoptotic cell removal in *C. elegans*. We found that loss of *cpl-1* function led to a strong accumulation of germ cell corpses, which resulted from a failure in degradation rather than engulfment. CPL-1 is expressed in a variety of cell types including engulfment cells and its mutation does not affect the maturation of cell corpse-containing phagosomes, including phagosomal recruitment of maturation effectors and phagosome acidification. Importantly, we found that phagosomal recruitment and incorporation of CPL-1 occurs prior to the digestion of cell corpses, which depends on factors required for phagolysosome formation. Using RNAi, we further examined the role of other candidate lysosomal proteases in cell corpse clearance but found that they did not obviously affect this process. Collectively, these findings establish CPL-1 as the leading lysosomal protease required for elimination of apoptotic cells in *C. elegans*.

Contact: xmeng@genetics.ac.cn

Identification and Characterization of Two Transthyretin-like Proteins in Cell Corpse Clearance in *C. elegans*

Haibin Wang

National Institute of Biological Sciences, Beijing, China

The clearance of apoptotic cells by phagocytes is essential for animal development and defects in this process cause self-tolerance and autoimmunity. Phosphatidylserine (PS) is exposed on the surface of dying cells and serves as a signal for engulfment. Our previous study shows that TTR-52, a transthyretin-like protein, functions as a bridge molecule that recognizes PS and mediates cell corpse engulfment through the phagocytic receptorCED-1. Here, we find that CEL-1 and CEL-2, the two family members of TTR-52, cluster specially around cell corpses, suggesting that they can recognize apoptotic cells like TTR-52. Interestingly, the labeling of apoptotic cells by CEL-1 and CEL-2 is not altered in *tat-1* mutants, which disrupt PS asymmetry and cause ectopic PS appearance on living cell surfaces, but affected by mutations in *ced-7*. We found that overexpression of CEL-1 or CEL-2 caused persistent cell corpses and blocked PS appearance on engulfing cells. We are now in the process of examining loss-of-function phenotypes in *cel-1cel-2* double mutants, and will report our progress in the meeting.

Contact: wanghaibin@nibs.ac.cn

LIN-3/EGF promotes the programmed cell death fate in *C. elegans* by transcriptional activation of the pro-apoptotic gene *egl-1*

Hang-Shiang Jiang, Yi-Chun Wu

Institute of Molecular and Cellular Biology, National Taiwan University, Taiwan

Programmed cell death (PCD) is the physiological death of a cell mediated by an intracellular suicide program. Several transcription factors have been identified to regulate the death fate of specific cells in *C. elegans*. However, whether the PCD fate, like some other cell fates, may be regulated by extrinsic signal(s) during development remains unknown. Here, we report that the epidermal growth factor (EGF)-like ligand LIN-3 acts as an extrinsic signal to promote the PCD fate of specific cells. Several *lin-3* mutants showed reduced numbers of cell corpses and, in contrast, *lin-3* overexpression caused an increased number of cell corpses. By four-dimensional microscopy analysis, we found that the *lin-3* mutation caused partially penetrant cell survival of the niece/sister cells of hyp8/9 hypodermal cells, g1 gland cells, and l1 interneurons that would normally die in the wild-type. Moreover, overexpression of *lin-3* resulted in ectopic cell deaths, indicating that an appropriate level of LIN-3 is required for the precise control of PCD fate. Interestingly, mis-expression of *lin-3* in intestine also resulted in ectopic PCD, suggesting that LIN-3 may act at a distance to promote the PCD fate. The LIN-3 signal is transduced through its receptor, LET-23, to activate the LET-60-MPK-1 pathway and the downstream transcription factor LIN-1. Genetic studies indicate that the LIN-3 signaling acts upstream of the core PCD execution pathway, raising the possibility that LIN-3 may act through the transcription factor LIN-1 to regulate the transcription of core PCD genes. Using Quantitative real-time reverse transcriptase PCR and *Pegl-1::gfp* reporter, we found that the level of *egl-1* transcripts was less abundant in *lin-3* and *let-23* mutants than in wild-type animals. Using EMSA and transgenic bypass experiments, we demonstrated that LIN-1 can directly bind to the *egl-1* promoter *in vitro* and that this binding is important for *lin-3* to elicit its cell death-promoting function *in vivo*. Our results provide the first evidence that LIN-3, an extrinsic signal, can promote the PCD fate in *C. elegans* and reveal the molecular basis for its death-promoting function. We conclude that the level of LIN-3 signaling is important for the precise fine-tuning of the life-versus-death fate of cells.

Contact: gemini_midori@yahoo.com.tw

Assisted suicide: a caspase- and engulfment-dependent cell death

Holly Johnsen[1], Bob Horvitz[1,2]

[1]Department of Biology, MIT, USA
[2]HHMI, USA

Programmed cell death occurs during the development of many organisms. The *C. elegans* cell-death pathway has been extensively studied and is evolutionarily conserved. During programmed cell death, caspases are activated in the dying cell. The cell corpse is engulfed by a neighboring cell and degraded. Almost all *C. elegans* cell deaths are “suicides”—they are cell-autonomous and caspase-dependent and can occur even in engulfment-defective animals.

During development of the *C. elegans* male tail, the cells B.alapaav and B.arapaav are generated during the late L3 stage. During the early L4 stage one of these cells undergoes programmed cell death, and the other survives and adopts an epithelial fate. These two cells form an equivalence group; the decision of which cell dies and which survives is stochastic. We have found that this decision is made during the L3/L4 lethargus. The cell that dies is engulfed by the neighboring cell P12.pa. In contrast to most *C. elegans* cell deaths, the B.al/rapaav cell death is engulfment-dependent; we and others have found that if engulfment is blocked by a mutation in one of the genes in the engulfment pathway, both B.alapaav and B.arapaav survive. Furthermore, we have found that if the engulfing cell P12.pa is ablated, the B.al/rapaav death fails to occur in approximately 60% of animals. These observations suggest that cell interactions between B.alapaav and B.arapaav as well as between B.al/rapaav and P12.pa are involved in this cell death, consistent with the idea that P12.pa “murders” B.al/rapaav.

We found that when the B.al/rapaav cell death is blocked by engulfment defects or P12.pa ablation, the undead cell still initiates the cell-death pathway. Similar to cells that are about to die, the undead cell looks unusually round by Nomarski and electron microscopy and exposes phosphatidylserine on its surface. By contrast, an undead B.al/rapaav in *ced-3* mutants appears healthy, suggesting that the cell-death pathway fails at a point after caspase activation in engulfment mutants. *egl-1* and *ced-3* are expressed in the undead cell and are required for the B.al/rapaav cell death, suggesting that the core cell-death pathway is required but not sufficient for this cell death. We suggest that this death is an "assisted suicide." We hope our studies will provide insight into new mechanisms of programmed cell death, cell-cell signaling, and cell-fate determination within equivalence groups.

Contact: hjohnsen@mit.edu

Influence of *wago-4* and *wago-5* on *C. elegans* germ cell apoptosis

Martin Keller[1,2], Deni Subasic[1,2], Anneke Brummer[3], Shivendra Kishore[3], Yibo Wu[4], Mihaela Zavolan[3], Ruedi Aebersold[4], Michael O. Hengartner[1]

[1]Institute of Molecular Life Sciences, University of Zurich, Switzerland
[2]Molecular Life Sciences PhD program, Life Science Zurich Graduate School, ETH/University of Zurich, Zurich, Switzerland
[3]Biozentrum Basel, University of Basel, Switzerland
[4]Institute of Molecular Systems Biology, ETH Zurich, Switzerland

Post-transcriptional control of mRNAs by RNA-binding proteins (RBPs) plays a prominent role in the regulation of gene expression. RBPs interact with mRNAs to control their biogenesis, splicing, transport, localization, translation, and stability. Defects in regulation can lead to a wide range of human diseases from neurological disorders to cancer. Many RBPs are conserved between *Caenorhabditis elegans* (*C. elegans*) and humans, and several RBPs are known to regulate apoptosis in the adult *C. elegans* germline. How these RBPs control apoptosis is, however, largely unknown. We identified *wago-4* and *wago-5* in an RNAi screen as novel regulators of germ cell apoptosis. In an *in-vivo* CLIP experiment we found that *wago-4* mRNA is bound by MINA-1, another novel apoptosis relevant RBP. To understand how these RBPs control apoptosis, their target mRNAs, RNA-binding motifs, and interaction partners will be identified in CLIP and coimmunoprecipitation experiments. Proteins that are differentially expressed in *wago-4* and *wago-5* mutants compared to wild-type worms will be identified using SILAC. This approach allows us to build a model of the germ cell apoptosis RNA regulon and thus broaden our understanding of how RBPs orchestrate cellular events.

Contact: martin.keller@imls.uzh.ch

ced-11 Is Required for the Morphological Appearance of Apoptotic Cell Corpses

Kaitlin Driscoll[1], Gillian Stanfield[2], Bob Horvitz[1]

[1]MIT, USA
[2]University of Utah, USA

Programmed cell death is a fundamental process required for proper development and tissue homeostasis in many organisms. Genetic analyses of programmed cell death in *C. elegans* led to the discovery of an evolutionarily conserved genetic pathway that regulates the activation of apoptosis. A cell dying by apoptosis undergoes a series of morphological changes resulting in the appearance of round refractile cell corpses, as visualized by Nomarski optics. *ced-11* was identified in a screen for mutations that alter the morphological appearance of cell corpses. The corpses of *ced-11* mutant embryos are non-refractile as visualized by Nomarski optics.

We have found that while mutations in *ced-11* do not prevent cells from dying, they can enhance the ventral-cord cell-death defect of weak alleles of other cell-death genes. This observation indicates that *ced-11* plays a role in the cell death process. *ced-11* acts downstream of the CED-3 caspase and appears not to have an effect on engulfment. *ced-11* encodes a protein with similarity to members of the TRP family of non-selective cation channels. As TRP channels are often permeable to calcium, we tested if *ced-11* regulates calcium during apoptosis. We used GCaMP3, a genetically-encoded calcium indicator, to monitor calcium in dying cells. In wild-type embryos refractile corpses that express GCaMP3 have bright fluorescence throughout the corpses. In *ced-11* mutants corpses that express GCaMP3 have bright fluorescence in the cytoplasm but a reduction of fluorescence in the nucleus. In addition, in wild-type embryos a long-lasting increase of fluorescence coincides with the onset of refractility of the corpses. By contrast, *ced-11* corpses have occasional transient bursts of fluorescence in the cytoplasm. This observation suggests that *ced-11* might act as a calcium-permeable channel to regulate the entry of calcium into the nuclei of cells undergoing apoptosis. Alternatively, *ced-11* might regulate the breakdown/integrity of the nuclear envelope and thus allow calcium into the nucleus of apoptotic corpses. We plan to determine how *ced-11* affects the entry of calcium into the nucleus of apoptotic corpses. Better understanding of the role of *ced-11* in apoptosis might help elucidate the role of calcium downstream of caspase activation and the mechanism of nuclear degradation in apoptotic cell death.

Contact: kbd@mit.edu

A role of a *C. elegans*- pyrimidine synthase in programmed cell death

Wan-Ying Lin[1], Hung-Tsai Kan[1], Hang-Shiang Jiang[1], Yi-Chun Wu[1,2,3,4]

[1]Institute of Molecular and Cell Biology, National Taiwan University, Taipei, Taiwan
[2]Genome and System Biology Degree Program, National Taiwan University, Taipei, Taiwan
[3]Research Center for Developmental Biology and Regenerative Medicine, National Taiwan University, Taipei, Taiwan
[4]Institute of Atomic and Molecular Sciences, Academia Sinca, Taipei, Taiwan

Programmed cell death (PCD) is a conserved cellular process, which is important for animal development. *C. elegans* is one of the most commonly-used model organisms in the studies of PCD. Extensive studies in *C. elegans* show that four key genes, *egl-1*, *ced-9*, *ced-4* and *ced-3* control the execution of PCD, and the activation of caspase CED-3 leads to the demise of the cell. However, only few substrates of CED-3 have been identified thus far. To identify more genes that participate in PCD, we undertook a genetic screen and isolated the *tp12* mutation. The *tp12* mutant has reduced numbers of embryonic cell corpses as compared to those of wildtype. A genetic complementation test reveals that *tp12* is an allele of *pyr-1*. PYR-1 is a homolog of the mammalian CAD, which stands for carbamoyl phosphate synthetase, aspartate transcarbamylase, and dihydroorotase, and is known to control the rate-limiting step during pyrimidine biosynthesis. Using 4-dimentional microscopy analysis, we found that the *pyr-1(tp12)* mutant, in a sensitized background, shows frequent loss of specific cells, the aunt cells of the hyp8/9 hypodermal cells and the excretory cell. Consistently, an extra hyp8/9 nucleus and large cysts which are seen in mutants with an extra excretory cell (Daniel P., 2012) are observed in the *pyr-1(tp12)* mutant in a sensitized background. These results suggest that *pyr-1* is involved in the death of the aunt cells of the hyp8/9 cells and the excretory cell. We are currently doing experiments to test how PYR-1 functions in programmed cell death and whether the pyrimidine synthase activity of PYR-1 is required for its role in programmed cell death.

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Contact: wylin617@gmail.com

Transition from nuclear division to endoreduplication during intestinal development is modulated by CDC-25.2 activity

Yong-Uk Lee[1], Miseol Son[1], Esther Yoon[1], Ichiro Kawasaki[1], Yhong-Hee Shim[1,2]

[1]Department of Bioscience and Biotechnology, Konkuk University, Seoul 143-701, Republic of Korea
[2]Institute of KU Biotechnology, Konkuk University, Seoul 143-701, Republic of Korea

CDC-25.2, a member of CDC-25 phosphatase family, promotes cell cycle by dephosphorylating inhibitory phosphates of the Cyclin-Dependent Kinases in *C. elegans*. In the absence of CDC-25.2, 4 specific intestinal cell divisions during the late embryogenesis and intestinal nuclear divisions (binucleations) during the early L1 larval stage are abrogated. However, the following intestinal nuclear endoreduplication at each larval stage of *cdc-25.2* (*ok597*) mutant seems to occur normally and their DNA became from 2C to 32C DNA. In *lin-23* (*e1883*) and *lin-35* (*rr33*) mutants, which encode a component of SCF ubiquitin-ligase complex and a retinoblastoma (Rb) homolog, respectively, number of intestinal nuclei was increased due to an additional nuclear division instead of L1 and L2 endoreduplication. These additional nuclear divisions in *lin-23* (*e1883*) and *lin-35* (*rr33*) mutants were effectively suppressed by *cdc-25.2* RNAi. Furthermore, we observed that the expression of *cdc-25.2* mRNA, which is restricted to the L1 stage in the wild type, was extended to the early L2 stage in *lin-35* (*rr33*) mutant. Therefore, we hypothesize that CDC-25.2 activity needs to be eliminated by the end of the L1 stage for successful transition of intestinal cell cycle mode from nuclear divisions to endoreduplications. We are currently investigating whether the expression of CDC-25.2 protein is also extended to the L2 stage in *lin-23* (*e1883*) mutant, because LIN-23 is an essential component of SCF ubiquitin-ligase complex required for protein degradation. To verify that *cdc-25.2* is indeed expressed and functioning in the intestine, a mCherry-tagged *cdc-25.2* transgene that is expressed under the control of intestine-specific *elt-2* promoter was introduced into worms of several different genetic backgrounds. When this transgene was introduced into wild-type background, the number of intestinal nuclei increased abnormally. Now we are examining whether this *elt-2* promoter driven transgene can rescue intestinal defects of *cdc-25.2* mutant. Because LIN-35 is known to transcriptionally repress Cyclin D expression, putative transcriptional regulators of *cdc-25.2* including LIN-35 were screened by using yeast one-hybrid system. From this analysis, the transcriptional regulation mechanism for timely specific expression of *cdc-25.2* will be elucidated.

Taken together, our study suggests that the activity of CDC-25.2 is finely regulated to control the transition from nuclear division to endoreduplication during intestinal development. This study was supported by a grant NRF-2013R1A1A2009090, NRF-2013R1A1A2009820, and 2014 KU Brain Pool Program of Konkuk University.

Contact: sms1258@naver.com

Elucidating the role of *nmy-2* in seam cell division patterns

Siyu Serena Ding, Alison Woollard

Department of Biochemistry, University of Oxford, UK

C. elegans seam cells are multipotent neuroectodermal cells that undergo both symmetric and asymmetric divisions during larval development, thus providing a paradigm for the stem cell mode of division. Reiterative seam cell asymmetric division typically produces an anterior daughter that rounds up and a posterior daughter that elongates after division. The anterior daughter then moves out of the seam line and differentiates whereas the posterior daughter further elongates to re-form the seam line and maintains the stem fate. Non-muscle myosin is fundamental to processes such as cellular reshaping and migration, hence it emerges as a potential regulator of seam cell asymmetric divisions. A precedent for the role of non-muscle myosin 2 (NMY-2) in *C. elegans* post-embryonic development has been established in the Q neuroblast lineage (Ou et al., 2010). This further sparked our interest in the role for NMY-2 in seam cells. While post-embryonically abrogating *nmy-2* function either using temperature sensitive mutants or by RNA interference failed to produce significant changes in seam cell numbers, combining the two treatments robustly reduced terminal seam cell number from 16 in wild-type animals to around 12 in treated animals. Progressive cell loss was observed in each round of asymmetric division. Our current focus is to understand the cell molecular processes by which *nmy-2* influences seam cell asymmetric divisions.

Contact: serena.ding@bnc.ox.ac.uk

Investigating the role of *pal-1*, the *Caenorhabditis elegans* homologue of *caudal*, in the development of the stem-like seam cells

Sophie P. R. Gilbert, Charles Brabin, Peter J. Appleford, Alison Woollard

University of Oxford, UK

pal-1 has previously been shown to have an important role in establishing posterior patterning during embryogenesis[1], as well as during male tail development[2]. Here we investigate a novel role for *pal-1* in posterior seam cell development. *pal-1* was identified in a small scale candidate RNAi screen to detect genes acting redundantly with *rnt-1*, a gene already known to be essential for maintaining proliferative seam cell divisions. Synthetic phenotypes of *pal-1* with *rnt-1* are observed in double mutants, indicative of a genetic interaction. Bimolecular fluorescence complementation (BiFC) analysis has provided further evidence for a physical interaction between PAL-1 and RNT-1 *in vivo*. Worms homozygous for a *pal-1* mutant allele *e2091* display an uneven distribution of seam cells along their length upon hatching, together with other seam defects. Analysis of this strain has previously identified two point mutations in the last intron of *pal-1* [2]; we have found that a wild-type copy of this intron is capable of driving much of the expression pattern of *pal-1*, including early larval seam expression. An intron bearing the two mutations fails only to express in the seam, suggesting a separate seam-specific role for *pal-1*. We have demonstrated that the last intron of *pal-1* contains an enhancer element (perturbed in *e2091*) required to correctly specify *pal-1* expression in the seam and enable correct seam cell development and orientation. To identify transcription factors that bind to this tissue-specific intronic enhancer we are carrying out a yeast one-hybrid screen using both the wild-type and *e2091* mutant version of the *pal-1* intron. We will also report a novel phenotype of *pal-1(e2091)* with respect to the L1 division of the seam cells which eludes to a separate mechanism of seam division at the L1 stage.

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Contact: sophie.gilbert@exeter.ox.ac.uk

The kinase module of the Mediator complex regulates EGFR signaling to influence cell fate decisions in the *C. elegans* vulva

Jennifer M Grants[1,2,3], Lisa TL Ying[2,3], Akinori Yoda[4,5], Hideyuki Okano[4,6,7], Charlotte C You[2,3], Hitoshi Sawa[4,8,9], Stefan Taubert[1,2,3]

[1]Department of Medical Genetics, Canada
[2]Centre for Molecular Medicine and Therapeutics, Canada
[3]University of British Columbia, Vancouver, Canada
[4]Osaka University Graduate School of Medicine, Japan
[5]Graduate School of Medicine, Kobe University, Japan
[6]CREST, Japan Science and Technology Corporation, Japan
[7]Keio University School of Medicine, Tokyo, Japan
[8]PRESTO, Japan Science and Technology Corporation, Japan
[9]Riken, Center for Developmental Biology, Kobe, Japan

The Mediator complex is a conserved transcriptional coregulator that acts in diverse signaling pathways. Several Mediator subunits regulate *Caenorhabditis elegans* vulval induction, a developmental process controlled by epidermal growth factor receptor (EGFR) signaling, but how Mediator regulates EGFR signaling is incompletely understood. Thus, we investigated the role of the Mediator's regulatory kinase module in this context. We show that the four Mediator subunits in the kinase module, cyclin dependent kinase 8 (CDK-8), cyclin C, DPY-22/MED12, and LET-19/MED13, negatively regulate EGFR signaling-dependent cell fate decisions in the vulva, as mutants display a multivulva (Muv) phenotype. Genetically, the kinase module acts downstream of the EGF Receptor, as loss of the kinase module suppresses the vulvaless (Vul) phenotype caused by an EGFR reduction-of-function allele. Mechanistically, the kinase module acts as a corepressor of LIN-1, a conserved Ets-family transcription factor that is a terminal effector in the pathway, as loss of the kinase module promotes ectopic expression of a LIN-1/Ets element and enhances the Muv phenotype of *lin-1/Ets* reduction-of-function. Furthermore, vulval induction in kinase module mutants occurs independently of SUR-2, a Mediator subunit that coactivates transcription downstream of the EGFR signaling pathway, as loss of the kinase module suppresses the Vul phenotype of *sur-2* mutants. We attribute this SUR-2-independent vulval induction to derepression of the MDT-27/MED3 Mediator subunit in kinase module mutants, as *mdt-27* knockdown suppresses the Muv phenotype of kinase module mutants. As yeast MED3 is phosphorylated by CDK-8 leading to ubiquitination and eventual turnover of the Mediator complex, our result suggests that *C. elegans* CDK-8 regulates the coactivator activity of the Mediator complex via the MDT-27/MED3 subunit. Altogether, our study demonstrates that the kinase module coordinates transcriptional regulation downstream of the EGFR signaling pathway by promoting transcriptional repression by a conserved Ets factor and by inhibiting transcriptional activation by the Mediator complex. These findings highlight the critical role of Mediator in maintaining a balance between gene activation and repression downstream of an important developmental signaling cascade.

Contact: jgrants@cmmt.ubc.ca

A noise-filtering genetic network regulates the timing of distal tip cell dorsalward turning

Yi-Chen Chen[1,2], Yi-Ting Cheng[3], Ching-Cher Sanders Yan[2], Chun-Yi David Lu[1], Yi-Chun Wu[3,4], Chao-Ping Hsu[2,4]

[1]Department of Chemistry, National Taiwan University, Taipei, Taiwan
[2]Institute of Chemistry, Academia Sinica, Taipei, Taiwan
[3]Institute of Molecular and Cellular Biology, National Taiwan University, Taipei, Taiwan
[4]Genome and Systems Biology Degree Program, National Taiwan University, Taipei, Taiwan

Gene expression processes, including transcription and translation, are inherently stochastic. The fluctuations in gene expression can lead to cell-to-cell variability and hence phenotypic heterogeneity in otherwise genetically identical cells. In *C. elegans*, the cell lineage and patterning during development are essentially invariant from animal to animal. However, little is known about how *C. elegans* copes with gene expression noise to ensure robustness of development. Combining both experiments and mathematical modeling, we show that a noise-filtering genetic network can precisely control the timing of distal tip cell (DTC) dorsalward turning in *C. elegans*. The DTC is a somatic cell located at the tip of each gonad arm, and guides the extension of the gonad arm during larval development. DTCs initially migrate towards the ends of the body during L2 and early L3. In late L3, DTCs turn 90 degrees, moving from the ventral to dorsal side. We and others showed that a genetic network consisting of *daf-12, lin-29, dre-1, lin-42* and *blmp-1* controls the timing of DTC dorsalward turning[1, 2,3]. Steroid hormone receptor DAF-12, zinc finger transcription factor LIN-29 and F-box protein DRE-1, promote DTC dorsalward turning, whereas zinc finger transcription factor BLMP-1 and period-like LIN-42 prevents DTCs from undergoing precocious dorsalward turning. Interestingly, the *blmp-1; daf-12* or *blmp-1; lin-29* double mutants showed phenotypic heterogeneity in DTC dorsalward timing. The phenotype of precocious, retarded or normal timing was observed in populations of these mutants. We hypothesized that such phenotypic heterogeneity may result from a noise-filtering defect in the gene regulatory network. To test this, we constructed a stochastic kinetic model that incorporated the empirical molecular and genetic data. This model supports a noise-filtering function in the gene regulatory network and successfully predicts the phenotypic heterogeneity of the mutants. Moreover, we validated our simulation by manipulating the noise levels of specific genes in both experiment and mathematical model and observing similar results. Our work shows that gene expression noise can be filtered out through a structured genetic network to ensure robustness of developmental events.

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Contact: dnalib@gmail.com

Regulation of Developmental Timing and Cell-Fate Determination by Heterochronic Proteins LIN-29 and MAB-10

Akiko Doi, Bob Horvitz

Massachusetts Institute of Technology, USA

During animal development, gene regulation needs to be temporally precise for proper cell-fate decisions to occur. The evolutionarily conserved *C. elegans* heterochronic pathway controls the temporal progression of development by regulating the activities of a sequence of genes. Components of this pathway control cell-fate decisions of proliferation versus differentiation, and mammalian homologs of these components play critical roles in stem cell regulation.

mab-10 was discovered in our laboratory to encode the *C. elegans* NGFI-A-binding protein (NAB) transcriptional co-factor. MAB-10 is involved in the terminal differentiation of the hypodermal stem-like seam cells and more generally in the larval-to-adult transition (Harris & Horvitz, *Development*, 138, 4051, 2011). LIN-29, the master regulator of the larval-to-adult transition, was shown to be an early growth response (EGR) protein that acts together with MAB-10 to control the expression of genes that regulate the onset of adulthood and terminal differentiation in the hypoderm. There is a striking parallel in mammals, in which EGR proteins interact with NAB proteins to cause terminal differentiation and the onset of puberty (Topilko et al., *Mol Endocrinol*, 12, 107,1998). Furthermore, EGR1, the mammalian homolog of LIN-29, has been shown to act as a barrier during reprogramming to human induced pluripotent stem cells (Worringer et al., *Cell Stem Cell*, 14, 40, 2014). Despite the importance of this pathway, mechanisms by which the terminal effectors LIN-29/EGR and MAB-10/NAB function and are regulated remain largely unknown.

We are studying the functions of LIN-29/EGR and MAB-10/NAB with the goal of understanding the mechanisms that control *C. elegans* developmental timing and providing insights concerning stem cell identity and development in mammals. Although MAB-10 has been identified as a co-factor of LIN-29, mab-10 mutants have a weaker phenotypic defect than *lin-29* mutants. This observation suggests the existence of additional genes in this pathway that either function as co-factors of LIN-29 or that act in parallel with MAB-10. To identify such factors, we have performed genetic screens for enhancers of the *mab-10* mutant phenotype. We are currently characterizing these mutations. We hope that these studies will identify new molecules involved in stem cell regulation and facilitate advances in regenerative medicine.

Contact: adoi@mit.edu

Analysis of the function and dysfunction of the ALS gene *C9ORF72* using *C. elegans*

Anna Corrionero, H. Robert Horvitz

HHMI, USA
Dept. Biology, MIT, USA

An expansion of a GGGGCC hexanucleotide repeat in an intronic region of a gene of unknown function, *C9ORF72* (*chromosome 9 open reading frame 72*), is the most common known genetic cause of familial amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (DeJesus-Hernandez et al., 2011; Renton et al., 2011). Among the mechanisms proposed for the pathogenic effects of the *C9ORF72* hexanucleotide expansion in the etiology of ALS is the loss of wild-type *C9ORF72* gene function.

The *C. elegans* genome contains an uncharacterized gene homologous to *C9ORF72*, *F18A1.6*. Both C9ORF72 and F18A1.6 show structural similarities to DENN domain-containing proteins, guanine exchange factors for Rab GTPases (Levine et al., 2013; Zhang et al., 2012). However, the molecular function of C9ORF72 and F18A1.6 remains unknown.

We have observed that *F18A1.6* mutants show slow embryonic development. A translational reporter of *F18A1.6* is expressed during embryogenesis, localizing in the cytoplasm. We are using a candidate-gene approach to identify genes with a similar abnormal embryonic development and that might genetically interact with *F18A1.6*.

Our goals are to determine the molecular function of F18A1.6, its role during embryonic development and to establish if C9ORF72 and F18A1.6 are functionally related. We hope to help elucidate how the most common ALS-causing mutation, a non-coding hexanucleotide repeat expansion in a conserved gene of unknown function, exerts its pathogenic effects.

Contact: annacs@mit.edu

The miR-58 family restricts expression of PMK-2 p38 MAPK to the nervous system

Daniel J. Pagano, Elena R. Kingston, Dennis H. Kim

MIT, USA

Of the hundreds of microRNAs identified in *Caenorhabditis elegans*, a single microRNA, miR-58, accounts for nearly half of the total microRNAs at all developmental stages of the animal, but its function has remained enigmatic. We have identified a requirement for the miR-58 family of microRNAs in restricting the expression of *pmk-2*, which encodes a p38 mitogen-activated protein kinase (MAPK), to the nervous system of *C. elegans*. Whereas PMK-2 functions in the nervous system to regulate neuronal development and behavioral responses to pathogenic bacteria, the miR-58, miR-80, miR-81, and miR-82 microRNAs function redundantly to destabilize *pmk-2* mRNA in non-neuronal cells with dramatic, switch-like potency. Our data support a physiological role for a relatively abundant, constitutively expressed microRNA in the maintenance of tissue-specific gene expression in *C. elegans*.

Contact: dpagano@mit.edu

Vulval bursting is caused by dysregulation of a single *let-7* target, LIN-41

Magdalene Rausch, Matyas Ecsedi, Helge Grosshans

Friedrich-Miescher-Institute for Biomedical Research, Switzerland

The *let-7* microRNA (miRNA) is a classical example of a heterochronic regulator in *Caenorhabditis elegans*. Mutations in *let-7* lead to reiteration of L4-specific divisions of the stem cell-like seam cells and failure to secrete an adult specific cuticle. Consistent with the canonical view that miRNAs function by coordinately regulating sets of target mRNAs, dysregulation of several targets has been implicated in this phenotype, among them LIN-41 (TRIM71 in humans), DAF-12, and LET-60/RAS. However, the cellular and molecular basis of the most striking *let-7* mutant phenotype, vulval bursting at the L4-to-adult transition, has remained unexplored. Inappropriate seam cell differentiation and subsequent alterations in the hypodermis-to-vulva connection have been proposed as a possible cause of bursting. On the other hand, dysregulation of LET-60, which plays a crucial role in vulva precursor cell (VPC) fate specification, could be an intrinsic cause for this phenotype.

To elucidate the cause of vulva rupturing, we studied the spatial expression and activity pattern of *let-7*. We find that this miRNA is not only expressed and functional in the *C. elegans* vulva, but that its expression in this organ is indeed required to avoid vulval bursting. Nonetheless, *let-7*-mediated regulation of *let-60* in the vulva is minor and not required for successful completion of vulval development. Consistently, early VPS specification occurs properly in *let-7* mutants. By contrast, LIN-41 is extensively dysregulated in the vulva upon loss of *let-7* activity. Indeed, we demonstrate that uncoupling of *lin-41* from *let-7* through targeted mutation of the endogenous *let-7* binding sites in the *lin-41* 3' untranslated region suffices to phenocopy *let-7* vulva phenotypes. Hence, regulation of this single target is sufficient to explain a major physiological function of a miRNA. Equally interesting, whereas the *let-7/lin-41* miRNA/target pair regulates self-renewal and differentiation in the seam cells as well as mammalian systems, its role in the vulva seems to be distinct and involve control of a late step of morphogenesis. Hence, they might work as a flexible regulatory module that can be wired into distinct molecular pathways.

Contact: magdalene.rausch@fmi.ch

In vivo toxicity of nanomaterials are regulated by microRNA

Yunli Zhao, Dayong Wang

Southeast University, China

The molecular basis for *in vivo* engineered nanomaterials (ENMs) toxicity is still largely unclear. In the present study, we employed the *in vivo Caenorhabditis elegans* assay system to perform the SOLiD sequencing analysis to identify the possible miRNAs targets of mult-walled carbon nanotubes (MWCNTs) and Graphene oxide (GO), after prolonged exposure from L1-larvae to young adult. We identified 55 differentially expressed miRNAs (21 up-regulated miRNAs and 34 down-regulated miRNAs) in MWCNTs and 31 differentially expressed miRNAs (23 up-regulated and 8 down-regulated) in GO exposed nematodes compared with control. The identified dysregulated miRNAs were confirmed by qRT-PCR assay, and their expression was concentration-dependent in MWCNTs and GO exposed nematodes. The bioinformatics analysis on targeted genes for the identified dysregulated miRNAs in ENMs exposed nematodes demonstrate that the dysregulated miRNAs may be involved in the control of many biological processes. Moreover, we used the available mutants for identified dysregulated miRNAs to perform the toxicity assessment by evaluating the functions of primary (intestine) and secondary (neuron and reproductive organs) organs. Functions of the identified miRNAs in regulating the GO toxicity on lifespan were confirmed in the available miRNAs mutants. We detected 5 miRNAs mutants with the susceptible property and 3 miRNAs mutants with the resistant property to MWCNTs toxicity. With *mir-259* and *mir-51* mutants as the examples, results data imply that MWCNTs translocation was strengthened by susceptible miRNAs mutants, whereas MWCNTs translocation was inhibited by resistant miRNAs mutants. Moreover, we provide the evidence to raise a hypothesis that GO may reduce lifespan through influencing the functions of insulin/IGF signaling, TOR signaling, and germline signaling pathways controlled by miRNAs. For the underlying mechanism, we hypothesize that both physiological state of intestine and defecation behavior were involved in the formation of susceptible or resistant property of specific miRNAs mutants. Our results will be helpful for understanding the molecular basis for future chemical design to reduce ENMs toxicity and finding clues for useful surface modifications to reduce ENMs toxicity.

Contact: yunli201@126.com

RACK-1 regulates *let-7* microRNA expression and terminal cell differentiation in *Caenorhabditis elegans*

Yu-De Chu[1], Wei-Chieh Wang[1], Shi-An A. Chen[2], Yen-Ting Hsu[1], Meng-Wei Yeh[1], Frank J. Slack[3], Shih-Peng Chan[1,2,3]

[1]Graduate Institute of Microbiology, College of Medicine, National Taiwan University, Taiwan
[2]Genome and Systems Biology Degree Program, College of Life Science, National Taiwan University, Taiwan
[3]Department of Molecular, Cellular and Developmental Biology, Yale University, USA

The *let-7* microRNA (miRNA) regulates cell cycle exit and terminal differentiation in the *C. elegans* heterochronic gene pathway. Low expression of *let-7* results in retarded vulva and hypodermal cell development in *C. elegans* and has been associated with several human cancers. Previously, the versatile scaffold protein Receptor for Activated C Kinase 1 (RACK1) was proposed to facilitate recruitment of the miRNA-induced silencing complex (miRISC) to the polysome and to be required for miRNA function in *C. elegans* and humans. Here, we show that depletion of *C. elegans* RACK-1 by RNAi increases *let-7* miRNA levels and suppresses the retarded terminal differentiation of lateral hypodermal seam cells in mutants carrying the hypomorphic *let-7(n2853)* allele or lacking the *let-7* family miRNA genes *mir-48* and *mir-241*. Depletion of RACK-1 also increases the levels of precursor *let-7* miRNA. When Dicer is knocked down and pre-miRNA processing is inhibited, depletion of RACK-1 still leads to increased levels of pre-*let-7*, suggesting that RACK-1 affects a biogenesis mechanism upstream of Dicer. No changes in the activity of the *let-7* promoter or the levels of primary *let-7* miRNA are associated with depletion of RACK-1, suggesting that RACK-1 affects *let-7* miRNA biogenesis at the post-transcriptional level. Interestingly, *rack-1* knockdown also increases the levels of a few other precursor miRNAs. Our results reveal that RACK-1 controls the biogenesis of a subset of miRNAs, including *let-7*, and in this way also plays a role in the heterochronic gene pathway during *C. elegans* development.

Contact: sh90152@hotmail.com

An hnRNP Q/R homolog HRP-2 regulates *let-7* miRNA function in *Caenorhabditis elegans*

Yu-De Chu, Xiao-Shin Wei, Huan-Yao Chiu, Shih-Peng Chan

National Taiwan University College of Medicine, Taiwan

Heterogeneous nuclear ribonucleoproteins (hnRNPs) have been demonstrated to regulate transcription, pre-mRNA splicing, mRNA export, translation and turnover. Recent studies have also shown that a few hnRNPs are involved in miRNA biogenesis and function. Here, we report that an hnRNP Q/R homolog HRP-2 regulates the *let-7* miRNA activity in *C. elegans*. In *C. elegans*, the *let-7* miRNA controls cell cycle exit and terminal differentiation of hypodermal cells at the larva-to-adult developmental switch by down regulation of LIN-41, which represses the adult transcription factor LIN-29. miRNA-induced silencing complexes (miRISCs) containing the *let-7* miRNA bind to the 3′ untranslated region (3′ UTR) of *lin-41* mRNA and trigger translational repression and mRNA degradation. We found that reduction of HRP-2 by RNAi enhanced *let-7*-directed silencing of a *gfp* reporter fused with the *lin-41* 3′ UTR without alteration of *let-7* levels. In addition, we demonstrated that HRP-2 was associated with the *lin-41* 3′ UTR and also the Argonaute ALG-1 protein. Taken together, we propose that mRNA-bound HRP-2 negatively regulates miRISC function. (Support: National Health Research Institutes. NHRI-EX102-10151SI)

Contact: shihpengchan@ntu.edu.tw

Molecular mechanisms of heterochromatin segregation during *C. elegans* differentiation

Daphne S Cabianca[1], Susan M Gasser[1,2]

[1]Friedrich Miescher Institute for Biomedical Research, Maulbeerstrasse 66, CH-4058 Basel, Switzerland
[2]University of Basel, Faculty of Natural Sciences, CH-4056 Basel, Switzerland

The nucleus is a spatially organized cellular compartment that accommodates chromatin-related processes, yet very little is known about the role of nuclear organization in chromatin dynamics and tissue development. Euchromatin and heterochromatin occupy distinct domains within the nucleus of interphase cells with heterochromatin positioned at the nuclear and nucleolar periphery.

Our lab previously demonstrated that heterochromatin repression and perinuclear anchoring in undifferentiated embryonic cells is triggered by an H3K9 methylation-dependent pathway. However, when H3K9 methylation-deficient embryos differentiate into worms, the peripheral anchoring of a fluorescently tagged heterochromatic reporter transgene array is restored, arguing that heterochromatin anchoring is orchestrated by different, unknown mechanisms during cell differentiation.

Aiming to identify these pathways, we designed a targeted RNAi screen to impair the function of 108 potential “chromatin anchoring” candidates (16 SET-, 17 Chromo-, 2 MBT-, 7 Tudor- and 25 PHD- containing proteins, 36 factors related to the nuclear envelope and 5 to chromatin processes). We scored a fluorescently tagged heterochromatin reporter and scored for its subnuclear positioning in L1 tissues using high-resolution microscopy. Remarkably, we could observe that already in control conditions the degree of reporter anchoring to the nuclear envelope differs among distinct groups of cells of the same tissue—most notably nuclei of the intestine. Moreover, the screen allowed us to identify factors that can selectively affect anchoring in defined cells but not others, within the same tissue. This further supports the notion of cell type-specific regulation of chromatin localization. Validation and characterization of the RNAi screen hits using mutants is ongoing. We are investigating the genome-wide positioning of endogenous heterochromatin and RNA profiles in specific tissues and cells of interest of wt and mutant worms. These approaches, combined to tissue functionality assays, will allow us to test whether heterochromatin mislocalization affects tissue homeostasis and differentiation.

Contact: daphne.cabianca@fmi.ch

Phenotype and mRNA expression analyses of sphingosine kinase gene *sphk-1* and sphingosine-1-phosphate phosphatase gene *F53.C3.13* in *Caenorhabditis elegans*

Chiharu Rokushima, Yoshiyasu Ushida, Aimi Tomita, Takehiro Oshime, Haruka Yamaji, Hisao Kojima, Masahiro Ito

Department of Bioinformatics, College of Life Sciences, Ritsumeikan University, Japan

Sphingolipids are one of the major lipid components of eukaryotic cell membrane. It is known that among other sphingolipid metabolites, sphingosine, ceramide, and sphingosine-1-phosphate (S1P) are important lipid mediators that regulate cell survival, proliferation, differentiation, and motility. Particularly, S1P exerts various effects such as cell proliferation activity, cell movement, and cell differentiation for various cell types such as lymphocytes and vascular endothelial cells.

In the present study, we focused on sphingosine kinase (*sphk-1*) and S1P phosphatase (*F53.C3.13*), which catalyzes the synthesis of sphingosine and the degradation into sphingosine, in *C. elegans*. Phenotypes were observed using a Nomarski microscope. mRNA expressions were analyzed by using quantitative RT-PCR against Δsphk-1(*ok1097*) and ΔF53.C3.13 (*ok1861*) strains. Furthermore, comparative analysis of mRNA expressions was performed at three developmental stages: embryonic, larval 1 (L1) and adult stages.

Embryonic lethalities of mutants showed high values: 40.7% in Δsphk-1 and 44.7% in ΔF53C3.13. These phenotypes in adult stage were observed to be vulva-less and abnormal line of eggs. Subsequently, we measured and compared the mRNA expressions of *sphk-1* and *F53C3.13* genes with that of wild type at three developmental stages. mRNA expression of *sphk-1* at L1 stage was lowest. Compared to L1, mRNA expression at embryonic stage was 1.7 times and that at adult stage was 2.5 times. Next, mRNA expression of *F53.C3.13* at L1 stage was also lowest. mRNA expression at embryonic stage was 2.0 times and that at adult stage was 1.5 times. From these results, we hypothesize that *sphk-1* and *F53C3.13* genes were involved in development on embryonic and adult stages.

Contact: sj0013sr@ed.ritsumei.ac.jp

Gene expression and phenotype analyses of a ceramide kinase gene *T10B11.2* in *Caenorhabditis elegans*

Yoshiyasu Ushida, Chiharu Rokushima, Haruka Yamaji, Hisao Kojima, Masahiro Ito

Department of Bioinformatics, College of Life Sciences, Ritsumeikan University, Shiga, Japan

Sphingolipids had been considered as the lipids only involved in maintaining the structure of the biomembrane for a long time. However, in recent years, it has become apparent that sphingolipids are involved in intracellular signaling. Ceramide, a central component of sphingolipid metabolism, has attracted increasing attention as a signaling molecule which controls apoptosis, cell proliferation, cell differentiation, insulin resistance and inflammatory reaction. Furthermore, it has been shown that ceramide induces apoptosis in *Caenorhabditis elegans* germ cells. A major metabolite of ceramide is ceramide 1-phosphate (C1P), which is biosynthesized by ceramide kinase. In *C. elegans*, T10B11.2 protein is homologous to human ceramide kinase (51% similarity).

In the present study, we aimed to identify the function of a ceramide kinase gene T10B11.2. Mutant phenotypes of*ΔT10B11.2* (*ok1252*) were observed against embryogenesis, larval 1 (L1), and young adult stages. Furthermore, mRNA expressions of *T10B11.2* in the N2 strain were determined by using quantitative real-time reverse transcription PCR (qRT-PCR) assay and the ΔΔCt method.

Phenotypes of *ΔT10B11.2* (*ok1252*) strain was observed several different morphological defects such as abnormal blastomere morphology, the transformation of the ABp or EMS cell sides into flatness, the development of space between the blastomere and the eggshell in embryogenesis stage, and vulva-less appearance in adult stage. The embryonic lethality of *ΔT10B11.2* (*ok1252*) strain was higher (45.8%). Furthermore, we revealed that the *T10B11.2* expression level was highest in embryogenesis stage and lowest in L1 stage, and the relative expression ratio among embryogenesis, L1, and young adult stages was found to be 2.5:1:1.2. These results hypothesize that the function of T10B11.2 is important for the determination of cell fate in embryogenesis of *C. elegans*.

Contact: sj0007pi@ed.ritsumei.ac.jp

Feedback Modulation of Chemoreceptor Gene Expression Mediated by Feeding State and NPR-1

Matthew Gruner[1], Dominic Valdes[1], Dru Nelson[1], Samuel H Chung[2,3], Christopher V Gabel[2,3], Alexander M van der Linden[1]

[1]Department of Biology, University of Nevada, Reno, NV 89557, USA
[2]Department of Physiology and Biophysics, Boston University School of Medicine, 72 E. Concord Street, Boston, MA 02118, USA
[3]Boston University Photonics Center, 8 Saint Mary's Street, Boston, MA 02215, USA

Animals dramatically modify their chemosensory behaviors to attractive and noxious chemical stimuli in the environment depending on their food availability and feeding state. This could allow them to alter and optimize their food-search strategies to increase their survival and reproduction. Although these state-dependent behaviors have long been thought to arise from plasticity in central processes, it is now evident that changes in the periphery are also major contributions to these behavioral transitions. In fact, dynamic changes in the gene expression of chemoreceptors specialized in detecting environmental stimuli is observed in fish, insects and nematodes, and may underly some of these state-dependent changes in chemosensory behaviors. However, the mechanisms by which information from food and feeding state are translated into changes in expression levels of chemoreceptor genes, and how these changes may contribute to an altered chemosensory response in any animal are unknown.

To elucidate the mechanism underlying dynamic changes in chemoreceptor gene expression, we have developed an *in vivo* reporter assay in *C. elegans* for monitoring the expression of a candidate chemoreceptor gene, *srh-234*, in the ADL sensory neuron type as a function of feeding state; *srh-234* is highly expressed in fed animals, but not in starved animals. Using this reporter assay, we found that activity of the neuropeptide Y receptor, NPR-1, in RMG modulates *srh-234* expression in ADL via gap-junctions in response to starvation. In addition, using genetic and physical manipulation, we show that sensory inputs from external food into ADL transduced by the TRPV channel, OCR-2, modulate *srh-234* expression, which in turn may regulate NPR-1 by altering the output of ADL. Sensory and circuit inputs into ADL are conveyed through a common calcium-dependent signaling pathway to modulate *srh-234* expression. These results suggest that dynamic changes in the expression levels of *srh-234* are regulated by the direct sensation of food presence mediated by ADL sensory neurons, which in turn may regulate state-dependent feedback modulation of *srh-234* expression from RMG interneurons.

To further investigate the transcriptional mechanisms underlying the state-dependent modulation of *srh-234* expression, we performed a candidate gene approach of transcriptional regulators. We found that loss-of-function (lf) mutations in *mef-2* (MEF2), and genes encoding for bHLH factors (*hlh-30, mxl-3, hlh-2*) alter *srh-234* expression levels. In addition, we found that lf mutations in the insulin-like receptor, *daf-2*, and insulin-like peptides in *daf-28*-expressing cells (ASI, ASJ) also modulate *srh-234* expression. These results suggest that, in addition to NPR-1, insulin signals from neurons other than ADL modulate *srh-234* expression, possibly through the action of MEF-2 and bHLH factors. Together, our results provide insight into the neural and molecular mechanisms of how expression changes in chemoreceptor genes may contribute to changes in chemosensory behavior as a function of feeding state.

Contact: avanderlinden@unr.edu

Regulation of the *bed-3* gene

Takao Inoue, Jin Yang, Jason Wei Han Tan, Zhengyang Xie, Kah Yee Goh

National University of Singapore, Singapore

We are studying the regulation of the *bed-3* gene which encodes a BED type zinc finger transcription factor. *bed-3* regulates vulval cell division and molting. In addition, we found that *bed-3* is required for male tail development. The modENCODE project identified a possible BLMP-1 binding site in the intron 3 of *bed-3*, within a 2kb region previously identified as having vulva and hypodermis specific enhancer activity. Using EMSA and *in vivo* enhancer assays, we found that BLMP-1 directly and positively regulates the expression of the *bed-3* gene through this enhancer. We are also interested in the region upstream of *bed-3*, which appears to be devoid of protein coding genes (up to approximately 10 kb upstream), and contains a number of predicted long non-coding RNA genes and enhancers. Using reporter assays, we found that some of these non-coding RNAs appear to be expressed in a tissue-specific manner. From the analysis of deletion mutants generated by MosDEL, we found that this region is required for egg laying and male tail development. These deletions failed to complement the Egl phonotype of a *bed-3* mutant, however qPCR assays found no obvious effect of these deletions on the total *bed-3* transcript level. We are now carrying out FISH experiments to determine the effect of deletion on *bed-3* transcripts.

Contact: bchti@nus.edu.sg

Combined use of chloroform and chymotrypsin as an effective strategy for the proteomic analyses of hydrophobic proteins

Hisao Kojima[1], Tetsushi Itami[1], Toshiya Hayano[2], Masahiro Ito[1]

[1]Department of Bioinformatics, College of Life Sciences, Ritsumeikan University, Japan
[2]Department of Biomedical Sciences, College of Life Sciences, Ritsumeikan University, Japan

In recent years, proteomic analyses have been performed in the nematode, *Caenorhabditis elegans* (Tohsato *et al.* 2012). In the analyses, conventional proteomic methodologies have revealed the average protein detection to be about 1500, which are mostly hydrophilic proteins. In particular, hydrophobic proteins have been rarely identified, despite the fact that they comprise 30% of the coding proteins in the nematode genome. Therefore, the present study describes a novel organic solvent-based extraction method in order to improve the efficiency of detecting hydrophobic proteins, such as multi-transmembrane proteins in *Caenorhabditis* species.

In the present study, total proteins were extracted from adults of the tropical nematode, *Caenorhabditis briggsae* with a solvent mixture of chloroform/methanol/M9 buffer (1:2:0.8, v/v/v). After worm debris and organic solvents were removed, the obtained crude protein sample was rinsed thrice using 50 mM NH_4HCO_3 to remove hydrophilic proteins. Subsequently, the remaining hydrophobic protein fraction was dissolved in water using invitrosol, which has been previously reported to have little influence on mass spectrometry. Further, the quantified (100 µg) hydrophobic proteins were denatured, reduced, alkylated, and digested by chymotrypsin. The resultant digested peptides were fractionated by a cation-exchange cartridge, desalted, and analyzed by a liquid chromatography-tandem mass spectrometry.

Our novel proteomic assay facilitated the identification of a significant number of proteins which could not be detected by the conventional method, and further yielded significantly abundant hydrophobic proteins with a grand average hydropathy score greater than zero. Expectedly, a significantly higher number of transmembrane proteins were detected in our study. However, taken together, only 40% of proteins have been detected both by the conventional as well as the novel proteomic methodologies, till date. Therefore, our novel proteomic assay confirmed a number of the identified peptides to be transmembrane peptides, thus validating the combined use of chloroform and chymotrypsin in the detection of membrane proteins. The present study highlights an effective strategy for the proteomic analysis of hydrophobic proteins.

Contact: hkojima@fc.ritsumei.ac.jp

A genome-wide systematic screen for genetic modulators of embryonic development

Patricia Giselle Cipriani[1,2], Firoz Ahmed[1,2], Amelia White[3], Huey-Ling Kao[1], Eliana Munarriz[1], Katherine Erickson[1], Jerome Reboul[1], Jessica Lucas[1], Indrani Chatterje[1], Noah Smit[1], Kristin Gunsalus[1,2], Fabio Piano[1,2]

[1]New York University, USA
[2]New York University Abu Dhabi, UAE
[3]Memorial Sloan Kettering Cancer Center, USA

Large-scale loss-of-function studies in multiple organisms have revealed that a minority of expressed genes are essential on their own. Double perturbations can be used to identify *in vivo* roles for non-essential genes and to elucidate functional relationships within and between molecular modules. To identify modulators of essential gene functions in embryonic development, we have used RNAi to perform genome-wide enhancer and suppressor screens of 24 temperature-sensitive alleles that affect diverse processes in embryogenesis. To score for possible interactions, we have assayed embryonic lethality in more than 80 genome-wide screens. We have archived over 3 million images of mixed-stage populations. We have analyzed the images using our algorithm DevStaR, which combines computer vision and machine learning approaches to obtain quantitative readouts of embryonic lethality. Putative interactions identified in primary screens were re-assayed twice in quadruplicate, and scored both manually and by DevStaR. We will present our semi-automated screening pipeline and findings from the analysis of our suppressor network, which links over 500 genes through more than 900 interactions.

Contact: pgc212@nyu.edu

Proteomic and Transcriptomic Analysis of Mutation of the Maternal Gene *spn-4* in *Caenorhabditis elegans*

Aimi Tomita[1], Hiroki Yamashita[1], Takehiro Oshime[1], Haruka Yamaji[1], Narumi Enna[1], Hisao Kojima[1], Toshiya Hayano[2], Masahiro Ito[1]

[1]Department of Bioinformatics, College of Life Sciences, Ritsumeikan University, Shiga 525-8577 Japan
[2]Department of Biomedical Sciences, College of Life Sciences, Ritsumeikan University, Shiga 525-8577 Japan

In *Caenorhabditis elegans*, the polarity of early embryogenesis is determined by several maternal genes. Cell fate is determined by translational factors of the maternal gene products that regulate translational control of downstream genes. We focused on a maternal gene, *spn-4*. *spn-4* encodes a protein (SPN-4) containing an RNA-binding domain of the RNP type and is required for normal cytokinesis and spindle orientation in early embryogenesis. SPN-4 binds the 3′-UTR region and forms a complex with POS-1. This complex is required for maternal *glp-1* mRNA translation in anterior blastomeres of early embryogenesis. Furthermore, SPN-4 regulates translation of other maternal genes, such as *nos-2*, *pal-1*, *skn-1*, and *zif-1*. However, many of the molecular mechanisms related to maternal genes remain unknown.

We performed quantitative proteomic analysis by using isobaric tags for relative and absolute quantitation (iTRAQ) to identify a group of genes whose translation is regulated by SPN-4. iTRAQ analysis was performed 3 times on each of 2 samples corresponding to embryonic and adult stages. The proteins were analyzed by using a volcano plot. Next, the patterns of mRNA expression were checked by NEXTDB, the *in situ* hybridization pattern database, to narrow the search to maternal genes. These maternal genes were analyzed by quantitative RT-PCR (qRT-PCR) and next-generation sequencer.

In total, 1,082 proteins were identified at least twice by iTRAQ analysis. The volcano plot showed that the expression levels of 54 proteins were significantly up- or downregulated at the embryonic stage. In addition, NEXDB analysis of the expression patterns indicated that 44 of the genes are maternal. qRT-PCR analysis showed that the protein expression of 21 of the genes were either decreased or increased, indicating that these genes were translationally regulated by SPN-4.

Contact: sj0003ri@ed.ritsumei.ac.jp

Quantitative proteomic and transcriptomic analysis of the maternal gene *mex-3* in *Caenorhabditis elegans*

Takehiro Oshime[1], Hiroki Yamashita[1], Aimi Tomita[1], Haruka Yamaji[1], Narumi Enna[1], Hisao Kojima[1], Toshiya Hayano[2], Masahiro Ito[1]

[1]Department of Bioinformatics, College of Life Sciences, Ritsumeikan University, Japan
[2]Department of Biomedical Sciences, College of Life Sciences, Ritsumeikan University, Japan

In *Caenorhabditis elegans*, maternal genes determine cell fates until the 12-cell stage, but the molecular networks of most maternal gene products remain unknown. We focused on the maternal gene *mex-3* because it is involved in regulation of translation. MEX-3 regulates translation of many maternal genes, such as *pal-1*, *glp-1*, etc., by binding (A/G/U) (G/U) AGN (0-8) U (U/A/C) UA, which is present in the 3′ -UTR region of mRNA. However, the relationships between genes in this network, including the MEX-3 binding site and MEX-3, remain unknown. MEX-3 is involved in development of muscle in early embryogenesis. We aimed to elucidate the molecular mechanisms of cell fate determination and the relationships between MEX-3 and development of function in early embryogenesis.

First, adult- and embryonic-stage individuals of wild-type (N2) and *mex-3* (*or20*) mutants were analyzed by proteomics using isobaric tags for relative and absolute quantitation (iTRAQ). A volcano plot, a type of scatter plot for differential expression analysis, was used to identify proteins with minimal fluctuation in expression level. These proteins were classified as upregulated or downregulated by comparison of protein expression between *mex-3* (*or20*) and N2 individuals. Maternal genes were chosen from the upregulated and downregulated groups using *in situ* hybridization patterns from NEXTDB and protein and mRNA expression data from WormBase. mRNA expression of the downregulated maternal genes was analyzed using quantitative RT-PCR (qRT-PCR) of N2 and *mex-3* (*or20*) embryos. The downregulated maternal genes were classified into 2 groups: those with high gene expression and those with unaltered gene expression by comparison of mRNA expression between *mex-3* (*or20*) and N2.

In present study, 1,474 proteins were quantitatively identified using iTRAQ shotgun proteomic analysis; a volcano plot was used to further narrow the chosen proteins to 946, based on expression level fluctuations. The 26 upregulated proteins and 25 downregulated proteins were defined as maternal genes. Eleven genes showed unaltered expression levels. Seven genes that showed unaltered expression included a MEX-3 binding site. We suggest that the translation of these 7 genes at least may be involved in positive regulation of MEX-3.

Contact: sj0001pr@ed.ritsumei.ac.jp

Quantitative proteomic and transcriptomic analysis of mutation of the maternal gene *mex-1* in *Caenorhabditis elegans*

Hiroki Yamashita[1], Takehiro Oshime[1], Aimi Tomita[1], Hisao Kojima[1], Toshiya Hayano[2], Masahiro Ito[1]

[1]Department of Bioinformatics, College of Life Sciences, Ritsumeikan University, Japan
[2]Department of Biomedical Sciences, College of Life Sciences, Ritsumeikan University, Japan

Maternal genes in *Caenorhabditis elegans* are transcribed into mRNA in the gonads of the adult stage. These translational products play a role in determining cell fate in the early embryogenesis. Regulatory cascades are one of important systems for determining cell fate. Translational factors which are translated from maternal mRNA bind the 3'-UTR of many mRNAs to regulate their translation. The *muscle excess-1* (*mex-1*) gene is a maternal gene. Deletion of *mex-1* triggers various phenotypes such as embryonic lethal, sterile, abnormal gonads and so on. The *mex-1* gene encodes a protein, MEX-1, which is expressed in germ cell cytoplasm and P granules. In addition, MEX-1 is an RNA-binding protein with tandem zinc finger domains of the CCCH type and it plays a role in determining cell fate. It is very likely that MEX-1 regulates translation of various other maternal genes because MEX-1 binds to the 3'-UTR of the maternal gene *mom-2* (*More of MS-2*) to upregulate translation of MOM-2.

The aim of this research was to investigate the mechanism of translational regulation. We performed proteomics using isobaric tags for relative and absolute quantitation (iTRAQ) and transcriptomics using next generation sequencing to identify genes whose translation is regulated by MEX-1 and to specify their function by comparing N2 and *Δmex-1* (*or286*).

Quantitative proteomic analysis using iTRAQ was performed three times. The number of identified proteins was 1708, 1813, and 1654, respectively. For subsequent analyses, we targeted 1630 proteins which were identified in at least two of the three analyses. Using a volcano plot, we identified 103 proteins that were specifically expressed in *Δmex-1* (*or286*) and 68 proteins were classified as maternal gene products from NEXTDB; 22 of the genes lacked mRNA expression data. In addition, we identified 43 genes in which the mRNA expression was unchanged between N2 and *Δmex-1* (*or286*) by transcriptomics using the next generation sequencing. Our results suggest that MEX-1 regulates translation of maternal genes, including the 43 genes identified here.

Contact: sj0012rf@ed.ritsumei.ac.jp

Chemotaxis Simulation of *Caenorhabditis Elegans* Using an Active Cord Mechanism in an Actual Environment

Shinya Nishikawa, Zu Soh, Yuichi Kurita, Toshio Tsuji

Hiroshima University, Japan

Caenorhabditis elegans (*C. elegans*) responds to various environmental stimuli such as temperature and chemicals. For responses to chemicals such as NaCl, it has been shown that both the pirouette (Pierce-Shimomura et al. 2008) and weathervane (Iino and Yoshida, 2009) mechanisms are involved in chemotaxis. To demonstrate this internal process, Izquierdo and Beer (2013) recently succeeded in identifying a minimal neural network that can adjust the direction of the computational worm model performing the weathervane mechanism. However, computer simulation approaches appear unable to verify its robustness in an actual environment. This problem was studied by Morse et al. (1998) more than a decade ago. They proposed a method using a rear wheel driven mobile robot to simulate the indeterminate noise caused by non-uniform friction and chemical diffusion. Their robot sensed brightness instead of chemical density using a photocell equipped sensor tip, and the output of the neural network model oriented the robot to approach the light source. The robot, however, was not designed to perform undulating motion and thus could not simulate a head swing that would significantly change the sensor input. In this study, we propose a model construction method using an Active Cord Mechanism (ACM-R5, Hibot, Tokyo) (Yamada et al. 2005) with the aim of demonstrating the calculation performed in the neural network of the worm. The ACM-R5 has 9 modules, and the joint angles between each module are manipulated by a laptop computer through a Wi-Fi connection. Therefore, the ACM-R5 is able to simulate the undulating motion and an omega turn followed by a sequential long-reversal similar to a pirouette mechanism. This enabled the simulation of the parallel use of both the pirouette and weather vane mechanisms for chemotaxis in an actual environment. To simulate chemotaxis, we employed a light source as a substitute for the chemical, similar to a previous study (Morse et al. 1998). The brightness of the environment was captured by a wireless video camera attached to the head module of the ACM-R5, and input to the neural network model was implemented in a laptop computer. The computer then sent the joint angles to orient the robot to the light source. Through simulation, we confirmed that the trained neural network model can robustly direct the robot to the light source. We now plan to discuss the calculation mechanisms using this trained neural network model.

Contact: s-nishi@bsys.hiroshima-u.ac.jp

WDDD: Worm Developmental Dynamics Database

Koji Kyoda[1], Hatsumi Okada[1], Tomoko Sugimoto[1], Kenichi Henmi[1], Shihoko Yashiro[1], Shuichi Onami[1,2]

[1]Laboratory for Developmental Dynamics, RIKEN Quantitative Biology Center, Japan
[2]National Bioscience Database Center, Japan Science and Technology Agency, Japan

Animal development is controlled by dynamic changes in multicellular structure and gene expression. A collection of quantitative data of cell division dynamics under a wide variety of gene perturbations would provide a rich resource for understanding the mechanism of development. We developed the Worm Developmental Dynamics Database (WDDD: http://so.qbic.riken.jp/wddd/), which is a free open database for a collection of quantitative data of cell division dynamics in early *Caenorhabditis elegans* embryos with single genes silenced by RNA-mediated interference (RNAi) (Kyoda et al., 2013). The database provides 50 sets of quantitative data for wild-type embryos and 136 sets of quantitative data for RNAi-treated embryos corresponding to 72 of the 97 essential embryonic genes on chromosome III. Each set of quantitative data contains the three-dimensional (3D) coordinates of the outlines of nuclear regions and the dynamics of the outlines over time from 1-cell to 8-cell stage at intervals of 20 seconds. The database also provides sets of the corresponding 4D differential interference contrast (DIC) microscope images. Using information in the database, we developed several computational methods to analyze early embryogenesis of *C. elegans* such as statistical method for RNAi phenotype analysis. These results demonstrate that the database enables computational biologists to have a novel opportunity for obtaining new insights into the mechanism of development. The database can be searched based on the name of open reading frame or gene targeted by RNAi. To make easy access to database contents for experimental biologists, each set of quantitative data and DIC microscope images can be synchronously viewed through a web browser without the use of a special software program. Moreover, the database provides results of the RNAi experiments including embryonic lethal analysis for all 97 essential embryonic genes on chromosome III. We plan to expand the database to include data for all embryonic essential genes on all chromosomes and five sets of data for each gene as well as the results of computational RNAi phenotype analysis.

Contact: kkyoda@riken.jp

Exploring the limit: the robustness landscape of embryonic PAR polarity

Yen Wei Lim, Fumio Motegi

Temasek Lifesciences laboratory, Mechanobiology Institute, National University of Singapore, Singapore

Cell polarization is critical for proper cell function and architectures. A hallmark of cell polarity in animal cells is the asymmetric distribution of partition defective (PAR) proteins into two distinct domains on the cell cortex. Segregation of PARs relies on self regulation through reciprocal cortical exclusion between two groups of PAR proteins - anterior PARs (aPAR) and posterior PARs (pPAR). The self-organizing PAR system dynamically shapes the cellular asymmetry with high accuracy in space and time, but the general principles that secures the robustness and adaptability of PAR polarity remains elusive.

Here, we explore to map the robustness landscape of PAR polarization topologies, identifying parameter variations in PAR protein levels capable of driving functional cellular asymmetry in one-cell stage *C. elegans* zygote. We systematically modified levels of aPAR (PAR-6) and pPAR (PAR-2) by a combination of tuned expression of transgenes, a mutation in the endogenous *par* gene, and a second mutation that increases and decreases PAR-6 level (*lgl-1* and *nos-3,* respectively). Asymmetric PAR segregation is highly stable under a wide range of changes in relative PAR-6/PAR-2 levels: 1) mutual segregation of aPAR/pPAR was extensively robust over wide ranges of higher PAR-6/lower PAR-2 conditions with non-linear changes in aPAR/pPAR boundary position. 2) In contrast, lower PAR-6/higher PAR-2 condition caused sharp loss of mutual exclusion but remained to keep asymmetric aPAR domain with homogeneous pPAR distribution. Quantitative measurement of PAR behaviors in zygotes where aPAR/pPAR ratio was off-balance showed remarkable modification of cortical PAR-6 concentration in response to different PAR-2 levels, suggesting a dynamic balance of cortical PAR levels that compensate aPAR/pPAR unbalance. We will further discuss potential mechanisms that allow PAR system to produce robust polarization with tolerance to relative PAR level variations.

Contact: erica.limyenwei@gmail.com

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