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Book of Abstracts
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Keynote I: C. elegans research: a view of the next 50 years

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Zoom link: https://hkust.zoom.us/j/98281551372?pwd=ZUJGZGV2czlhZFI3S0hKZ0gzKzNGQT09
Meeting ID: 982 8155 1372
Passcode: 149344

Over the past 50 years, we as a community have learned a great deal about *C. elegans*, making many fundamental and applied discoveries in development, neuroscience, physiology, and evolution. I will discuss my view on the next 50 years from the perspective of a worm biologist proud of the accomplishments of my and other laboratories, and from the perspective of WormBase from which I see how much more there is to know and understand. *C. elegans* is poised to make major contributions to systems biology, human genetics, and nematology. Systematic studies are fundamental to our progress and the need to study systems biology questions, both to understand cell type and the nervous system. For example, a full set of gene knockouts will help understand gene function, and bipartite expression systems, such as cGAL-UAS, can make the nervous system more genetically accessible. Human genetics increasingly needs our help to understand gene function, to embed genes in functional pathways, and to understand the consequences of variants of known or suspected importance, in for example autism spectrum disorder and Alzheimer’s Disease and related dementia. *C. elegans* also has much to offer insights into nematode biology, but the direct study of gene function in other worms is crucial to understand their interesting and important biology and can help us think like a worm. For example, the insect parasite with a microbial symbiont *Steinernema hermaphroditum* can be treated quite like *C. elegans*, and along with *Bursaphelenchus okinawaensis* provides another tractable clade IV worm.

An astacin metalloprotease is required for predation in the nematode *Pristionchus pacificus*

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Behavioral evolution in animals requires modification of many gene functions. The genetic mechanisms for the acquisition of evolutionarily novel behaviors and related traits are largely unknown. While the model nematode *Caenorhabditis elegans* feeds on bacteria, the satellite species *Pristionchus pacificus* exhibits predatory feeding behavior toward other nematodes, which is an evolutionary novel behavior. Previously we showed that serotonin and a subset of serotonin receptors modulate the tooth movement that is required for opening the prey cuticle; however, evolutionary mechanism of predation is still unclear. Using a forward genetic approach, we found that the astacin metalloprotease *Ppa-nas-6* is required for predation in *P. pacificus*. *Ppa-nas-6* mutants were defective in control of tooth movement during predation and processing of larval cuticle during molting, specifically in the mouth part anterior to the pharynx. In *C. elegans*, *nas-6* is required for the molting of the grinder, a feeding apparatus at the posterior part of the pharynx, which is absent in *P. pacificus*. Rescue experiments of *nas-6* in *P. pacificus* and *C. elegans* suggest that alteration of spatial expression patterns rather than the changes in molecular function of *nas-6* can be a key to acquiring the predation-related traits. Reporter analyses with *Ppa-nas-6* promoter in both species suggest the alteration of
A sphingolipid-mTORC1 nutrient-sensing pathway regulates animal development by an intestinal peroxisome relocation dependent gut-brain crosstalk

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Animals have developed many signaling mechanisms that alter cellular and developmental programs in response to changes in nutrients and their derived metabolites, many of which remain to be understood. Effective assays in model organisms are often essential to explore nutrient-sensing mechanisms that commonly require cross-tissue coordination under physiological conditions, which are impractical to study in immortalized cell cultures. We recently uncovered that glucosylceramides, the core structural components of glucosylated sphingolipids, act as a critical nutrient signal for the overall amino acid level to promote development by activating the intestinal mTORC1 signaling pathway. However, how the intestinal GlcCer-mTORC1 activity regulates development throughout the whole body is unknown. Through a large-scale genetic screen, we found that the peroxisome is critical for antagonizing the GlcCer-mTORC1-mediated nutrient signal. Mechanistically, deficiency of glucosylceramide, inactivation of the downstream mTORC1 activity, or prolonged starvation relocated peroxisomes closer to the intestinal apical region to release peroxisomal-beta-oxidation derived hormones that target chemosensory neurons to arrest the animal development. Our data illustrated a new gut-brain axis for orchestrating nutrient-sensing dependent development in Caenorhabditis elegans, which may also explain why glucosylceramide and peroxisome become essential in metazoans. (This article has been accepted in principle by Cell Reports)
found, however, that MOM-5 and Ror1/CAM-1 double-receptor deficient embryos showed abnormal POP-1 polarity showing that these Wnt receptors have redundant functions. We also observed abnormal POP-1 polarity in a double mutant lacking the Wnt-binding domain of MOM-5 (ΔCRD) and cam-1 null mutation. It supports that CAM-1 functions in the mid-stage embryo parallel to MOM-5. Next, we revisited the function of MOM-5 in gastrulation, which has been reported to act as a Wnt receptor for endoderm internalization. Unexpectedly, we observed that the mutant lacking the MOM-5 (ΔCRD) showed normal gastrulation. In contrast, double mutants between MOM-5 (ΔCRD) and null mutation of the CAM-1 resulted in abnormal gastrulation. It suggests that, in gastrulation, CAM-1 compensates for the loss of MOM-5’s Wnt binding activity. Our study suggests that CAM-1 supports MOM-5 function in different modes at different developmental events, and it underlies the robustness of C. elegans embryogenesis.

Dynamic evolution of telomeric-repeat motifs in the phylum Nematoda

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Telomeres are made up of tandem arrays of telomeric-repeat motifs (TRMs) and telomere-binding proteins (TBPs), which address the difficulties of end-protection and end-replication. TRMs are usually highly preserved due to the sequence specificity of TBPs, although significant TRM alterations have been observed in several taxa but not in Nematoda. We used public whole-genome sequencing data to analyze putative TRMs of 100 nematode species to investigate TRM evolution in Nematoda. We discovered that six distinct branches included specific novel TRMs, suggesting frequent TRM evolution in Nematoda. We concentrated on one of the six branches, the Panagrolaimidae family, to validate TRM evolution by collecting nematode species and obtaining whole-genome sequencing data. We also de novo assembled five high-quality draft genomes of Panagrolaimidae species and these genomes showed densely clustered arrays of the novel TRM. With reference to the telomere evolution in Caenorhabditis elegans, we comprehensively analyzed subtelomeric regions in the genomes to determine how the unique TRM evolved. We found that the novel TRM was used to preserve telomere integrity by alternative lengthening of telomeres even in species that employ the canonical TRM. We propose a hypothetical scenario in which some pre-existing TBPs may be capable of binding both canonical and novel TRMs, resulting in pre-adaptation of the novel TRM and aiding its evolution in the genus Panagrellus.

Intrinsic Sex and Vulval Cues Shape Sexually Dimorphic Branching and Behaviors of PVP interneurons in C. elegans

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Sex has tremendous impacts on assembling neural circuits that lead to sexually dimorphic behaviors. Previous studies highlight that intrinsic sex autonomously controls sexually dimorphic morphogenesis, but whether external cues contribute to sexually dimorphic development remains unclear. In this study, we show that PVP cholinergic interneurons display sexually dimorphic axonal branching near the vulva at the late larval stage in hermaphrodites but not in males. The sexually dimorphic branching requires the hermaphrodite fate of PVP neurons, but feminized PVP neurons in males do not form PVP branches, suggesting that additional hermaphroditic cues are essential. Genetically ablation of the vulva but not HSNs (hermaphrodite-specific neurons) leads to the loss of PVP branches. Moreover, ectopic vulva in hermaphrodites and males is sufficient to locally promote branch formation, indicating that the vulva serves as an instructive cue to induce branch formation. Interestingly, the local F-actin is enriched in the vulva region and precedes the future branching sites. Functional characterizations of PVP neurons suggest that sexually dimorphic branches participate in the hermaphrodite-specific egg-laying circuit. Moreover, matured branches exhibit either wing- or rod-like structure, which is highly dynamic according to nutritional status via the insulin pathway, revealing a possible adaptive role of PVP neurons. Our study thus provides mechanistic insight into how intrinsic sexes and external growth factors orchestrate to sculpt sexually dimorphic morphology for circuit wiring.

**Development / 46**

**A lipid-mTORC1 mediated amino acid sensing in C. elegans developmental regulation**

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How animals regulate their development in response to nutrient availability is a fascinating question. Here, we developed a *Caenorhabditis elegans* model to study the impact of amino acid deficiency on development. We found a leucine-derived monomethyl branched-chain fatty acid and its downstream metabolite, glycosphingolipid, critically regulates postembryonic development by mediating the mTORC1-dependent overall amino acid sensing pathway. We further found that intestinal peroxisomal positioning and hormone secretion were pivotal in mediating this lipid/mTORC1 signal to orchestrate global development. These studies present a new example that how nutrients are deeply involved in developmental decision-making.

**Neurobiology & Gene Expression / 95**

**Nuclear hormone receptor NHR-49 in the body cavity neurons mediate pathogen avoidance in C. elegans**

**Authors:** Saebom Kwon\(^1\); Kyoung-hye Yoon\(^2\)

**Co-authors:** Hee Kyung Lee \(^1\); Jessica Antonio \(^2\)
Both fight and flight are important to survive in a harmful environment. In *C. elegans*, the nuclear hormone receptor NHR-49 is a functional homolog of mammalian PPAR (peroxisome proliferator-activated receptor), and serve as an important regulator of fat metabolism. In addition to altered lipid composition, *nhr-49* mutants display a pleiotropy of defects, including shorter lifespan, impaired starvation response, and increased susceptibility to oxidative stress and pathogenic bacteria, hinting at the diverse roles that lipids play in the body. While trying to understand how NHR-49 controls immunity, we found that a significant part of the mutant’s susceptibility to *Pseudomonas aeruginosa* (PA14) was due to defective avoidance response to the pathogenic lawn. Restoring NHR-49 in the neurons significantly improved the avoidance behavior, whereas intestinal rescue did not. Among the neurons, we found that restoring NHR-49 expression in cholinergic and glutamatergic neurons was sufficient for the increased avoidance. Genetic studies showed that NHR-49 acted downstream of the TGFβ/DAF-7-mediated chemosensory detection of PA14, as well as the induction of NPR-1 ligand neuropeptides previously shown to elicit pathogen avoidance. Since NPR-1 is known to signal through a neuronal circuit that includes the oxygen-sensing neurons, we restored NHR-49 selectively in the oxygen-sensing body cavity neurons, AQR, PQR and URX neurons. We found that this was sufficient for the increased avoidance, but avoidance was not due to preference for any oxygen concentrations. We are currently trying to understand how NHR-49’s role as a lipid regulator influences neuronal function and behavior.
male mating efficiency, influencing motor events during contact with a hermaphrodite. Our findings indicate sex-specific roles of this peptide in feeding and reproduction in *C. elegans*, providing further insight into neuromodulatory control of sexually dimorphic behaviours.

**Neurobiology & Gene Expression / 85**

**A novel type of synaptic plasticity underlies salt chemotaxis learning in C. elegans.**

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The contexts such as physiological state or past experience often switch whether to move toward or avoid environmental sensory cues. In *C. elegans*, such attraction-avoidance switch is regulated by nPKC homolog PKC-1: The activities of PKC-1 in each sensory neuron determine the direction of migration on the stimulus gradients sensed by that neuron. Indeed, when animals learn salt concentration they experienced in the cultivation plate, the activity of PKC-1 in salt sensory neuron ASER is dynamically regulated, and thus animals migrate toward the learned concentration. However, the mechanism of how PKC-1 switches the direction of migration remains unclear. Here we describe the molecular pathway and synaptic plasticity driven by PKC-1, which underlies salt concentration learning.

Using neuron-specific phosphoproteomics, we identified unc-64/ syntaxin 1 as a downstream factor of PKC-1. The phosphorylation of unc-64 affects glutamate release from ASER in absence of a stimulus (tonic release). Furthermore, we investigated how the tonic release affects neurotransmission from ASER to 1st layer interneuron AIB which controls reversal. In vivo and ex vivo Ca2+ imaging of AIB revealed that the amount of tonic release reverses (inhibitory ↔ excitatory) the response of AIB to stimulus-induced input.

Finally, we investigated the characteristics of inhibitory and excitatory receptors. The inhibitory receptor, AVR-14, appeared to be much more sensitive than the excitatory receptor GLR-1. Based on these observations, we proposed a model in which differential sensitivities are used to decode the presynaptic tonic release level. Our results reveal that the learning in *C. elegans* is based on postsynaptic response reversal induced by the change in tonic release level, i.e. the novel type of synaptic plasticity.

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**The CCAAT-box transcription factor NF-Y complex mediates neuronal specification in C. elegans**

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Neuronal differentiation is coordinated through a cascade of gene expression via interactions between trans-acting transcription factors and cis-regulatory elements of their target genes. However, the mechanisms by which transcriptional regulation determines neuronal cell fate are not fully understood. In *C. elegans*, the IL1 sensory/inter/motor neurons consist of six neurons that are peptidergic as well as glutamatergic. To identify molecular mechanisms by which IL1s are terminally differentiated, we performed mutagenesis screens and isolated *nfya-1* mutants, in which *flp-3* neuropeptide gene expression is decreased in IL1. *nfya-1* encodes nuclear transcription factor Y alpha subunit, which is highly conserved from yeast to humans. We found that NFYA-1 is expressed in and localized to the nuclei of IL1, and NFYA-1 expression in the IL1 neurons of *nfya-1* mutants restores *flp-3* expression. Other IL1-expressed genes, including *eat-4* vesicular glutamate transporter gene and *unc-8* DEG/ENaC cation channel gene, are differentially affected in *nfya-1* mutants, suggesting that NFYA-1 regulates the expression of distinct terminal differentiation marker genes in IL1. In addition, we found that mutations in other subunits of NFY, NFYB-1, and NFYC-1, and a *C. elegans* paralog, NFYA-2, affect *flp-3* expression in IL1. We then performed promoter analysis of IL-1-expressed genes and identified a motif necessary and sufficient for *flp-3* expression that is similar to the mammalian CCAAT box and directly bound by the NF-Y complex. Taken together, our data indicate that NFYA-1 regulates neuronal subtypes specification via directly regulating a set of terminal-differentiation marker genes.

**Neurobiology & Gene Expression / 14**

**Mechanosensitive Piezo Channel, PEZO-1, regulates food deglutition in C. elegans**

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The PIEZO ion channel is an evolutionarily conserved mechanosensitive channel (Coste et al., 2010). Mammalian genomes encode two PIEZO genes, Piezo1 and Piezo2, of which functions have been shown to be involved in mechanosensation (Woo et al., 2014, Nonomura et al., 2017, Li et al., 2014, Rode et al., 2017). *C. elegans* genome has a single PIEZO gene, *pezo-1*, which encodes 14 isoforms (Bai et al., 2020). The molecular function of PEZO-1 in *C. elegans* has yet to be fully determined. To examine *pezo-1* function, we grouped 14 isoforms depending on the mRNA length and observed their expression patterns. The promoter region of long isoforms is specifically expressed in the pharyngeal-intestinal valve, which is predicted to mediate food swallowing (Avery and Thomas, 1997). Next, to examine whether *pezo-1* has a role in food swallowing, we fed animals with OP50-sized GFP-microsphere and found that *pezo-1* mutant animals show excess accumulation of GFP-microsphere in the anterior part of the intestinal lumen. Expression of long isoform PEZO-1 or mouse PIEZO1 under the control of valve cell-specific promoter restores the food swallowing defect of *pezo-1* mutant animals. We also observed that when GFP-microspheres are fully accumulated at the anterior part of the intestine, the pharynx is pulled posteriorly to push GFP-microspheres down into the posterior intestine. We named this as a pharyngeal plunge. We next found that the pharyngeal-intestinal valve exhibits calcium transient during pharyngeal plunge and the optogenetic activation of valve cells induces the pharyngeal plunge. Moreover, elevated pressure in the anterior part of the intestinal lumen by microinjecting buffer solution causes pharyngeal plunge, not in *pezo-1* mutant but wild-type animals. Ectopic expression of PEZO-1 in a *C. elegans* chemosensory neuron confers head bending-dependent responses. These results demonstrate that the *C. elegans* PIEZO channel regulates pharyngeal plunge and provides insights to understand the function of the mammalian PIEZO channel shown to be expressed in the esophagus.
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**Keynote II: Making the CUT: How an unusual homeobox gene family controls panneuronality**

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Much progress has been made in understanding the gene regulatory programs that drive individual neuron fates, meaning, that distinguish one neuron type from another neuron type. In contrast, it has remained enigmatic how genes that are expressed in all neurons of the nervous system, but not elsewhere ("panneuronal genes") become restricted to all cells of the nervous system and not beyond. Forward genetic screens to identify such factor(s) have remained remarkably unsuccessful. I will describe here how our genome wide expression pattern analysis of homeobox genes has identified a subfamily of homeobox genes, the CUT genes, as critical regulators of panneuronal identity. Six members of this family act in a dosage-sensitive manner to directly control the expression of panneuronal proteins, ranging from synaptic vesicle proteins to neuropeptide-processing proteins. The identification of panneuronally expressed CUT genes prompts the question of how their expression is controlled. One of the CUT genes appears to be controlled in a particularly unusual manner. Its genomic locus produces two isoforms, one the CUT homeobox transcription factor, the other a Golgi-localized protein; this structure is conserved from worm to humans. I will discuss ongoing work that attempts to understand the regulation of this locus.

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**Sex Pheromone Sensation and Habituation in C. elegans males require SRD-1 and Insulin Signaling Components**

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Passcode: 491864

Males of Caenorhabditis species are required to locate their mating partner prior to identification of vulva for spicule insertion. We previously reported that reproductively mature virgin females secrete sex pheromones from their somatic gonad to attract males (J.R. Chasnov et al., 2007). Reverse genetics approach, calcium imaging and behavioral assays suggested that sex pheromones are detected via a GPCR in the male AWA neuron. With evidences gleaned from AWA specific gene profiling along with reporter assays, RNAi analysis and ectopic gene expression, we reported SRD-1 as the putative sex pheromone specific GPCR in C. elegans males (Xuan Wan et al., 2019). As much as sex pheromone sensation is vital for locating mating partners, we observe that constant pheromone stimuli turn the males insensitive to pheromones (olfactory habituation). We characterize this phenomenon as species specific, occurring in males of C. elegans unlike C. remanei. SRD-1 orthologs are found in all Caenorhabditis species, behavioral experiments using srd-1 mutants carrying orthologous and chimeric SRD-1 revealed that pheromone habituation in C. elegans males is dependent on the cytoplasmic domain of SRD-1 receptor. With reverse genetics approach, we identify GPC-1 (G gamma subunit) as one of the putative regulators for sex pheromone habituation. Tissue and cell specific rescue of GPC-1 in C. elegans males.
substantiate the requirement for a functional nervous system, exclusively AFD and AIY neurons, to enable successful habituation to constant sex pheromone stimuli. To discern the downstream target of GPC-1, we referred to the regulatory relationship between GPC-1 and DAF-16 in life span extension (Lans and Jansen, 2007). We find that mutations in daf-16 suppress the habituation defects of gpc-1 null mutants, thereby underpinning DAF-16 as a downstream regulator of GPC-1 in sex pheromone habituation.

Upon identifying the involvement of DAF-16 in sex pheromone habituation, we probed into Insulin components and identified daf-2, age-1 mutants with pheromone sensation defects while akt-1 mutants displayed habituation defects. Similar to habituation cascade, we find that mutations in daf-16 also suppressed pheromone sensation defects observed in daf-2 mutants. Hence, Insulin signaling could potentially be a dual regulator for both pheromone sensation and habituation cascade with DAF-16 as a putative common downstream target.

Evidences from genetics, behavioral and global epistasis experiment suggest that pheromone sensation and habituation pathways may invoke nuclear translocation of DAF-16 in AWA neuron to control SRD-1 expression level. Chemo-attraction assays coupled with imaging the expression of SRD-1 receptor in insulin pathway mutants would help unveil and verify the regulatory relationship between DAF-16 and SRD-1 in specific cell(s) for the manifestation of chemosensation and habituation phenomenon.

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The contribution of AWC neuron to the perception of volatile sex pheromone component in C. elegans male

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Pheromones act as an important means of communication to unite individuals for successful reproduction. Though ascarosides are water-soluble pheromones that regulate development and sexual attraction in Caenorhabditis elegans, C. elegans hermaphrodites also produce volatile sex pheromones that attract males only when their self-sperms are depleted. In marked contrast to the ascarosides, for which over 140 compounds have now been characterized in nematodes, the chemical composition of volatile attractants has remained a mystery. Here, we identify one of the volatile sex pheromones secreted by the sperm-depleted C. elegans hermaphrodites and show males require AWC neuron to percept to it. We found two major volatile sex pheromone candidate compounds by using 2DGC-TOFMS, one of which is successfully identified as X based on a comparison of its mass spectrum and chromatographic retention time. We then performed volatile chemotaxis assays with specific mutants to clarify which neurons are responsible for the perception of X in C. elegans males. As a result, ceh-36(ks86) and njls79 mutant males are less attractive to X, indicating C. elegans males require AWC neuron to percept to X. We anticipate that this study will contribute to a better understanding of the sex pheromone communication mechanisms, the molecular and neuronal basis of chemosensory behaviors. It will also help to understand how volatile sex pheromones emerged across animal kingdom in the process of evolution.
Inter-tissue communication of mitochondrial stress response and aging

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The ability of the nervous system to coordinate the systemic mitochondrial proteostasis is essential for the overall fitness of an organism under stress conditions and during aging. We found that neuronal mitochondrial stress communicates stress signals to peripheral tissues via a secreted Wnt ligand EGL-20, coordinating mitochondrial proteostasis between neurons and the intestine. Furthermore, we found that worms that experienced neuronal mitochondrial stress pass on a stress memory to their descendants by propagating the elevated copy number of mtDNA through the germline for more than 50 generations. The Wnt pathway is necessary for the inheritance of this stress memory. The descendants with stress memory exhibited greater tolerance to stresses and lived longer but grew more slowly and produced fewer offspring as tradeoffs.

Key words: Mitochondrial stress, Aging

Characterization of yolk-related subcellular structures in C. elegans and their age-associated changes by electron microscopy

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Vitellogenins, the precursors of yolk proteins, are the most abundantly expressed proteins in C. elegans hermaphrodites during adulthood. After being synthesized in the intestine, vitellogenins are transported to oocytes and passed on to embryos and larvae. Vitellogenins or yolk proteins play an important role in post-embryonic development (Rompay, Scientific Reports, 2015) and adult senescent pathologies (Ezcurra, Current Biology, 2018). The subcellular structures of these abundant proteins are not well characterized, especially the ones in the intestine. We have created knock-in alleles to label the six vitellogenins (VIT-1 to VIT-6) with fluorescent protein tags and shown that they colocalized with each other. Using immuno-electron microscopy and conventional electron microscopy, we find that the yolk organelles (YOs) in the gonad and vitellogenin-containing vesicles (VVs) in the intestine are both uniformly medium electron density vesicles enclosed by a lipid bilayer. Intestinal vitellogenins are also found on rough ER and the Golgi apparatus. In the body cavity, there are pockets of yolk proteins between the invaginated basal membrane of the intestinal cells. The above morphological evidence suggests that classic exocytosis mediates vitellogenin secretion. In old worms, we see an increase in the size of VVs and fusion of VVs in the intestine cells, and an increase of high-density yolk in the body cavity. In old worms, yolk proteins are also found mislocalized in the hypodermis, gonad sheath, and oviduct. In summary, this study provides
an accurate documentation of the changes of the subcellular structures that contain vitellogenins or yolk proteins during *C. elegans* aging.

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**Regulatory mechanism of cold-inducible diapause**

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Diapause is widely used as a criterion of a genetic screen for aging genes in *C. elegans*. We recently discovered a novel type of diapause that is induced by low-temperature stimuli. At 9℃, N2 animals could develop into adult worms, but a mutation of *hsf-1*, a heat shock transcription factor 1, caused developmental arrest at the L1 stage. This growth arrest phenotype was recovered when *hsf-1* mutants were shifted to the normal temperature conditions (20℃). Therefore, we named the phenotype "Cold-Inducible Diapause (CID)". The *hsf-1* mutant animals could survive in CID for at least a month with maintaining fertility.

We also found that the CID entry was regulated by several longevity genes, such as *daf-16*, *xbp-1*, *skn-1*, and *hlh-30*. These longevity genes completely inhibited the CID entry at 9℃. CID is also controlled by the neural circuit, especially tyraminergic signaling. A loss of function mutation of *tdc-1*, a key enzyme of tyramine synthesis, prevented the CID entry in *hsf-1* mutants. However, octopamine that is converted from tyramine did not affect the CID entry.

Furthermore, we isolated non-CID mutants from *hsf-1* mutants background by EMS mutagenesis, and found that 30% of obtained non-CID mutants were longer-lived than *hsf-1* background mutants. By MutMap analysis, we identified novel regulators of CID: *smg-1* and *smg-2*, that were reported as aging genes previously. The results suggest that CID can be used as a tool to screen aging genes. We are now exploring other longevity genes using CID as a criterion and investigating the regulatory mechanism of CID in detail.

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**Deregulation of alternative 3′ splice site selection contributes to physiological aging in C. elegans**

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Aging is associated with changes in biological processes at multiple levels, including the transcriptomic level. Age-associated changes scale with two types of aging: chronological and physiological
aging. Here we comprehensively analyzed transcriptomic features that scaled with physiological and chronological aging using *C. elegans*. We first showed that noncoding RNAs and intron-derived transcripts were generally upregulated during aging. We also found that the usage of distal 3′ splice sites (3′ ss) increased during aging. Next, to dissect the transcriptomic features associated with physiological and chronological aging, we compared transcriptomes of wild-type and long-lived daf-2/insulin/IGF-1 receptor mutant animals at various ages. We showed that age-dependent upregulation of noncoding RNAs and intron-derived transcripts were similar between wild-type and *daf-2* mutant animals, and scaled with chronological ages. In contrast, we showed that the usage of distal 3′ ss scaled with physiological ages, because the age-dependent upregulation was decelerated in *daf-2* mutants. We also noted that mRNAs with different biological functions, including RNA processing, were downregulated with physiological aging. We then sought to identify factors that mediated age-dependent increases in transcriptomic deregulation. We showed that differential expression of particular RNA-processing factors contributed to physiological aging via modulating the usage of 3′ ss. Overall, our study identified the age-dependent alteration of features that match physiological and chronological aging and demonstrated that the usage of distal 3′ ss is associated with physiological aging. Our study will provide novel insights into dissecting aging at the transcriptomic level in various organisms, including *C. elegans*.

Hyper-activation of the sensory neuron leads to decreased stress tolerance and lifespan in *C. elegans*

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Neuronal activity can influence a broad range of physiological functions, including longevity and brain tumor growth. The mechanisms by which neuronal activity affects stress resistance and longevity are poorly understood. Here, we demonstrate a case of neuronal activity modulating organismal stress tolerance and lifespan in *C. elegans*, in which hyper-activation of thermosensory neurons by high temperature can markedly decrease worms’ heat tolerance, proteostasis, and lifespan. *cmk-1* mutant with lower thermosensory neuron activity or decreasing thermosensory neuron activity in WT through genetic methods shows improved heat tolerance and longer lifespan. The abovementioned beneficial effects via reduced neuronal activity are mediated by releasing of INS-1 neuropeptide from the distinct downstream neural circuit and subsequent activation of DAF-16/FOXO in the intestine. Our findings indicate that hyper-activation of certain neuron populations can have adverse consequences, thereby providing an explicit example of neuronal activity regulating organismal physiology through brain-gut signaling.

Long-term ibuprofen exposure induces reproduction decline via impairment of spermatogenesis in non-target organism *C. elegans*
Ibuprofen, a non-steroidal-inflammatory drugs (NSAIDs), is one of the most commonly used drugs. Due to its large consumption, continuous exposure to low concentrations of ibuprofen has gained increasing attention for their potential risk to the environment. There is increasing evidence that ibuprofen can cause adverse impact on reproduction, but few studies have investigated adverse effects of ibuprofen on spermatogenesis. This study assessed effects of ibuprofen on spermatogenesis in male *C. elegans*. We show that ibuprofen exposure affected spermatogenesis, decreasing the total brood size, germ cell number, and sperm activation, and also increasing the sperm malformation rate. Moreover, spermatogenesis and TGF-beta related genes might be involved in ibuprofen-induced reproductive toxicity via affecting the quality and quantity of spermatid. Our findings suggest the impairment of spermatogenesis of ibuprofen exposure and the consequent decline in male fertility, leading to potential risks to ecological health, especially to non-target organisms.

Protein disulfide isomerase PDI-6 regulates Wnt secretion to coordinate inter-tissue UPRmt activation and lifespan extension

Coordination of inter-tissue stress signaling is essential for organismal fitness. Neuronal mitochondrial perturbations activate the mitochondrial unfolded-protein response (UPRmt) in the intestine via the mitokine Wnt signaling in Caenorhabditis elegans. Here, we found that the protein disulfide isomerase PDI-6 coordinates inter-tissue UPRmt signaling via regulating the Wnt ligand EGL-20. PDI-6 is expressed in the endoplasmic reticulum (ER) and interacts with EGL-20 through disulfide bonds that are essential for EGL-20 stability and secretion. pdi-6 deficiency results in misfolded EGL-20, which leads to its degradation via ER-associated protein degradation (ERAD) machinery. Expression of PDI-6 declines drastically with aging, and animals with pdi-6 deficiency have decreased lifespan. Overexpression of PDI-6 is sufficient to maintain Wnt/EGL-20 protein levels during aging, activating the UPRmt, and significantly extending lifespan in a Wnt- and UPRmt-dependent manner. This study reveals that protein disulfide isomerase facilitates Wnt secretion to coordinate the inter-tissue UPRmt signaling and organismal aging.

Histone H3K36 dimethyltransferase SET-18 increases mitochondrial ROS and promotes aging by activating NADase tir-1 in Caenorhabditis elegans
The mitochondrial free radical theory of aging (MFRTA) implies the key role of mitochondria in aging control. Accumulating evidence shows mutation or RNAi of some histone lysine methyltransferases and demethylases extend or shorten lifespan. However, whether and what signaling transmitted from histone methylation marks in chromatin to mitochondria that determines longevity remains elusive to date. In our previous report, we identified \textit{C. elegans} SET-18, a SET and MYND domain containing protein (SMYD) homologous, as a novel H3K36me2 methyltransferase enriched in muscle, and found it shortened lifespan. In this study, we discovered that SET-18 promoted mitochondrial fragmentation and increased mtROS level during aging, and \textit{tir-1} (a mammalian NADase \textit{sarm1} homologous) was a novel target gene activated by SET-18-mediated H3K36me2 modification. Disrupting TIR and SAM domains of TIR-1 decreased NAD+ level of worms, suggesting TIR-1 had NADase activity \textit{in vivo}. Significantly, we found that these two domains were critical for the roles of SET-18 in raising mtROS and shortening worm lifespan. As NAD+ is a major metabolite generated in mitochondria, our findings reveal that the hydrolysis of NAD+ is an unreported signaling that histone lysine methyltransferase transmits from chromatin to mitochondria to determine lifespan.

Keywords: SET-18; H3K36me2 modification; NADase; mitochondrial ROS; aging

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Axonal mitochondria regulate gentle touch response through control of actin dynamics

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Mitochondria are present in the cell body, along neuronal processes and are often enriched at energy demanding regions of a neuron. They are important for ATP production and buffering cytosolic calcium. Along the neuronal processes of \textit{C. elegans} touch receptor neurons(TRNs), we observe that about 80-90% of axonal mitochondria are present at actin rich regions in L4 and young adults. The distribution of axonal mitochondria and F-actin change together across development. L1 animals lack uniform mitochondrial distribution which is achieved at L2 stage. This transition coincides with changes in the distribution of F-actin rich regions and actin dynamics. We investigated the relationship between F-actin and axonal mitochondria. On constitutively depolymerizing F-actin with a genetic tool-deAct, the largely uniform mitochondrial distribution is lost. We used \textit{ric-7} mutants that lack mitochondria in axons to assess the relationship between mitochondria and actin. All \textit{ric-7(£)}, alleles tested lacked F-actin dynamics along the axons. Artificially driving mitochondria along only the TRN neuronal processes in \textit{ric-7} mutants through a motor attached to mitochondria restored uniform mitochondrial distribution and actin dynamics. The anti-apoptotic protein, CED-9, has been shown to regulate actin at synapses via mitochondria. However, we do not observe any role for CED-9 in axonal actin dynamics. To understand the importance of axonal mitochondria and F-actin, we assessed gentle touch responses of animals. L1 and deAct animals with a random mitochondrial distribution and no F-actin/F-actin dynamics respectively have reduced gentle touch responsiveness. Additionally, \textit{ric-7(£)} animals with no mitochondria along the neuronal process show reduced gentle touch responsiveness. However, artificially driving mitochondria into TRN...
processes restores gentle touch responsiveness suggesting that mitochondrially derived F-actin are important for gentle touch response. Expressing a constitutively active form of RHO-1 in TRNs of ric-7(lf) mutants restored actin dynamics and gentle touch responsiveness. Thus, mitochondria along the neuronal process of TRNs are important for F-actin dynamics in vivo and mitochondria mediated F-actin dynamics is necessary and sufficient for gentle touch response.

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**FLP-17 regulates a novel oviposition behavior that increases maternal reproductive fitness in low oxygen environments**

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Parental behaviors are an evolutionary adaptation for the benefit of offspring survival. Here, we identified a novel oviposition behavior when cultivated in 3D environments that increases the survival of the young. *C. elegans* lays eggs directly on the OP50 lawn with no discernable pattern in 2D. In 3D, however, the mothers display a stereotypical behavior remaining near the edge of the spherical bacterial colony, temporarily leaving the area to lay her eggs far away from the bacteria resulting in eggs surrounding the colony. We show mutants of *flp-17*, which encodes a FMRF-like neuropeptide expressed solely in the BAG sensory neurons, and *egl-6*, which encodes the cognate receptor of FLP-17 in the HSN motor neurons, were defective for oviposition behavior, laying eggs close to the bacteria. Transgenic rescue of *flp-17* restores normal oviposition behavior. In addition, genetic ablation of the BAG and HSN neurons in *egl-46* and *egl-1* mutants, respectively, inhibits oviposition behavior. Previous studies have shown that FLP-17 is involved in both O2 sensation, and we demonstrate that due to the embedded bacteria, worms are exposed to a low oxygen environment in 3D. We tested egg-laying in mother worms in 2D and determined that 7% O2 could induce an oviposition behavior in a *flp-17*-dependent manner. *flp-17* mutants had significantly decreased brood size in 3D compared to 2D, and we wondered how oviposition behavior could increase reproductive fitness. We placed eggs inside and outside an OP50 lawn in low oxygen, and most of the eggs inside the lawn died, whereas nearly all the eggs outside survived. Overall, we describe an oxygen-sensing circuitry mediated by a neuropeptide and its cognate receptor that alters egg laying behavior to increase survival of the young in toxic environments. We speculate that oviposition behavior is an adapted product of evolution in the natural habitat of *C. elegans*.

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**UNC-104 anterograde bias is regulated by ubiquitination**

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UNC-104 gets degraded at synapses through ubiquitin-mediated pathways depending on its ability to bind cargo. Ubiquitination is also known to regulate protein function without leading to degradation. Thus we investigated whether, in addition to degradation, ubiquitination of UNC-104 can alter its function. To identify E3s that ubiquitinate UNC-104, we carried out a Touch Receptor Neuron-specific RNAi screen of 230 neuronally enriched E3 ubiquitin ligases. We identified *fbxb-65* as a potential regulator of ubiquitination of UNC-104. We observe UNC-104 has an anterograde bias in an ablation-based assay and an UNC-104 particle tracking assay. In RNAi of the E1 ubiquitin-activating enzyme, *uba-1*, and *fbxb-65*, UNC-104’s anterograde bias is increased due to an increased anterograde flux likely due to increased UNC-104 bound to cargo. These observed alterations of UNC-104 movement likely result in an increased anterograde displacement of RAB-3, a UNC-104 cargo, in the neuronal process in *uba-1* and *fbxb-65* RNAi. This suggests that in *uba-1* and *fbxb-65* knockdown, increased ATP-dependent UNC-104 transport is likely due to a greater fraction of cargo bound UNC-104. Together, these data suggest that ubiquitination may regulate UNC-104’s ability to bind cargo and in turn maintains an equilibrium of UNC-104’s anterograde and retrograde flux.

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**Study of PhosphatidylSerine Receptor (PSR) pathway in dendrite regeneration using C. elegans.**

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Neurons are interconnected with each other via synapses to form functional circuitry. When damaged, it can lead to partial or complete loss of function. One therapeutic approach is to encourage the regrowth and rewiring of broken neurites into a functional circuitry. Although the mechanisms of axonal regeneration are studied in detail, our knowledge of dendrite regeneration is limited. We use PVD neurons in *C. elegans* as a model for studying dendrite regeneration as they have elaborate dendrites. Previous studies using PVD neurons showed that primary dendrites can regenerate independent of major axonal regeneration pathways (Brar et al 2022). Also, the same study revealed that dendrite regeneration is dependent on RacGTPase CED-10 and RhoGEF TIAM-1. Since CED-10 is activated by PhosphatidylSerine Receptor (PSR) during phagocytic clearance, we hypothesised that PS might get accumulated near the site of dendrotomy. Using PS sensor AnnexinV::GFP, we observed PS accumulation at the injured tip of dendrite. Among various mutants we tested, the loss of integrin receptor *ina-1* which works upstream to GEF during phagocytosis perturbed dendrite regeneration. Two important aspects of dendrite regeneration namely ‘extent of regrowth and ‘reconnection between proximal and distal tip’ were assayed. We found that both the extent of regrowth and total branch length are affected in *ina-1* mutant similar to both *ced-10* and *tiam-1* mutants. Therefore, it is possible that *ina-1*, *tiam-1* and *ced-10* could be acting in a pathway to regulate dendrite regeneration. We will present how PS exposure is related to CED-10 pathway in dendrite regeneration.
The roles of AMPK and downstream effectors in swimming exercise mediated enhancement in axon regeneration

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Inefficient axon regeneration in the human nervous system impairs functional recovery after an accidental injury. With aging, both the function and axon regeneration capacity of the nervous system decline. The beneficial effects of physical activity are well established. Functional restoration through a rehabilitation approach is a promising therapeutic direction. As physical exercise has complex systemic effects, identifying the specific downstream effectors might be useful to help design better therapeutic strategies. A swimming-related exercise paradigm in *C. elegans* shows many features of physical exercise as seen in mammalian systems (Laranjeiro et al., 2017).

Using the gentle touch neuron (PLM) of *Caenorhabditis elegans*, which is responsible for posterior gentle touch sensation, we previously found that a single swimming session of 90 minutes duration after axotomy of PLM improved behavioral recovery through axon regeneration (Kumar et al., 2021). The benefit of exercise is dependent on the metabolic energy sensor AMPK/AAK-2. The same exercise regime also ameliorates the aging-associated decline in touch response in the day-5 stage. We found at least 12 hours is required after the swim session for behavioral improvement. Moreover, the alpha subunit of metabolic sensor AAK-2 is required for mediating the beneficial effects of swimming exercise. We tested several downstream effectors of AMPK and found DAF-16 and MDT-15 are required for the beneficial effect of swimming exercise. We are currently studying the tissue-specific requirement of AMPK and its downstream effectors using molecular genetics and RNAi experiments.

References:

Unraveling the importance of Extracellular Vesicles of Caenorhabditis elegans against candidate bacterial pathogen

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Extracellular vesicles (EVs) are membrane-bound vesicles which include exosomes and microvesicles. By transferring nucleic acid, protein, and small molecules, they are involved in various biological activities including molecular transport and stress responses. These vesicles are secreted by various biological systems. The cargos in EVs vary depending on their biological origin. Caenorhabditis elegans is a nematode model organism which is widely used for studying host-pathogen interaction and aging-related diseases. In C. elegans extracellular vesicles play a major role in the cuticle development, larval development, signaling, metabolism, aging, and immune regulation. In this study, the EVs secreted by C. elegans during normal and pathogen-exposed condition were analyzed for their role during bacterial infection. The isolated EVs were confirmed through scanning electron microscope imaging. Further, the presence of CD63 biomarker was identified using Western blotting. Protein profiling of isolated EVs through LC-MS/MS and MALDI-ToF/ToF analyses showed the presence of several immune-regulatory proteins in addition to tetraspanins. The role of isolated EVs of C. elegans was studied by analyzing its anti-bacterial and anti-virulent activities against candidate bacterial pathogen. It was found that EVs of C. elegans formed the zone of inhibition against Klebsiella pneumoniae and the same was validated by using the EVs biogenesis-deficient mutant strain VC4714 (vps-4). In addition, the isolated EVs showed anti-virulent activity against K. pneumoniae infected worms. The impact of EV protein/metabolite on C. elegans during bacterial infection was studied using protein-metabolite interaction under in-vitro conditions. Further studies are needed to understand the molecular interaction of EV cargo with whole protein of C. elegans.

Key words: Extracellular vesicles, Caenorhabditis elegans, anti-bacterial activity, anti-virulent activity, Protein and Metabolite

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Cultivating C. elegans in its true ecological niche: a peek into host-microbiome interaction and its role in nematode growth and reproduction

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In nature, organisms live in constant contact with other biological organisms sharing a complex ecological interaction from predator-prey, host-parasite, and other symbiotic relationships. Studies have shown that host-microbe interaction helps define processes affecting host development, reproductive fitness, and animal behavior. Here we sought to understand Caenorhabditis elegans ecology, behavior, and physiology while observing it in a simulated natural habitat in the laboratory. Since it has been reported that environmental changes affect animal behavior and such changes in behavior are better observed in an organism’s natural behavior, we developed a soil-fruit-based nematode habitat that will simulate the true ecological niche for C. elegans in the laboratory. In this preliminary study, C. elegans were subjected to varying environmental factors such as temperature, humidity, light, and a microbial community to help determine which are optimal for C. elegans growth and reproduction. We also did a preliminary observation of the ecological succession occurring in the soil...
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Dissecting Neuro-Immune Regulation in Caenorhabditis elegans

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Tightly regulated immune responses are paramount for host health, since ineffective responses can exacerbate infections while hyperactivation can be deleterious to hosts. Recent evidences from vertebrates demonstrate previously unanticipated roles for the nervous system in immune regulation. However, understanding neuroimmune regulation has been challenging owing to the complexity of vertebrate nervous and immune systems. These complexities could be overcome by using models with a simple, well-characterized nervous system, such as the nematode Caenorhabditis elegans. C. elegans has 302 neurons (1/3rd of somatic cells), each of whose connections are mapped, making it ideal for studying the nervous system. These neurons perform a variety of complex functions such as olfaction and chemo-sensation. The largest chemosensory organ in the worm is constituted by 12 neuron pairs, namely the amphid sensory neurons, and aids in perceiving environmental stimuli. Based on previous understandings that C. elegans mounts pathogen-specific immune responses, we hypothesized that the amphid sensory neurons assist in pathogen-sensing, and subsequently regulate immune responses. Using neuronal ablation lines, we show that the amphid sensory neurons differentially regulate immune responses in C. elegans during various infections. Most of these neurons play pathogen-specific roles and in particular, we have identified a neuron that negatively regulates immunity in the worm by directly suppressing key immune pathways and evolutionarily conserved stress response regulators, laying foundations for systemic immune homeostasis. Overall, our study demonstrates for the first time, direct roles for the amphid neurons in immune regulation and could facilitate better understandings in neuroimmune regulations in higher animals.

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A volatile ketone from oyster mushrooms disrupts plasma membrane integrity and triggers organismal death in nematodes

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Carnivores have evolved diverse strategies to efficiently capture their prey. The oyster mushroom Pleurotus ostreatus is a carnivorous fungus that attacks and paralyzes nematode prey via their sensory cilia. However, the chemical identity of the nematocidal toxin produced by P. ostreatus and
its killing mechanism had been unclear. Through genetic screens of *P. ostreatus* to isolate mutants displaying loss of toxicity (lot) to the nematode *Caenorhabditis elegans*, we discovered that spherical toxocysts present at intervals along fungal hyphae are required to paralyze nematodes. Toxocysts are fragile structures and, when disrupted, immediately lost their nematocidal activity, implying that the active compound is volatile in nature. GC-MS analyses detected 3-octanone in the toxocysts, and treatment of *C. elegans* with 3-octanone recapitulated phenotypes of rapid paralysis, calcium influx and neuronal cell death arising from fungal contact. We found that 3-octanone disrupts nematode cell membrane integrity in multiple tissues, resulting in massive extracellular calcium influx into the cytosol and mitochondria. Calcium influx caused cell death that propagated throughout the entire organism. Finally, we demonstrate that structurally-related compounds also display prominent biotoxicity in *C. elegans*, with the carbon number of the ketone being crucial. Our work reveals that the oyster mushroom has evolved a specialized structure, the toxocyst, to accumulate a natural ketone to a high local concentration that disrupts the plasma membrane integrity, leading to cell and organismal death in nematodes.

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**Keynote III: Natural variation in *C. elegans*: ecology, viral susceptibility and non-standard heredity**

**Author:** Marie-Anne Felix

1 Ecole Normale Supérieure

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*C. elegans* does not only grow in laboratories. Natural populations of *C. elegans* relatives can be found in ephemeral rotting vegetal material such as rotting stems, flowers and fruits. By surveying *Caenorhabditis* populations in an orchard and a wood over several years, we probed its metapopulation structure and outcrossing rate. With other labs, we discovered >60 new species in the *Caenorhabditis* genus and thus provide a framework for genomic, phenotypic and ecological studies.

Several natural pathogens were isolated, including the first viruses that infect *C. elegans* or *C. briggsae*. Using genetic association (GWAS) and recombinant inbred lines approaches, our team identified causal polymorphisms. We also find that different bacteria strongly affect the susceptibility of *C. elegans* to the Orsay virus.

In *C. elegans*, non-standard genetic heredity includes the transmission of small RNAs affecting histone modifications and gene expression. Our work shows that *C. elegans* harbors natural genetic variation in such non-genetic inheritance phenomena, including the mortal germline phenotype and memory of RNA interference.

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**How to escape a fungal predator if you were *C. elegans***?

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Between the host and pathogens, cell adhesion upon contact usually is the first step toward the establishment of infection. Similarly, predation between the fungal predator and the nematode prey begins with immediate adhesion of the nematodes to the fungal traps. The nematophagous fungus *Arthrobotrys oligospora* preys on nematodes when nutrients are scarce in the environment. This fungus develops complex adhesive nets to trap nematodes when nematodes contact the trap hyphae. The adhesive nets contain extensive layers of extracellular polymers, which are critical for traps to adhere to nematodes cuticle. However, the exact molecular targets on nematodes and the adhesion interaction are still unclear. To address this question, we conducted forward genetic screens in *C. elegans* and isolated the mutants that became resistant to *A. oligospora* trapping. Using genetic mapping and whole-genome sequencing, we identified that mutations in a nuclear hormone receptor would enable the nematodes to escape from traps. This transcription factor is expressed in the intestine, seam cell, and the hypodermis. Through tissue-specific rescue, we demonstrated that the site of action of the *nhr* gene is in hypodermis and seam cells. To identify the target genes regulated by the *nhr* transcription factor, we conducted RNAseq analyses to compare the transcriptome between the wild-type and the *nhr* mutant and identified more than 60 collagen genes were downregulated in the *nhr* mutant. Since the collagen proteins are the major components of the nematode cuticle, it suggests that altered nematode surface (cuticle) could result in the escape behavior. Our study reveals that collagens are some of the major targets that mediate adhesion between the fungal traps and the nematode prey.

**Metabolism & Pathogenesis / 18**

**Control of PI(4,5)P2 homeostasis via non-vesicular lipid transport regulated by PDZD-8 and TEX-2**

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Cellular membranes maintain unique lipid compositions that are important for their functional identity. PI(4,5)P2 is enriched in the plasma membrane where it contributes to local activation of key cellular events, including actomyosin contraction and cytokinesis. However, how cells prevent PI(4,5)P2 from accumulating in intracellular membrane compartments, despite constant intermixing and exchange of lipid membranes via membrane trafficking, remains poorly understood. Using the *C. elegans* early embryo as our model system, we show that the evolutionarily conserved lipid transfer proteins (PDZD-8 and TEX-2) and PI(4,5)P2 phosphatases (UNC-26/synaptojanin and OCRL-1) act together to prevent the build-up of PI(4,5)P2 on endosomal membranes. In the absence of these four proteins, large amounts of PI(4,5)P2 accumulate on endosomes, leading to embryonic lethality due to ectopic recruitment of proteins involved in actomyosin contractility. PDZD-8 localizes to sites of contact between the endoplasmic reticulum and late endosomes and regulates endosomal PI(4,5)P2 levels via its lipid harboring SMP domain. Accumulation of PI(4,5)P2 on endosomes is accompanied by impairment of their degradative capacity. Thus, cells use multiple redundant systems to maintain endosomal PI(4,5)P2 homeostasis. Mutations in human homologs of UNC-26 and OCRL-1 are linked to Parkinson’s disease and Lowe syndrome, respectively. Our study reveals an exciting possibility that manipulating the activity of the lipid transfer proteins may help alleviate disease progression in patients with dysfunction in these proteins.
Modeling microbe-host interaction in neurodegenerative diseases

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Growing evidence indicates that gut microbiota plays a critical role in regulating the progression of neurodegenerative diseases such as Parkinson’s disease. The molecular mechanism underlying such microbe–host interaction is unclear. In this study, by feeding Caenorhabditis elegans expressing human α-syn with Escherichia coli knockout mutants, we conducted a genome-wide screen to identify bacterial genes that promote host neurodegeneration. The screen yielded 38 genes that fall into several genetic pathways including curli formation, lipopolysaccharide assembly, and adenosylcobalamin synthesis among others. We then focused on the curli amyloid fibril and found that genetically deleting or pharmacologically inhibiting the curli major subunit CsgA in E. coli reduced α-syn–induced neuronal death, restored mitochondrial health, and improved neuronal functions. CsgA secreted by the bacteria colocalized with α-syn inside neurons and promoted α-syn aggregation through cross-seeding. Similarly, curli also promoted neurodegeneration in C. elegans models of Alzheimer’s disease, amyotrophic lateral sclerosis, and Huntington’s disease and in human neuroblastoma cells.

Temperature acclimation in C. elegans regulated by brain-gut coupling: from thermosensation to metabolic changes in gut fat.

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Acclimation to the higher or lower temperature is an essential function to survive in nature. The nematode C. elegans has temperature acclimation, a phenomenon in which it becomes acclimated to a temperature change in just a few hours, resulting in altered survival after cold stimulus at 2°C for 48 hours. Three pairs of thermosensory neurons involved in temperature acclimation: ASJ, ADL, and ASG in the head. However, downstream interneuron of thermosensory neurons and further downstream event to alter cold tolerant of the body has been unknown. Here, we show that neural circuits for processing temperature signals can alter the accumulation of fat in the gut and facilitate acclimatization to new temperatures. We found that cyclic AMP response element-binding protein (CREB) facilitates temperature acclimation at ASJ sensoryneuron and RMG interneuron simultaneously. Interestingly, the tail interneuron PVQ is involved in transmitting temperature information from ASJ to RMG to control temperature acclimation. CREB also modulates thermal responsivity of thermosensoryneuron ASJ depending on past cultivation temperature. Next focusing on the downstream events to drive the body cold tolerant or not directly, we found that intestinal triglycerides were more accumulated in the 15°C cultivated worms, which are cold tolerant to 2°C, than in the 25°C-cultivated non-cold tolerant worms. This metabolic change in the gut fat was under controlled by...
ASJ-PVQ-RMG temperature signaling. Thus, we proposed a new experimental model for brain-gut coupling in temperature acclimation of animal (Motomura, Ohta et al., in press).

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Dietary bacteria repress C. elegans fat content by increasing de novo phosphatidylcholine synthesis and lysosome-related organelle biogenesis

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Diet, which contains micro- and macro- nutrients, regulates organisms’ metabolism. However, how dietary micronutrients modulate gene expression and organelle dynamics in host to regulate fat metabolism is not fully understood. Here, we take advantage of C. elegans and its dietary bacterial strains C. aquatica DA1877 (DA) and the standard E. coli OP50 (OP) to study host-microbial diet interactions. By taking lipidomics and transcriptomics approaches, we found that the DA, compared to OP, diet reduces the fat level in C. elegans by transcriptional down-regulation of fat-7. fat-7 encodes delta(9)-fatty-acid-desaturase and has been shown to be important for lipogenesis. To identify genes responsible for the DA-induced repression of fat-7, we undertook a genetic screen for mutations that increased fat-7::GFP expression in DA. We found that genes involved in B12 metabolism and biosynthesis of S-adenosyl methionine (SAM) and phosphatidylcholine (PC) are required for fat-7 downregulation and fat reduction. Specifically, high PC reduces fat-7 expression through transcription factor SBP-1/SREBP. Interestingly, the SAM-PC axis leading to fat-7 downregulation is also important for increased lysosome-related organelles (LROs) observed in worms fed DA. An experiment using fluorescent fatty acid labeling suggests that LROs are important for fatty acid storage. In addition, mutations that reduce LRO biogenesis suppress DA-mediated fat reduction. These results reveal a novel role of fat-7 in promoting the fat level by increasing LRO biogenesis. We propose that high B12 in the DA diet represses the fat level in C. elegans through the SAM-PC pathway and LRO biogenesis.

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A chromodomain protein mediates heterochromatin-directed piRNA expression

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piRNAs play significant roles in suppressing transposons, maintaining genome integrity, and defending against viral infections. How piRNA source loci are efficiently transcribed is poorly understood. Here, we show that, in C. elegans, transcription of piRNA clusters depends on chromatin microenvironment and a chromodomain-containing protein UAD-2. piRNA clusters form distinct focus in germline nuclei. We conducted a forward genetic screening and identified UAD-2 that is required for piRNA focus formation. In the absence of histone 3 lysine 27 methylation or proper chromatin remodeling status, UAD-2 is depleted from the piRNA focus. UAD-2 recruits the upstream sequence transcription complex (USTC), which binds the Ruby motif, to piRNA promoters and promotes piRNA generation. Vice versa, the USTC complex is required for UAD-2 to associate with the piRNA focus. Thus, transcription of heterochromatic small RNA source loci relies on coordinated recruitment of both the readers of histone marks and the core transcriptional machinery to DNA.

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Inter-tissue microRNA signalling controls the ageing of C. elegans tissues

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The inter-tissue signalling is essential to the systematic decline of physiological functions in ageing. Recent studies highlight secreted microRNAs as a new class of intercellular signalling molecules. We previously discovered in C. elegans that a secreted microRNA from the intestine disrupts autophagy in the body wall muscle during ageing. To systematically explore the role of inter-tissue microRNA signalling in worm ageing, we isolated cells from five key somatic tissues in young and aged worms and profiled the age-dependent mRNA and microRNA transcriptomic changes within these tissues. In addition, we further profiled the change of miRNAs transcription in these tissues and the alteration of miRNAs levels in purified worm EVs. Combining these datasets, we found a complex miRNA trafficking network across worm tissues, which is regulated by ageing through miRNAs transcription and selective secretion. This inter-tissue miRNA trafficking network, together with the autonomous transcriptional regulation, modulates gene expression in tissues and thus coordinates ageing throughout the body.

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INPP5K and Atlastin-1 maintain the non-uniform distribution of endoplasmic reticulum-plasma membrane contacts in neuron

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In neurons, the endoplasmic reticulum (ER) extends throughout all cellular processes, forming multiple contacts with the plasma membrane (PM) to fine-tune neuronal functions. However, the mechanisms that regulate the distribution of neuronal ER-PM contacts remain elusive. In this study, we used the C. elegans DA9 motorneuron to explore the molecular mechanisms that maintain the distribution of neuronal ER-PM contacts. We developed a novel strategy for visualizing ER-PM contacts in live C. elegans neurons using a split GFP approach, and found that neuronal ER-PM contacts are highly enriched in the somatodendritic region and generally absent from the axon. Using a forward genetic screen, we identified that two proteins involved in ER shaping, the dynamin-like GTPase ATLN-1 (human Atlastin-1) and the inositol 5-phosphatase CIL-1 (human INPP5K), help to maintain the non-uniform, somatodendritic enrichment of neuronal ER-PM contacts. Genetic and cell biological assays revealed that CIL-1 acts upstream of ATLN-1 to maintain the balance between tubules and sheets of the ER at cell cortex and restrict the distribution of ER sheets to somatodendrites. In mutants with reduced activities of CIL-1 or ATLN-1, ER sheets expand and invade into the axon. This was accompanied by the ectopic formation of axonal ER-PM contacts and defects in axon regeneration following laser-induced axotomy. Mutations in Atlastin-1 and INPP5K have been linked to various neurological disorders, including hereditary spastic paraplegia and intellectual disability. The unique distribution of neuronal ER-PM contacts maintained by these proteins may support neuronal resilience during the onset and progression of these human disorders.

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**UBR-1 Regulates Rhythmic Behavior through Inhibitory Glutamate Signaling**

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The E3 ubiquitin ligase UBR-1 has been typically considered as the most important component in the process of protein ubiquitination. Mutations in the human UBR-1 are associated with the Johanson-Blizzard Syndrome (JBS), which is an autosomal recessive disorder. However, the molecular mechanisms of UBR-1 regulation are poorly understood. Here we report that UBR-1 regulates the defecation motor program mediated by inhibitory glutamate neurotransmission in Caenorhabditis elegans. In particular, loss of UBR-1 leads to reduced expulsion frequency, which could be compensated by removing vesicular loading of glutamate in GABAergic neurons. In addition, two conserved glutamate-gated chloride channels GLC-2 and GLC-3 receive DVB’s glutamate signals to inhibit intestinal muscles and DVB neuron, respectively. Thus, we propose a dual function model in enhancing neuronal activity for UBR-1 through balancing a co-transmission of excitatory GABAergic and inhibitory glutamatergic signalings, which may provide new insights into JBS disease.
CHDP-1 is required for dendritic development and transport in C. elegans neurons

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Cortical actin, a thin layer of actin network underneath the plasma membranes, plays critical roles in numerous processes such as cell morphogenesis and migration. Neurons often grow highly branched dendrite morphologies, which is critical for neural circuit assembly. It is still poorly understood how cortical actin assembly is controlled in dendrites and whether it is critical for dendrite development, maintenance and function. In the present study, we find that knock-out of C. elegans chdp-1, which encodes a cell cortex localized protein, causes dendrite formation defects in the larval stages and spontaneous dendrite degeneration in adults. Actin assembly in the dendritic growth cones is significantly reduced in the chdp-1 mutants. PVD neurons sense muscle contraction and act as proprioceptors. Loss of chdp-1 abolishes proprioception, which can be rescued by expressing CHDP-1 in the PVD neurons. In the high-ordered branches, loss of chdp-1 also severely affects assembly of the microtubule cytoskeleton, transport of intracellular organelles and secretion of neuropeptides. This study will help us to understand the mechanisms and functions of neural cytoskeleton assembly in dendritic development and transport.

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Electric shock causes a fear-like persistent behavioral response in the nematode Caenorhabditis elegans

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Behavioral persistency reflects internal brain states, which are the foundations of multiple brain functions. However, experimental paradigms that enable genetic analyses of behavioral persistency and its associated brain functions have been limited. By using a new experimental paradigm, we revealed novel persistent behavioral responses caused by electric stimuli in the nematode Caenorhabditis elegans. We first discovered that, when the animals on bacterial food are stimulated with alternating current of 30 V, their movement speed suddenly increases more than 2-fold, which persists for several minutes even after electric stimulation is terminated. Interestingly, a different response is observed when a stronger electric stimulus is applied: with 75 V, the speed does not increase on average during the stimulus but does increase immediately after the stimulus removal, which lasted for a few minutes. Genetic analyses demonstrated that multiple types of voltage-gated channels are required for the response, possibly as the sensors, and neuropeptide signaling regulates the duration of the persistent response. Additional behavioral analyses suggest that the animal’s response to electric shock is scalable and has a negative valence. These properties, along with persistency, have been recently regarded as essential features of emotion, suggesting that the animal’s response to electric shock may express a form of emotion, such as fear. Currently, we are analyzing the neurons in which the voltage-gated channels function, and related whole brain activities. Combining
findings from this series of experiments, we hope to elucidate the underlying molecular and neural mechanisms of behavioral persistency which may be related to emotion.

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**Keynote IV: Asymmetry and sexual dimorphism of the PVD neurons**

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Meeting ID: 977 2340 3873
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The *C. elegans* bilateral PVD neuron pair develop a complex yet stereotypical dendritic branching pattern. This ordered structure is maintained in early adulthood, yet gradually accumulates additional processes and becomes disorganized. Little is known regarding the bilateral differences in this homeostatic process. We show that early adult animals display robust dorso-ventral and left-right asymmetries for additional branching presence. Using mutant analysis, we demonstrate that some asymmetries do not depend on the initial degree of hyperbranching. Further, these asymmetries do not reflect enhanced plasticity, as determined by a similar outgrowth response to dendritic damage. Additionally, we show that male PVD neuron structure retains some asymmetries seen in the hermaphrodite, but presents additional, sexually dimorphic, traits.

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**Genetic pathways for biogenesis of precursors synaptic vesicles**

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Synaptic vesicles are defined organelles at synapses essential for synaptic transmission. Synaptic vesicle proteins are trafficked in vesicles from the cell body into axons using the synaptic vesicle motor, UNC-104. A Genetic enhancer and suppressor screens using an allelic series of cargo binding defective synaptic vesicle motor, UNC-104/KIF1A, mutants led to the identification of a hierarchical pathway for biogenesis of precursors of synaptic vesicles. UNC-16/JIP3 and LRK-1/LRRK2 respectively regulate the exclusion of non-synaptic vesicle proteins like golgi resident enzymes and lysosomal proteins. JIP3 and LRK-1 both also regulate the inclusion of synaptic vesicle proteins to form the final UNC-104/KIF1A dependent precursor of synaptic vesicle protein transport carriers that exists the cell body. The genetic pathway also identified a synaptic vesicle-lysosome post-golgi compartment through which sorting and cell body retention of unsorted precursors of synaptic vesicles occurs. This sorting from the synaptic vesicle-lysosome compartment depends on both LRK-1/LRRK2, the clathrin adaptor protein AP3 and the active zone molecule SYD-2/Liprin-α. This genetic pathway is also critical for polarized distribution of synaptic vesicle proteins such that they are targeted to axons and not dendrites.
CSR-1 RNA interference pathway restricts holocentromere protein CENP-A/HCP-3 localization in Caenorhabditis elegans

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CSR-1 is an argonaute of a RNA interference pathway that is important for chromosome segregation in C. elegans. Live-cell imaging revealed that CSR-1 depletion slows down spindle pole separation in a kinetochore-dependent manner. In csr-1(RNAi) embryos, the kinetochores may be misattached to the microtubules and chromosome segregation is disrupted. On the holocentromeres, there are increased levels of some kinetochore proteins, including the centromeric epigenetic mark, CENP-A or HCP-3. Without affecting HCP-3 expression level, HCP-3 density is higher on stretched chromatin fibers in CSR-1-depleted embryos. The increased HCP-3 deposition on chromatin after CSR-1 depletion is at least partially independent of HCP-3 loading factors, KNL-2 and LIN-53, suggesting a non-classical, improper HCP-3 loading pathway. Negative regulation of HCP-3 holocentromere loading by CSR-1 required its slicer activity and the b isoform. CSR-1 acts as a HCP-3 repressor for its chromosomal occupancy, shedding light on the role of RNAi pathways in specifying the localization of centromere proteins.

Phosphoregulation of DSB-1 mediates control of meiotic double-strand break activity

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In the first meiotic cell division, proper segregation of chromosomes in most organisms depends on chiasmata, exchanges of continuity between homologous chromosomes that originate from the repair of programmed double-strand breaks (DSBs) catalyzed by the Spo11 endonuclease. Since DSBs can lead to irreparable damage in germ cells, while chromosomes lacking DSBs also lack chiasmata, the number of DSBs must be carefully regulated to be neither too high nor too low. Here, we show that in Caenorhabditis elegans, meiotic DSB levels are controlled by the phosphoregulation of DSB-1, a homolog of the yeast Spo11 cofactor Rec114, by the opposing activities of PPH-4.1 phosphatase and ATL-1 kinase. Increased DSB-1 phosphorylation in pph-4.1 mutants correlates with reduction in DSB formation, while prevention of DSB-1 phosphorylation drastically increases the number of meiotic DSBs both in pph-4.1 mutants as well as in the wild type background. C. elegans and its close relatives also possess a diverged paralog of DSB-1, called DSB-2, and loss of dsb-2 is known...
to reduce DSB formation in oocytes with increasing age. We show that the proportion of the phospho-
ylated, and thus inactivated, form of DSB-1 increases with age and upon loss of DSB-2, while non-phosphorylatable DSB-1 rescues the age-dependent decrease in DSBs in dsb-2 mutants. These results suggest that DSB-2 evolved in part to compensate for the inactivation of DSB-1 through phosphorylation, to maintain levels of DSBs in older animals. Our work shows that PPH-4.1, ATL-1, and DSB-2 act in concert with DSB-1 to promote optimal DSB levels throughout the reproductive lifespan.

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G1 cyclins regulate reproductive fitness

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As an organism ages, different tissues undergo decline in functionality at different rates. In humans, female reproductive aging is accelerated during mid-life leading to poor oocyte quality as well as increased chances of infertility, miscarriages and birth defects. The reproductive fitness of an organism has been shown to be regulated by the nutrient sensing Insulin/IGF-1 signaling (IIS) pathway. Worms with lowered insulin signaling (active DAF-16/FOXO) display better oocyte quality and extended reproductive span. Here, we show a non-canonical role of two G1 phase cyclins- cyd-1/cyclin D and cye-1/cyclin E, in regulating reproductive fitness. In the absence of these G1 cyclins, oocyte quality in worm deteriorates, resulting in severe defects in oocyte morphology and increased cases of dead eggs. These worms have shorter reproductive span and show increased uterine tumors. Under such circumstances, if DAF-16 is activated, as in under low IIS, it acts as a quality control checkpoint and completely prevents entry of germ cells to oogenesis, thus leading to sterility. Interestingly, we found that while cye-1 regulates oocyte quality (and germline arrest under low IIS) in a germline cell-autonomous manner, cyd-1 is required in the vulva to regulate oocyte quality (and germline arrest under low IIS) cell non-autonomously. Moreover, the sterility under low IIS upon knock-down of cyd-1 was rescued by loss of f3r-1, while loss of lin-35/Rb rescued sterility due to cye-1 depletion. Taken together, our study shows a novel role for G1 cyclins in regulating oocyte quality and role of active DAF-16/FOXO to act as a checkpoint to monitor oocyte quality and prevent production of faulty progeny.

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Centrosome maturation requires phosphorylation-mediated sequential domain interactions of SPD-5

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Centrosomes consist of two centrioles and the surrounding pericentriolar material (PCM). The PCM expands during mitosis in a process called centrosome maturation, in which PCM scaffold proteins play pivotal roles in recruiting other centrosomal proteins. In *Caenorhabditis elegans*, the scaffold protein SPD-5 forms a PCM scaffold in a polo-like kinase 1 (PLK-1) phosphorylation-dependent manner. However, how SPD-5 assembles the PCM scaffold is still unclear. To understand the mechanism by which SPD-5 is localized to the centrosomes and participates in PCM scaffold formation, we conducted a domain analysis of SPD-5 in vivo, and identified three functional domains. Here, we propose a model for SPD-5 scaffold assembly during centrosome maturation through sequential interactions of these domains mediated by PLK-1-dependent phosphorylation. Firstly, SPD-5 is recruited to centrioles through a direct interaction between its centriole localization (CL) domain and the centriolar protein PCMD-1. Then, intramolecular and intermolecular interactions between the SPD-5 phospho-regulated multimerization (PReM) domain and the PReM association (PA) domain are enhanced by phosphorylation by PLK-1, which leads to PCM scaffold expansion. Our findings suggest that the sequential domain interactions of scaffold proteins mediated by PLK-1 phosphorylation are an evolutionarily conserved mechanism of PCM scaffold assembly.