# ACeS 2019

## The second Australian *C. elegans* Symposium

## October 23–25

Queensland Brain Institute
The University of Queensland

Sponsored by the Clem Jones Centre for Ageing Dementia Research at the Queensland Brain Institute, The University of Queensland

THE UNIVERSITY OF QUEENSLAND
AUSTRALIA

# Second Australian *C. elegans* Symposium, 2019

**Queensland Brain Institute, The University of Queensland**

Organized by: Massimo A Hilliard, CJCADR, Queensland Brain Institute, The University of Queensland
Brent Neumann, Biomedicine Discovery Institute, Monash University
Roger Pocock, Biomedicine Discovery Institute, Monash University
Peter Boag, Biomedicine Discovery Institute, Monash University

| Day 1 Wednesday 23 October | |
|---|---|
| 11am–2pm | **Registration**, Level 3 Reception |
| 2–2.15pm | **Welcome**, Level 7 Auditorium, Queensland Brain Institute, The University of Queensland, Australia |
| 2.15–3pm | **Session 1: Keynote lecture 1**<br>Chair: Roger Pocock, Monash University |
| 2.15pm | **Re-engineering *C. elegans*—genome defence mechanisms and non-Mendelian inheritance**<br>A/Prof Christian Frøkjær-Jensen, King Abdullah University of Science and Technology (KAUST), Saudi Arabia. |
| 3–4.45pm | **Session 2: Mechanisms of Development I**<br>Chairs: Alyson Ashe, The University of Sydney<br>Arnaud Ahier, The University of Queensland |
| 3pm | **Insights into the function and evolution of nematode RNAi pathways**<br>Miguel Almeida, Institute of Molecular Biology, Mainz, Germany. |
| 3.15pm | **NHL-2, a versatile player in RNA biology of *C. elegans***<br>Joshua Anderson, Biomedicine Discovery Institute, Monash University, Melbourne, Australia. |
| 3.30pm | **Afternoon tea**, Level 7 Terrace |
| 4pm | **Invited talk: GCNA interacts with Spartan and Topoisomerase II to regulate genome stability**<br>Gregory Davis, School of Biomedical Sciences, Federation University, Churchill, Australia. |
| 4.30pm | **Disruption of mitochondrial factor SDHA-2 affects sperm motility and male fertility**<br>Rachel Woodhouse, The University of Sydney, New South Wales, Australia. |
| 4.45–7pm | **BBQ**, Level 7 Terrace |

| Day 2 Thursday 24 October | |
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| 9–9.45am | **Session 3: Keynote lecture 2**<br>Chair: Massimo Hilliard, The University of Queensland |
| 9am | **Modeling non-cell-autonomous mechanisms of neuronal proteotoxicity**<br>A/Prof Sandra Encalada, The Scripps Research Institute, La Jolla, California, USA. |
| 9.45–10.45am | **Session 4: Synapse Formation and Function**<br>Chair: Zhaoyu Li, The University of Queensland |
| 9.45am | **Invited talk: Functional development and ageing in *C. elegans* neuromuscular junction**<br>Jie Liu, Biomedicine Discovery Institute, Monash University, Melbourne, Australia. |
| 10.15am | **A dual role for the UNC-13 M domain in $Ca^{2+}$-triggered neurotransmitter release**<br>Haowen Liu, CJCADR, QBI, The University of Queensland, Brisbane, Australia. |
| 10.30am | **$Ca^{2+}$-triggered neurotransmitter release requires two $Ca^{2+}$ sensors in *C. elegans***<br>Lei Li, CJCADR, QBI, The University of Queensland, Brisbane, Australia. |
| 10.45–11.15am | **Morning tea**, Level 7 Terrace |
| 11.15am–12.30pm | **Session 5: Ageing, Degeneration and Repair I**<br>Chairs: Linda Dansereau, Garvan Institute of Medical Research<br>Steffen Nørgaard, Copenhagen University |
| 11.15am | **Dopamine transporter function is co-regulated by secreted ferritin**<br>Gawain McColl, The Florey Institute of Mental Health, Melbourne, Australia. |
| 11.30am | **Oxidative stress: identification and study of a novel molecule with a neuronal protective function**<br>Alessandra Donato, CJCADR, QBI, The University of Queensland, Brisbane, Australia. |
| 11.45am | **Developing tools for Alzheimer's disease research**<br>Neha Sirwani, La Trobe University, Melbourne, Australia. |
| 12.00pm | **Ursolic acid promotes neurite outgrowth and protects against axon degeneration in *Caenorhabditis elegans***<br>Wenyue Wang, Biomedicine Discovery Institute, Monash University, Melbourne, Australia. |
| 12.15pm | **The α-tubulin acetyltransferase MEC-17/αTAT1 is essential for robust axonal regeneration**<br>Jean-Sébastien Teoh, Biomedicine Discovery Institute, Monash University, Melbourne, Australia. |
| 12.30–1.30pm | **Lunch**, QBI reception lawn, front of building |
| 1.30–3pm | **Session 6: Sensory Function and Behaviour**<br>Chairs: Lilian Wong, Florey Institute of Neuroscience and Mental Health<br>Ramon Martinez-Marmol, The University of Queensland |
| 1.30pm | **Invited talk: Understanding the multiplex connectome of *C. elegans*: a focus on nociceptor sensitisation**<br>Yee Lian Chew, University of Wollongong, New South Wales, Australia. |
| 2pm | **Neuronal misexpression of fusogens results in neuron-neuron fusion and altered behaviour**<br>Rosina Giordano-Santini, CJCADR, QBI, The University of Queensland, Brisbane, Australia. |
| 2.15pm | **Neuronal regulation of diacetyl chemotaxis in *C. elegans***<br>Anubhuti Dixit, Amity Institute of Neuropsychology and Neurosciences, Amity University, Noida, India. |
| 2.30pm | **Nociceptive response and neural circuit**<br>Wei Wang, QBI, The University of Queensland, Brisbane, Australia. |
| 2.45pm | **ETS-5 regulates BAG-specific insulin signalling to control intestinal metabolism**<br>Ava Handley, Biomedicine Discovery Institute, Monash University, Melbourne, Australia. |
| 3–6pm | Poster session, Coffee and refreshments provided |
| 6–7.30pm | **Cocktail party**, Level 7 Terrace |

| Day 3 Friday 25 October | |
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| 9.30–10.30am | **Session 7: Ageing, Degeneration and Repair II**<br>Chair: Ramesh Narayanan, ANZAC Research Institute |
| 9.30am | **Invited talk: ATFS-1 localises to the mitochondria to protect mitochondrial DNA from damage**<br>Steven Zuryn, CJCADR, QBI, The University of Queensland, Brisbane, Australia. |
| 10.00am | **Role of Dynamin GTPases in axonal fusion**<br>Tarika Vijayaraghavan, Biomedicine Discovery Institute, Monash University, Melbourne, Australia. |
| 10.15am | **The metalloprotease ADM-4 promotes regenerative axonal fusion**<br>Xue Yan Ho, CJCADR, QBI, The University of Queensland, Brisbane, Australia. |
| 10.30–11.15am | **Morning tea**, Level 7 Terrace |
| 11.15am–12pm | **Session 8: Mechanisms of Development II**<br>Chair: Ina Kirmes, The University of Queensland |
| 11.15am | **Bringing together what belongs together – The *WormJam* international research community for *C. elegans* systems biology and metabolic modelling**<br>Horst Joachim Schirra, Centre for Advanced Imaging, The University of Queensland, Brisbane, Australia. |
| 11.30am | **Understanding the genetic basis of organismal susceptibility to xenobiotics using *Caenorhabditis elegans* and *Drosophila melanogaster***<br>Nida Moin, CSIR-Indian Institute of Toxicology Research. |
| 11.45am | **Loss of SAX-7 suppresses axonal defects of *ctbp-1* mutant animals**<br>Tessa Sherry, Biomedicine Discovery Institute, Monash University, Melbourne, Australia. |
| 12pm | **Prizes and concluding remarks** |

# Re-engineering *C. elegans*—genome defence mechanisms and non-Mendelian inheritance

Christian Frøkjær-Jensen.

*King Abdullah University of Science and Technology (KAUST).*

Cells face the problem of regulating the expression of endogenous genes while at the same time identifying and silencing foreign genes such as transposable elements. This is a particular challenge in germ cells where any deleterious effects of unrestrained transposition could have deleterious consequences for all future progeny. Silencing mechanisms in the *C. elegans* germline are mediated by small RNAs and repressive chromatin. However, pathways that prevent silencing are less well understood. We have characterized a very abundant non-coding DNA sequence (named Periodic An/Tn clusters, PATCs) that makes up approximately 10% of the *C. elegans* genome which appears to play a role in "self" recognition. We have demonstrated that the inclusion of PATCs in transgenes can prevent transgene silencing in repressive chromatin domains and repetitive extra-chromosomal arrays. PATCs scale with their chromatin environment over evolutionary time suggesting that a substantial fraction of the non-coding genome of *C. elegans* is under selective pressure. We will show how we have used the ability to generate silencing resistant transgenes to engineer a novel mode of inheritance, which is not Mendelian. This mode of inheritance allows the creation of well-defined mosaic animals, "transfer" of mitochondria, and rapid homozygosing of strains at all loci.

# Insights into the function and evolution of nematode RNAi pathways

Miguel Vasconcelos Almeida[1], Sabrina Dietz[1], Andrea Hildebrandt[1], Christian Renz[1], António M. d. J. Domingues[1], Hahn Witte[2], Ralf J. Sommer[2], Helle D. Ulrich[1], Julian König[1], Falk Butter[1], and René F. Ketting[1].

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[2] *Max Planck Institute for Developmental Biology, Department of Evolutionary Biology, Max-Planck-Ring 5, 72076 Tübingen, Germany.*

Argonaute proteins bind small RNAs (sRNAs) and together regulate gene expression across all domains of life. In eukaryotes, Piwi-interacting RNAs (piRNAs) and endogenous small interfering RNAs (endo-siRNAs) are the main sRNA pathways silencing transposable elements. The nematode *Caenorhabditis elegans* expresses multiple classes of sRNAs, including 21U-RNAs, which are considered the piRNAs of *C. elegans*, and endo-siRNAs. GTSF1 proteins are evolutionarily conserved factors that are required, in the animal germline, for normal fertility and transposable element silencing, by physically interacting with Piwi Argonautes. Given the lack of conserved factors acting in the 21U-RNA/piRNA pathway, we wanted to dissect the role of the single GTSF-1 ortholog in *C. elegans*. We found that *gtsf-1* mutants display fertility defects similar to its orthologs in mouse and flies. Surprisingly, we found that GTSF-1 is not required for transposable element silencing, nor for 21U-RNA biogenesis and function. Instead, we have shown that GTSF-1 is required for the biogenesis of endo-siRNAs by interacting, via its CHHC zinc fingers, with the RNA-dependent RNA Polymerase RRF-3. Importantly, GTSF-1 is required for the assembly of the protein complex that assists RRF-3 in the biogenesis of endo-siRNAs. We propose that a common denominator of GTSF1 function may be to promote the assembly of multi-subunit effector complexes, in the context of sRNA pathways. To address whether the participation of GTSF-1 proteins in the endo-siRNA pathway is conserved in nematodes, we initiated comparative studies of GTSF-1 homologs in additional nematode species. We identified and mutated *gtsf-1* orthologs in *C. briggsae* and *Pristionchus pacificus*, and found that GTSF-1 is required for normal fertility in these two species, similar to its orthologs. However, GTSF-1 is required for endo-siRNA biogenesis only in *P. pacificus*, further demonstrating the evolutionary plasticity of GTSF-1 proteins.

# NHL-2, a versatile player in RNA biology of *C. elegans*

Joshua Anderson, Rhys Colson, Nasim Saadati, Peter Boag.

*Development and Stem Cells Program, Monash Biomedicine Discovery Institute and Department of Biochemistry and Molecular Biology, Monash University, Melbourne, VIC, Australia.*

Small RNA pathways are functionally diverse and have critical roles in maintaining genome organisation and regulating gene expression. Generally, each small RNA pathway is categorised by the Argonaute the small RNA associate with, and tissue they perform their function(s) in (soma or germline). In addition to the Argonaute, other proteins are important for the effector complex function efficiently. One such co-factor is NHL-2, one of five TRIM-NHL family member proteins of *C. elegans*, originally identified as a miRISC modulator for *let-7* and *lsy-6* targets in the soma. We now show that in addition to the miRNA pathway NHL-2, plays an essential role in the *C. elegans* germline. In *nhl-2*(*ok818*) mutants there is aberrant accumulation of the repressive histone modification (H3K9me2) on germline autosome chromatin, defects in oocyte chromosome organisation and high level of embryonic lethality. We also found an unexpected role for NHL-2 in the nuclear RNAi pathway, and accompanying temperature-sensitive transgenerational fertility defect. Interestingly, NHL-2 physically associates with DRH-3, CSR-1 and HRDE-1, suggesting it may act in multiple points of small RNA pathways (with DRH-3 of the RdRP complex and with AGO effectors of the RISC). High throughput sequencing of small RNAs in *nhl-2*(*ok818*) mutants reveals a depletion of 22G-RNAs for a subset of CSR-1 and WAGO target genes. Moreover, alterations in the distribution of 22G-RNAs across CSR-1 target genes suggest a mechanism of NHL-2 action in 22G- RNA biogenesis, and implicate NHL-2 in distinct roles for germline versus somatic small RNA pathways. RNA binding assays demonstrate that NHL-2 is a bona fide RNA binding protein that specifically associates with U-rich sequences. Together, our results show that NHL-2 is a key factor in multiple facets of germline and somatic gene regulation.

# GCNA interacts with Spartan and Topoisomerase II to regulate genome stability

Gregory M. Davis.

*School of Health and Life Sciences, Federation University.*

Germ cell chromosomes are subjected to continuous activity that threatens their genomic integrity, including meiotic double stranded DNA breaks, homologous recombination, and a large exchange of histones. Due to this, genomic surveillance and repair mechanisms are vital in germ cells and ensure the survival of a species. While several forms of DNA damage repair maintain genomic homeostasis, the DNA-protein crosslink repair pathway (DPC) removes proteins that are inappropriately covalently crosslinked to DNA. This can occur from exposure to ionizing radiation, chemical crosslinkers, and trapped enzymatic intermediates. Topoisomerases modify DNA and are required for multiple aspects of chromosome biology, including initiating single stranded and double stranded DNA breaks via TOP-1 and TOP-2 respectively. Due to this, topoisomerase associated DPCs occur frequently and need to be resolved to prevent genomic lesions and mutations from occurring. TOP-1 DPCs are amended during S phase via co-expression of the highly conserved eukaryotic protein, Spartan/DVC-1. However, TOP-2 is highly expressed in the G2/M phase, and an understanding of how TOP-2 DPC repair is facilitated remains unknown. Using *C. elegans* and mouse embryonic stem cells, we show that the highly conserved germline and embryo enriched protein, GCNA-1, physically interacts and colocalises with TOP-2 on condensed chromosomes during M phase. In addition to this, CGNA-1 mutants are hyper sensitive to inhibition of TOP-2, but not TOP-1, suggesting a role for GCNA-1 in processing TOP-2 DPCs. Collectively, this study shows that GCNA-1 is a highly conserved protein that is essential for maintaining germline and embryonic genomic integrity.

# Disruption of mitochondrial factor SDHA-2 affects sperm motility and male fertility

Rachel Woodhouse and Alyson Ashe.

*The University of Sydney, School of Life and Environmental Sciences, Sydney, New South Wales, 2006, Australia.*

SDHA is a component of the succinate dehydrogenase (SDH) complex and plays a critical role in mitochondria in both the citric acid cycle and mitochondrial respiration. In the citric acid cycle SDHA converts succinate to fumarate. Additionally, this reaction contributes electrons to the electron transport chain, responsible for driving ATP synthesis. *C. elegans* have two orthologues of SDHA, SDHA-1 and SDHA-2. Here, we show that mutation in *sdha-2* results in dramatically reduced male fertility due to defective sperm activation. We found that *C. elegans* harbouring an *sdha-2* SNP produce a significantly diminished brood size, one fifth of that of wild-type animals. In vitro sperm activation assays reveal that most mutant sperm do not activate from spermatids to spermatozoa, failing to grow the pseudopod required for motility. We show that, as a result, mutant sperm fail to localise to the spermatheca in hermaphrodites, the site of oocyte fertilisation. Instead, sperm display aberrant localisation throughout the uterus. Mutant animals with a large deletion in *sdha-2* exhibit the same phenotype as the SNP mutant. We repaired the *sdha-2* SNP in the endogenous locus to wild-type sequence using CRISPR-Cas9, and demonstrate complete rescue of brood size and sperm activation. The SNP is located in the SDHA-2 FAD binding domain, implicating the conversion of succinate to fumarate. We are currently testing other components of both the citric acid cycle and the electron transport chain for sperm defects. Additionally, we performed an EMS suppressor screen and have identified several candidates in which mutations suppress the brood size defect, providing clues about the pathway by which SDHA-2-dependent sperm activation failure occurs. Our results demonstrate a role for SDHA-2 in sperm motility and male reproductive health. In humans, mutations in *sdha-2* are associated with Leigh Syndrome, and this strain may provide a new animal model of Leigh Syndrome.

# Modeling non-cell-autonomous mechanisms of neuronal proteotoxicity

Sandra Encalada.

*The Scripps Research Institute, La Jolla, California, USA.*

Non-cell-autonomous mechanisms of neurodegeneration occur in the proteinopathies, but how neuronal toxicity is generated from misfolded proteins expressed in non-neuronal tissues is unclear. Moreover, whether modifying protein aggregate levels via degradation at distal locales (i.e. non-cell-autonomously), can modulate degeneration of post-mitotic neurons, remains largely unknown, but could be an important strategy for reducing aggregation. Our lab generated *C. elegans* models of the transthyretin (TTR) amyloid diseases, where non-cell-autonomous toxicity is the default mechanism. Outside the brain and the eye, TTR is produced primarily by the liver -which remains unaffected- but sensory peripheral pain- and thermo-sensing neurons of the extremities are impaired and ultimately lost in TTR-driven Familial Amyloid Polyneuropathy (FAP). How peripheral neurons degenerate in FAP remains unknown, as neither mouse nor *Drosophila* TTR models have recapitulated non-cell-autonomous neuronal toxicity.

Expressing familial TTR mutations exclusively in the body-wall muscle of *C. elegans* resulted in non-cell-autonomous dendritic branching defects, axonal mitochondrial fragmentation, and intracellular trafficking impairments in nociceptive sensory and in touch neurons, rendering animals partially unresponsive to pain stimuli. Importantly, this neurotoxicity was enhanced by inactivating TTR degradation cell non-autonomously, which increased non-native oligomeric TTR aggregate load. TTR-mediated toxicity was attenuated pharmacologically with a small molecule that stabilizes TTR against dissociation, misfolding and aggregation, as in humans. To gain insight into mechanisms of neuronal toxicity in FAP models, we further characterized the subcellular phenotypes in animals expressing TTR in the muscle or in PVD neurons. We found that FAP-associated TTR mutations resulted in the mislocalization of axonal proteins to dendrites and vice versa, resulting in neuronal polarity reversals. Moreover, PVD dendritic branching in TTR transgenic animals was significantly reduced. Both of these phenotypes are also observed in PVD neurons of kinesin-1 *unc-116* (*e2310*) and dynein heavy chain-1 *dhc-1* (*or195ts*) molecular motor mutants. Our findings suggest that TTR aggregation targets molecular motor function to drive proteotoxicity. Moreover, our work reveals a critical role for modulation of TTR degradation within distal tissues, as well as suggesting that activation of autophagy or analogous lysosomal degradation mechanisms in these tissues should be considered as a strategy for treating the TTR amyloidoses.

# Functional development and ageing in *C. elegans* neuromuscular junction

Jie Liu.

*Biomedicine Discovery Institute, Monash University, Victoria.*

Synapses experience both structural and functional changes, called synaptic plasticity, in different development stages. However, the biological basis of functional development and ageing of synapses is not well understood. *C. elegans* neuromuscular junctions (NMJ), chemical connections between motor neurons and muscle cells, share fundamental molecular mechanisms of neurotransmission with that in humans, which offer an attractive platform for decoding functional changes of neurotransmitter in different developmental stages. Our previous research show age-related slow locomotion in *C. elegans* stems from the decreased neurotransmission of presynaptic motor neurons, which followed by age-related functional decline in postsynaptic muscle cells. Here, we further study the functional development of *C. elegans* NMJ form larval stage to adult. We find that gamma-aminobutyric acid (GABA) initially acts as a depolarization neurotransmitter in the L1 larvae and switches to hyperpolarizing muscle cell from the L2 stage. Interestingly, further investigation on the L1 NMJs shows that cholinergic motor neurons trigger ventral muscle contraction with extrasynaptic neurotransmitter release. Our results provide insights into the diversity of spatial pattern of neurotransmitter release and the translation of the physiological function of GABA in different life stages.

# A dual role for the UNC-13 M domain in $Ca^{2+}$- triggered neurotransmitter release

Haowen Liu[1], Lei Li[1], Yi Yu[1], Jing Tang[1], S. Sheoran[2], Janet E. Richmond[2] and Zhitao Hu[1].

[1] *Clem Jones Centre for Ageing Dementia Research, Queensland Brain Institute, The University of Queensland, Brisbane, Australia.*
[2] *Biological Sciences, University of Illinois Chicago, Chicago, IL.*

Synaptic vesicle (SV) priming and fusion require the Munc13 family of proteins, several of which (e.g. Munc13-1, Munc13-2, and ubMunc13-2) have been shown to be essential in regulating short-term synaptic plasticity. However, the underlying molecular mechanisms remain unclear. The nematode *C. elegans* expresses two UNC-13 isoforms, UNC-13L and UNC-13S (also called UNC-13MR). Here we report a novel dual function of the N-terminal M domain in *C. elegans* UNC-13MR, a Munc13-2 orthologue. Deleting the M domain in UNC-13MR led to a significant increase in tonic and evoked neurotransmitter release, as well as the size of the readily releasable vesicle pool, revealing an inhibitory function of the M domain in SV priming and fusion. The inhibitory effects of the M domain were eliminated in the absence of the C1 and C2B domains. This suggests that the M domain inhibits the C1-C2B module during synaptic transmission. Interestingly, we found that the M domain directly promoted SV fusion when fused to the MUNC2C fragment, which has been shown to be the minimal region required for priming and fusion. These findings reveal that the M domain regulates synaptic transmission via dual modes. However, it is still unclear how it switches between these modes under physiological conditions.

# $Ca^{2+}$-triggered neurotransmitter release requires two $Ca^{2+}$ sensors in *C. elegans*

Lei Li, Haowen Liu, Yi Yu, Jing Tang and Zhitao Hu.

*Queensland Brain Institute, The University of Queensland, Brisbane, Australia.*

Synaptic vesicles (SVs) fuse with plasma membrane to release neurotransmitters. Several members in Synaptotagmin family proteins have been recognized as $Ca^{2+}$ sensors to mediate SV release through their tandem C2 domains (C2A and C2B). Here we report that SV release at the *C. elegans* neuromuscular junctions (NMJs) requires two $Ca^{2+}$ sensors, SNT-1 and SNT-3. Stimulus-evoked excitatory postsynaptic current (EPSC) was reduced by 60% in *C. elegans* mutant lacking *snt-1*, a mouse *Syt1* homolog. Electrophysiological screening of all other Synaptotagmin isoforms have identified SNT-3 as a second $Ca^{2+}$ sensor required for $Ca^{2+}$-triggered neurotransmitter release. The remaining evoked release in *snt-1* mutant was completely abolished in *snt-1*; *snt-3* double mutants. However, SV release was normal in *snt-3* single mutants, suggesting that the SNT-3 serves as a $Ca^{2+}$ sensor only when SNT-1 is absent. Compared to SNT-1 which has an N-terminal transmembrane domain (TM), SNT-3 lacks a TM, leading to a diffused expression in the axons. Biochemical evidence showed that SNT-3 interacts with plasma membrane, and the C2 domains have a similar $Ca^{2+}$-binding affinity with SNT-1. These findings raised new questions of how SNT-3 exerts a $Ca^{2+}$ sensor function, and why SNT-3 is not functional when SNT-1 exists.

# Dopamine transporter function is co-regulated by secreted ferritin

Patricia M. Chege, Teng L. Lim, Nicole L. Jenkins, Kirsten Grant, Ashley I. Bush and Gawain McColl.

*Melbourne Dementia Research Centre, Florey Institute of Neuroscience and Mental Health and University of Melbourne, Parkville, 3052 Victoria, Australia.*

Inside the brain, dopamine functions as both a neurotransmitter and neuromodulator. Neurotransmission is restricted to communication between the pre- and postsynaptic neuron and is mediated by fast acting ligand-gated ion channels. Neuromodulation involves diffusion through neural tissue to affect slow-acting receptors of many neurons. Modulatory neurotransmitter activity can thereby extend well beyond the synapse. In higher animals, distinct dopamine pathways play major roles in reward-motivated behaviour and control of movement. Dopamine dysfunction is thought to be involved in mania and certain subtypes of depression. Understanding of complex behaviours requires a complete characterisation of all the key regulators of neurotransmitter activity. In *C. elegans* the key enzymes and proteins required for dopamine synthesis, activity and turnover are both well conserved and well characterised. However, we have identified a novel and potent regulator of dopamine activity. The highly-conserved iron storage protein, ferritin, regulates dopamine reuptake by affecting dopamine transporter-1 (DAT-1) function. By exploring how ferritin exerts this novel function we hope to identify new therapeutic opportunities for modulating normal dopamine activity during neurological or behavioural disturbances.

# Oxidative stress: identification and study of a novel molecule with a neuronal protective function

Alessandra Donato[1], Sean Coakley[1], Eva Kaulich[1,3], Hang Lu[2] and Massimo A Hilliard[1].

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[2] *School of Chemical & Biomolecular Engineering, Georgia Institute of Technology, Atlanta, GA 30332, USA.*

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Reactive oxygen species (ROS) are involved in biochemical activities of cells, including cell signaling and homeostasis. However, accumulation of ROS leads to oxidative stress, a recognized pathological factor in several neurodegenerative conditions for which there is still no cure. KillerRed is a genetically encoded red fluorescent molecule that generates ROS upon illumination with green light, in a temporally- and spatially-controlled manner. Here, we used the nematode *C. elegans* expressing KillerRed in the mechanosensory neurons as an experimental model system to identify novel genes involved in the cellular response to ROS damage *in vivo*. Through an unbiased forward genetic screen, we identified a mutant strain, *vd60*, that presents highly increased neurodegeneration after KillerRed activation compared to the wild-type strain. Genetic mapping, full genome sequencing, and rescue experiments, revealed that *vd60* is an allele of a novel and previously uncharacterized gene, predicted to encode a transmembrane protein. We demonstrate that this molecule acts cell-autonomously to protect the neurons from oxidative damage and, remarkably, its overexpression drastically suppresses ROS-induced neurodegeneration. Moreover, expression of the protein in dopaminergic neurons of wild-type animals, protects them from the degeneration induced by 6-hydroxidopamine (6-OHDA), suggesting that its neuroprotective role is effective in a KillerRed-independent system and in different neuronal types. Our results revealed a new component that regulate the neuronal response to ROS-induced damage, and have the potential to untangle some of the pathological mechanisms that threaten the nervous system.

# Developing tools for Alzheimer's disease research

Neha Sirwani[1], Kirsten Grant[2], Stephen Doyle[3], Warwick Grant[1] and Gawain McColl[2].

[1] *Department of Animal, Plant and Soil Sciences, La Trobe University, Bundoora, Australia.*
[2] *The Florey Institute of Neuroscience and Mental Health, University of Melbourne, Australia.*
[3] *Wellcome Trust Sanger Institute, Hinxton, Cambridge, United Kingdom.*

Alzheimer's disease (AD) is an age-related neurodegenerative disorder that is the most common cause of dementia in humans. A characteristic feature of AD is the formation of neuronal extracellular senile plaques composed of aggregates of fibrillar amyloid β (Aβ) peptides, with the Aβ1-42 peptide being the most abundant species. These Aβ peptides have been proposed to contribute to the pathophysiology of the disease; however, there are few tools available to test this hypothesis directly. In particular, there are no data that establish a dose-response relationship between Aβ peptide expression level and disease. We have generated a panel of transgenic *C. elegans* strains expressing the human Aβ1-42 peptide under the control of two pan-neuronal promoters, *snb-1* and *rgef-1*. In 2017, preliminary data on these strains were presented. These strains have now been characterized in much more detail. Phenotypic data shows strong age-related defects in motility, subtle changes in chemotaxis, reduced median and maximum lifespan and changes in healthspan indicators such as maximum speed and fecundity. Current work highlights the differences in the timing and level of Aβ expression between strains differing in copy number and promoter, and possible correlation between expression level or timing and the severity of the disease phenotype. This work provides a new tool to investigate the *in vivo* toxicity of neuronal Aβ expression and the molecular and cellular mechanisms underlying AD progression in addition to permitting, for the first time, a direct test of the dose-response relationship between Aβ peptide expression and disease. These strains may also be used in subsequent screens to develop novel therapeutics to treat Alzheimer's disease.

# Ursolic Acid promotes neurite outgrowth and protects against axon degeneration in *Caenorhabditis elegans*

Wenyue Wang, Zhicheng Xiao and Roger Pocock.

*Monash Biomedical Discovery Institute, Department of Anatomy and Developmental Biology, Monash University, VIC 3800, Australia.*

Correct development and maintenance of neuronal architecture is required for proper brain function. Nascent neurons connect with each other through axons and dendrites that derive from neurites. Therefore, outgrowth and structural maintenance of neurites is crucial for a well-organized neuronal network. After neural injury or in neurodegenerative diseases, axons distal to the injury site show cytoskeletal disassembly and degeneration, known as axonal degeneration. My research focusses on identifying natural compounds that may be used to control neuronal development and neurodegeneration. Ursolic acid (UA), a naturally occurring pentacyclic triterpenoid, was first identified in the epicuticular waxes of apples and exists in many fruits and herbs, such as cranberry and rosemary. Various reports have suggested the potential therapeutic application of UA in neural injuries, inflammation, cancer, and diabetes. In this study, we examined neuroprotective activities of UA in *Caenorhabditis elegans*. We found that UA reduces axon outgrowth defects of the PVQ interneurons in animals defective for the CED-10/Rac1 protein. UA also promoted axon outgrowth in other mutants that affect the Rac1 pathway in *C. elegans*. Further, we showed that the axon outgrowth-promoting effect of UA occurred during embryogenesis. In addition, we found UA to reduce axon degeneration in mutants lacking the MEC-17/α-tubulin acetyltransferase 1, which causes spontaneous, adult-onset axonal degeneration of the PLM mechanosensory neurons. Interestingly, UA treatment in the parental generation reversed the axon degeneration defects of descendants for several generations. To better understand how UA protects the nervous system, we performed RNA sequencing to identify the potential target pathways of UA. Dysregulated candidate genes were verified using RNA interference (RNAi) experiments. We identified 6 genes that may be involved in the protective function of UA: *asah-1*, *swt-3*, *swt-7*, *dhc-4*, *nhr-43* and *ZK1058.5*. Together, our findings show that UA can promote axon outgrowth and prevent axon degeneration. Therefore, UA has potential therapeutic applications in neurodegenerative diseases. Further work will identify the molecular mechanism through which UA protects the nervous system by analysing molecular pathways regulated by UA.

# The α-tubulin acetyltransferase MEC-17/αTAT1 is essential for robust axonal regeneration

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Injury to the nervous system frequently results in devastating conditions for which very limited treatment options exist. Remodelling of the microtubule network is important for the repair of severed axons as it allows for the development of growth-promoting structures including the growth cone. Therefore, regulators of microtubule dynamics, including microtubule associated proteins and post-translational modifications, have been suggested to play an essential role in axon regeneration. Indeed, recent studies support the involvement of microtubule associated proteins in promoting stable microtubules for efficient repair. The acetylation of α-tubulin increases the stability of microtubules and protects them from mechanical stresses by imparting structural elasticity. However, the role of this post-translational modification in promoting nerve regeneration remains poorly defined. To study the role of the α-tubulin acetyltransferase MEC-17 in the repair of *C. elegans* neurons, we performed UV-laser surgery to transect the axons of individual sensory neurons and monitored their regenerative capacity at multiple time-points. Additionally, we studied the genetic pathways in which *mec-17* acts using epistatic analyses. Our results suggest *mec-17* to be important for efficient axonal regrowth and axonal fusion, for which it genetically interacts with *mec-12/α-tubulin* to promote axon repair. Additionally, we will present data on the impact of MEC-17 on microtubule structure, on the proteins with which it interacts, and how its misregulation impacts the expression of interacting genetic pathways. Collectively our data reveals the importance of MEC-17 for robust axonal regeneration after injury.

# Understanding the multiplex connectome of *C. elegans*: a focus on nociceptor sensitisation

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Can mapping neural connectivity tell us how the brain works? A major goal of global brain research initiatives is the expensive and labour-intensive mapping of neural networks. Much of this focuses on mapping synaptic connections between neurons. However, a considerable amount of neuronal communication occurs via neuromodulators such as neuropeptides and monoamines, which can act outside synapses. Neuromodulator-dependent signalling clearly drives important behaviours. During my postdoc at the LMB, Cambridge, I focussed on investigating the functions of neuropeptide networks and circuits in *C. elegans*, using automated behavioural tracking, optogenetics, microfluidics-based calcium-imaging, and high-content phenotyping. We showed that locomotor and sensory sensitisation during behavioural arousal occurs in a two-step process of neuropeptide signalling: afferent neuropeptides first convey mechanosensory information from sensory neurons to central interneurons, and these neuroendocrine centres then release efferent signals to convey behavioural state information to the periphery. Projects currently ongoing in my independent laboratory at the University of Wollongong/Illawarra Health and Medical Research Institute aim to characterise the more specific signals that directly trigger sensitisation of peripheral nociceptors and the motor circuit. These data present the exciting possibility of using the worm as a fully-described prototype for multilayer neuronal connections in bigger brains, to advance knowledge on how synapses and neuromodulators work together to control goal-oriented, context-dependent behaviour.

# Neuronal misexpression of fusogens results in neuron-neuron fusion and altered behaviour

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Recent studies in vertebrates and invertebrates have shown that neuronal cell-cell fusion can occur following viral infection, axonal injury, or after bone marrow transplantation. Although the circumstances leading to these fusion events have been characterized, very little is known about their consequences at the physiological and behavioural level. Here, we have developed and validated a model to study the functional consequences of neuronal cell-cell fusion *in vivo* in *C. elegans*. The Left and Right Amphid Wing "C" chemosensory neurons (AWCL/R), located in the head of the animal, mediate attraction to specific odours, including benzaldehyde, 2-butanone, and 2,3-pentanedione. We show that overexpression of the fusogens EFF-1 or AFF-1 in the AWCL and AWCR leads to neuronal fusion, as confirmed using the photoconvertible protein Kaede. By analyzing the chemosensory response, we found that animals with fused AWCs neurons are still able to mediate attraction to odours, which confirms the robustness of their response. On the contrary, fusion between AWC neurons and the Amphid Wing "B" neurons (AWBs), which mediate avoidance to nonanone, impairs the chemosensory response to odours. Moreover, our results reveal that fused neurons are viable and retain their original neuronal fate; however, analysis of calcium transients showed that these neurons are electrically coupled, compromising neural circuits connectivity. To our knowledge, this is the first study that shows how neuronal fusion affects the function of the nervous system *in vivo*. These findings may change our approach to the study of the nervous system function in health and disease, and provide the molecular basis of possible shortcuts of neural circuits.

# Neuronal regulation of diacetyl chemotaxis in *C. elegans*

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*Caenorhabditis elegans* is considered as one of the best models to study the neuronal control of biological processes due to its simple nervous system and known connectome. An adult *C. elegans* hermaphrodite has 302 neurons in its nervous system, including 14 types of chemosensory neurons. Among these, two types of chemosensory neurons, called AWA and AWC Amphid neurons detect volatile attractants and are similar to vertebrate olfactory neurons. Diacetyl (2,3-butanedione), a by-product of bacterial fermentation, may signal the presence of food to worm. Previous studies have shown that diacetyl requires an AWA neuron specific G protein coupled receptor, ODR-10 receptor for diacetyl chemotaxis. Although initial events of diacetyl olfactory perception have been studied, regulation of AWA chemotactic response by other neurons and neurotransmitters are not understood yet. Present study examines the role of other sensory neurons in *C. elegans* chemotaxis to diacetyl. Taking advantage of connectome of *C. elegans*, we screened mutants affecting neurons wired to AWA neuron, in diacetyl chemotaxis assay. We found that ASE neuron inhibits diacetyl chemotaxis under normal conditions. Moreover, neuropeptidergic signalling, involving an ASE linked FMRFamide-related peptide (FLP), affected diacetyl chemotaxis. We hypothesize that ASE neuron, with the participation of FLP, inhibits diacetyl chemotaxis under normal condition to maintain homoeostasis. Overall, the study identifies that diacetyl perception by AWA neuron in *C. elegans* is regulated by ASE neuron.

# Nociceptive response and neural circuit

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Nociceptive responses are crucial for animals' survival in reacting to noxious stimuli. In *C. elegans*, avoidance response was generated in a dose dependent manner of stimulus strength. In the past, the mechanism of underlying nociceptive response was studied by examining single neuron or a small portion of nervous system. Whole brain imaging is a newly developed technique, which allow us to learn more information when taking comprehensive account of different populations of neurons. Here we use whole brain imaging with perfuse system to report a phenomenon when worms experiencing severe noxious stimuli.

# ETS-5 regulates BAG-specific insulin signalling to control intestinal metabolism

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Metabolic homeostasis is essential to life. The nervous system is the master metabolic coordinator that integrates input information such as external nutrient availability, levels of internal nutrient stores, and even past experiences. The outputs from the nervous system include changing food consumption, storing excess energy and nutrients, deciding what that excess should be stored as, and ultimately altering behaviour. Using *Caenorhabditis elegans* as a model we investigate how gene-regulatory mechanisms in the nervous system control organismal metabolism and behaviour. We recently discovered that the conserved transcription factor ETS-5/Pet1, acts from the BAG and ASG neurons to control intestinal fat levels and exploration behaviour. Precisely how ETS-5 functions in the nervous system to affect intestinal fat levels is unknown. However, we have shown that neuropeptide secretion from the BAG neurons is an important factor in this pathway. We hypothesised that ETS-5 transcriptionally regulates neuropeptide expression in the BAG neurons, and that these BAG-specific neuropeptides in turn regulate intestinal metabolism. To test this hypothesis, we screened BAG-expressed neuropeptides for exploration defects and identified INS-1. Although INS-1 is expressed in multiple neurons, we show that INS-1 expressed specifically in the BAG neurons regulates intestinal fat levels and exploration behaviour. Our subsequent analysis revealed that ETS-5 directly binds to, and regulates the *ins-1* promoter in the BAG neurons. This regulatory mechanism prevents fat storage and promotes exploration. Intriguingly, we found that excess nutrients in the intestine can reduce both ETS-5 and INS-1 levels within the BAG neurons, revealing a nutrient-dependent feedback loop. Together, this work reveals a neuron-intestinal signalling circuit that is critical for maintaining metabolic homeostasis.

# ATFS-1 localises to the mitochondria to protect mitochondrial DNA from damage

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Owing to its location within the highly oxidative microenvironment of the mitochondria, the mitochondrial genome (mtDNA) is faced with a constant barrage of molecular insult during life. Molecular damage to mtDNA threatens cellular viability and is associated with ageing and a wide range of heritable and acquired diseases. The polyploid nature of the mtDNA can act as a defence mechanism by buffering the effects of molecular lesions. However, it remains poorly understood whether other processes mitigate the accumulation of mitochondrial genomic damage. Here, we invoked spatiotemporally controllable mtDNA double-stranded breaks in *C. elegans* to screen for nuclear factors that could modify the penetrance and expressivity of mitochondrial genome lesions. We found that the nuclear-encoded factor ATFS-1 mediated dichotomous roles during mtDNA damage, depending upon its subcellular localisation. While mtDNA double-stranded breaks induced nuclear translocation of ATFS-1 and activated the conserved mitochondrial unfolded protein response, nuclear restricted ATFS-1 severely enhanced the penetrance of mtDNA lesions. Oppositely, ATFS-1 activity restricted to the mitochondria strongly suppressed cellular dysfunction caused by mtDNA double-stranded breaks. We found that mitochondrial ATFS-1 acted cell-autonomously to protect cell function from mtDNA damage, in a manner dependent upon its DNA binding capacity and the mitochondrial fusion gene *fzo-1*. Underpinning its protective role, mitochondrial-localised ATFS-1 reduced the accumulation of endogenous mtDNA damage over time, extending the functional longevity of energetically demanding cells while increasing their resistance to exogenous threats against the integrity of the mtDNA.

# The dynamin GTPase is required for regenerative axonal fusion

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Axonal fusion is an efficient regenerative process that restores neuronal function post-injury. It is a highly specific mechanism whereby a regrowing axon reconnects and fuses with its severed segment, restoring cytoplasmic and membrane integrity, and preventing the degeneration of the detached segment. While synaptic vesicle recycling has been linked to axonal regeneration, its role in axonal fusion remains largely unknown. Dynamin proteins are large GTPases that hydrolyse lipid binding membranes to carry out clathrin-mediated synaptic vesicle recycling. The role of dynamin in endocytosis was first discovered in *Drosophila melanogaster*, with disruption of the dynamin-like *shibire* gene causing blockage of synaptic vesicle transport in this species. In *C. elegans*, the dynamin related protein DYN-1 functions in apoptotic cell clearance by mediating a signalling pathway involving the ABC transporter CED-7, as well as the transmembrane receptor CED-1/LRP1 and its intracellular adapter CED-6/GULP in the engulfing cell. CED-7 and CED-6 were previously reported to be important for axonal fusion, functioning to mediate the reconnection between separated axon segments. Thus, we hypothesized that DYN-1 may also be necessary for axonal fusion. To test this, we conducted UV-laser axotomy and analysed axonal regeneration in the posterior lateral microtubule neurons (PLMs) in animals carrying the temperature-sensitive *dyn-1*(*ky51*) allele. We show that disruption of DYN-1 function by raising animals at higher, restrictive temperatures (20°C and 25°C) leads to dramatically reduced levels of axonal fusion. Importantly, we demonstrate that these defects are reversible at lower, permissive temperatures, with the level of axonal fusion being restored to wild-type levels at 15°C. Quantification of the length of regrowth under these conditions has revealed no defect in *dyn-1* mutants compared to wild-type animals at 15°C and 20°C but showed a significant defect at 25°C. Furthermore, we find that DYN-1 activity is only required from 16 hours post-axotomy for neuronal repair. Together, these results establish important roles for DYN-1 in regulating axonal regrowth and fusion.

# The metalloprotease ADM-4 promotes regenerative axonal fusion

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Axonal damage, such as that observed in nerve and spinal cord injuries, interrupts the communication between a neuron and its target tissue. Functional recovery is achieved when the regenerating axon re-innervates its original target tissue. However, despite progress in medicine, intervention to repair such damage is still not achievable. *C. elegans* and other invertebrate species have evolved a specific repair mechanism, whereby the proximal axonal fragment (still attached to the cell body) first regrows, reaches its own separated distal axonal fragment, and then through a membrane fusion event, known as axonal fusion, re-establishes the original axonal tract and neuronal function. Axonal fusion therefore represents an innovative and potentially highly-effective repair strategy for axonal injuries. Using a candidate gene approach, and the PLM mechanosensory neurons as a model system, we have identified ADM-4 as a key regulator of axonal fusion. ADM-4 is a member of the ADAM (A Disintegrin and Metalloprotease) family, and ortholog of the human ADAM17/TACE (Tumor necrosis factor Alpha-Converting Enzyme). *adm-4* loss of function leads to severe reduction of axonal fusion in PLM neurons without affecting axonal regrowth. We demonstrate that ADM-4 regulates axonal fusion by functioning cell-autonomously in PLM neurons. Furthermore, overexpression of this molecule selectively in the PLM neurons is sufficient to enhance axonal fusion in wild-type animals. We reveal dynamic changes in the subcellular localisation of ADM-4 after axotomy, and identify the metalloprotease domain as essential to promote axonal fusion. We have previously demonstrated that phosphatidylserine (PS) exposure on the damaged axon functions as a "save-me" signal, which allows for successful axonal fusion. We show that putative PS-binding sites in ADM-4 are required for axonal fusion, and propose that PS exposure induced by injury, activates ADM-4 metalloproteinase and promotes fusion. Thus, our results reveal an essential function for ADM-4 in promoting axonal repair by fusion, and provide a strong foundation for designing novel therapeutics for injuries to the nervous system.

# Bringing together what belongs together—The WormJam international research community for *C. elegans* systems biology and metabolic modelling

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*Caenorhabditis elegans* is an important model organism in metabolomics, developmental biology, genetics, and other life science disciplines. In recent years, metabolism has been increasingly recognised as an important contributor to lifespan, as well as the basis for resistance to toxic insults or adaptation to different environments. In this context, the ability to model metabolism *in silico*, facilitated by metabolic reconstructions of *C. elegans*, is important for the study and characterisation of this organism and its physiological processes. The *WormJam* genome-scale model (GSM) is a consensus metabolic reconstruction of *C. elegans*, created from the merger of three individual GSMs through the efforts of an international consortium called *WormJam* (short for *Worm Jamboree*). The *WormJam* GSM is currently the most comprehensive knowledgebase for *C. elegans* metabolism, and further curation will improve its accuracy for *in silico* simulation of metabolism. With the increasing recognition of metabolism as being of pivotal importance for ageing, development, or disease, the availability of a highly curated community-driven consensus GSM of *C. elegans* will lay the foundation for bringing this organism to the forefront of metabolism research. As such, work is currently underway to improve the structure of the metabolic network reconstruction whilst increasing the underlying knowledgebase through incorporating links to databases such as WormBase, the Chemical Entities of Biological Interest (ChEBI) dictionary, and the Rhea database of biochemical reactions. This presentation will cover the aims of *WormJam*, the current work being undertaken, and the proposed developments to the model over the next few years. It will also show an application of systems biology and the *WormJam* knowledgebase by using NMR-based metabolomics to characterise the mechanisms behind the resistance of *C. elegans* to the toxic gas phosphine, which is used world-wide as agricultural fumigant. The metabolic changes behind phosphine resistance hint at conserved mechanisms for global metabolic regulation.

# Understanding the genetic basis of organismal susceptibility to xenobiotics using *Caenorhabditis elegans* and *Drosophila melanogaster*

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The interaction between biotic and abiotic factors of the environment in maintaining ecological balance is a critical determinant of animal diversity. When the anthropogenic chemicals enter an ecological system, organisms thriving in that ecosystem show a distinct sensitivity to the pollutant. To understand the same, we exposed *Caenorhabditis elegans* (worm) and *Drosophila melanogaster* (fly) to Methyl parathion (MP), a commonly used acaracide and insecticide and assessed the organism-specific response. First, we determined the lethal concentration at which 50% of the population is dead and found $LC_{50}$ for *C. elegans* is 275 µM and that for *D. melanogaster* is 3.12 µM. This 90 fold difference in $LC_{50}$ suggested the sensitivity of *D. melanogaster* to the insecticide (MP) than *C. elegans* and also exemplified the varied tolerance/susceptibility of species from different habitats. Further, to understand the genetic basis for the organismal response, we used ecotoxicogenomics approach involving worms as well as flies exposed for 24h and 48h to 1/10th or 1/100th of $LC_{50}$ of MP. The microarray analysis revealed mis-regulation of nearly 8% of total genes in both *C. elegans* and *D. melanogaster* on exposure to MP. Of the total mis-regulated genes; only 66 genes were common between both these organisms while the remaining genes were organism specific. Gene ontology clustering of significantly mis-regulated genes revealed alterations in vital functional gene families in both organisms, such as response to the stimulus, metabolic process, ion-binding, membrane, and trans-membrane transport. Pathway clustering revealed that the metabolic pathway was majorly affected in both of these test organisms, on exposure to MP. However, significant deregulation of the fat metabolism was observed in *C. elegans* while carbohydrate metabolism was affected in *D. melanogaster*. These findings unravel that a complex genomic module governs the organismal response to xenobiotics through a combination of conserved and organismal specific molecules.

# Loss of SAX-7 suppresses axonal defects of *ctbp-1* mutant animals

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C-terminal binding proteins (CtBPs) are transcriptional co-repressors that are conserved across many species, including *Caenorhabditis elegans*. *C. elegans* CTBP-1 is expressed in the nervous system and hypodermis, and regulates several processes, including lifespan. We previously identified abnormal axonal morphology of dorsal SMD (SMDD) neurons in developing and adult *ctbp-1* mutant animals, highlighting a role for CTBP-1 in the development and maintenance of the nervous system. Further characterisation of SMDD axonal morphology revealed that *ctbp-1* mutant animals display longer axons than wild-type, suggesting that axon guidance and/or termination cues are disrupted. From the single *C. elegans ctbp-1* locus, two isoforms are transcribed: *ctbp-1a* and *ctbp-1b*. These transcripts encode distinct proteins: CTBP-1a, which contains an additional Thanatos-associated protein (THAP) domain, and the shorter CTBP-1b, which does not house this domain. Using isoform-specific mutations and rescue experiments, we found that CTBP-1a and not CTBP-1b is required for regulation of SMDD development and maintenance. To understand the mechanism by which CTBP-1 influences SMDD development, we performed epistasis experiments with known regulators of axon guidance and maintenance. We found that CTBP-1 functions independently from the SMD axon guidance pathway regulated by the L1 cell adhesion molecule LAD-2. Epistasis experiments were also performed to assess the roles of putative CTBP-1-target genes in SMDD development. We found that loss of SAX-7 (another L1 cell adhesion molecule) short isoforms in a *ctbp-1* mutant background reduces SMDD axon defects. Furthermore, overexpressing SAX-7 short cDNA in wild-type animals causes defective SMDD axons. These findings reveal distinct regulatory pathways that define parallel roles for SAX-7 and LAD-2 in SMDD axon development.

# Cell-Specific Mitochondrial Affinity Purification (CS-MAP) from *C. elegans* populations reveals mitophagy dependent somatic patterns of mosaicism

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Mitochondria are organelles that are universally found in eukaryotic organisms. Considered as the powerhouse of the cell, mitochondria generate most of the cell's energy in the form of adenosine triphosphate (ATP). In addition to providing cellular energy, mitochondria are involved in a plethora of other tasks, such as intracellular calcium signalling, apoptotic cell death, and innate immunity. Mitochondria contain their own DNA (mtDNA), which encodes key components of the oxidative phosphorylation (OXPHOS) metabolic pathway. mtDNA is susceptible to mutations that can be at the origin of severe and progressive metabolic diseases, encompassing neurodegenerative disorders. Here, we developed a new method called Cell-Specific Mitochondrial Affinity Purification (CS-MAP) that enables the isolation of pure mitochondria from a specific cell-type. Isolated mitochondria are intact and functional and can be used for various purposes. By analysing the genetic composition of cell-specific mitochondria, we determined the relative load of mtDNA mutations in each cell type of *C. elegans* over very large populations of animals. Pathogenic mtDNA mutations usually exist in a heteroplasmic state, whereby a mixture of wild type and mutant mtDNA molecules coexist within the same cell. We found that the heteroplasmy levels of a mtDNA mutation varied between tissues in a consistent manner, suggesting that the mtDNA mutational landscape within an organism is stereotypically determined. We show that this stereotyped pattern of heteroplasmy between tissues is generated by the cell-type-specific activities of PINK1-parkin-mediated mitophagy, which acts to selectively remove mtDNA mutations in a subset of tissue types. Interestingly, the nervous system is most dependent upon mitophagy for controlling mtDNA mutation levels and interference of mitophagy by neuronal proteotoxic stress can increase mtDNA mutation levels in the neurons. These results suggest that mitophagy deterministically drives differences in heteroplasmy between tissues, and points at the existence of a causal link between proteotoxicity, mitophagy, and mtDNA mutation levels in neurons.

# Effect of olanzapine and fluoxetine on body fat in *Caenorhabditis elegans*: a dose response study

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Mental illness is a major cause of healthcare burden worldwide. Treatment of mental disorders mainly relies on psychotherapy or prescribed medications (psychotropic medication). The use of these medications has rapidly increased in the last few decades and has been linked to overweight, obesity and metabolic disorders. The present study aims to examine the effects of olanzapine, a commonly prescribed antipsychotic and of fluoxetine, a popular antidepressant on fat content of the model organism *C. elegans*. Wild-type Bristol N2 strain (*Caenorhabditis* Genetics Centre) were grown on NGM plates seeded with *E. coli* (OP50). Worms were exposed to treatments from the L1 stage. Treatment included different doses of olanzapine and fluoxetine dissolved in 2% DMSO. Control group received 2% DMSO. Fat content was examined by taking images of stained worms (0.5 % Oil-red stain) during L4 stage. Images were analyzed for colour density using Image J software. The effect of various doses of olanzapine and fluoxetine were examined. Body fat content of *C. elegans* was increased by doses of up to 100 µM concentration. At higher doses, a reduction in body fat was observed. Our study demonstrates that *C. elegans* is a suitable model for investigations on obesogenic effects of psychotropic drugs but careful consideration must be given to the dosage used.

# Syndecan controls germline development by regulating Notch Receptor transcription

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Syndecans are transmembrane proteoglycans that influence multiple biological processes including growth factor signaling, cell adhesion and cytoskeletal organisation. In *C. elegans*, loss of *sdn-1* leads to reduced progeny and germline stem cell numbers. These observations hint at a role for *sdn-1* in germline stem cell development. We previously showed that SDN-1 negatively regulates TRP $Ca^{2+}$ channels to maintain physiological levels of cytosolic $Ca^{2+}$. Our current study shows that deletion of the TRP-2 channel restores the decreased germline stem cell numbers observed in *sdn-1* mutants, suggesting that this regulatory mechanism also controls germline function. To further examine the downstream effects of the SDN-1/TRP-2 axis, we analysed the transcriptome of *sdn-1* mutant germlines and found that multiple stem cell proliferation and meiosis promoting genes are downregulated in the absence of *sdn-1*. Of particular interest was the reduction in *glp-1* mRNA, which encodes the GLP-1/Notch Receptor, crucial for germline development. Deletion of the TRP-2 channel reverses *glp-1* downregulation observed in *sdn-1* mutant animals, providing further evidence for the importance of this regulatory mechanism. Syndecan, as a transmembrane protein, has not been previously shown to be associated with transcriptional regulation. However, our recent data suggest that SDN-1 controls gene expression, specifically *glp-1* expression through a highly conserved motif on the *glp-1* promoter. This identifies a novel regulatory mechanism controlling Notch Receptor expression during germline stem cell development.

# Transgenerational epigenetic inheritance: initiation, establishment and maintenance have different genetic requirements

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It has recently become clear that in some circumstances Lamarck may have been right. There are a growing number of examples where a clear case can be made for the inheritance from parent to offspring of environmentally acquired gene expression changes. However, the mechanism by which this inheritance occurs is not clear. We have developed a sensor in *C. elegans* in which RNAi-induced silencing of a GFP transgene is robustly inherited for multiple generations in the absence of the initial RNAi trigger. The visible nature of this phenotype provides an exquisitely sensitive system, in which we separate individual animals according to their silencing status and measure effects in these distinct groups. Using this approach, we have shown that transgenerational epigenetic inheritance can be broken down into three steps: initiation, establishment and maintenance. In order to determine the genetic requirements for each step we are testing a panel of genes in this assay and will report on our current results. Generally speaking, histone lysine methyltransferases tend to be involved in establishment, while genes associated with chromatin compaction, chromatin readers, and small RNA-associated genes tend to be required for maintenance. A coherent working model will be presented encapsulating our recent findings.

# A non-canonical TGF-β pathway drives neuronal guidance

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Correct development of biological systems requires strict regulation of cellular processes such as cell migration, proliferation and differentiation. The transforming growth factor beta (TGF-β) pathway is a major player in these processes, however its role in neuronal migration and axon guidance is not fully understood. We discovered that SMA-6, a TGF-β receptor type I homologue in *Caenorhabditis elegans* acts from the hypodermis to control neuronal migration and axon guidance of the hermaphrodite-specific neurons (HSNs). Conversely, DAF-4 which is the sole TGF-β receptor type II homologue is not required for HSN development. In the canonical TGF-β pathway, the type I receptor requires phosphorylation by the type II receptor in order to function. However, we show that a TGF-β receptor type I can act independently of a TGF-β type II receptor in neuronal development, and hence identify a non-canonical mode of action. We also found that TIG-2, the human bone morphogenetic protein-7 (BMP7) homologue, binds to and functions genetically upstream of SMA-6 to direct HSN guidance. Further, the SMAD transcription factors, SMA-2, SMA-3 and SMA-4, act downstream of SMA-6 to regulate HSN development. To identify TGF-β pathway target genes important for regulating HSN development, we conducted RNA sequencing to compare the transcriptomes of wild-type, *sma-6* and *sma-3* mutants. Differential expression analysis identified 13 candidate genes some of which have SMAD binding sites. Finally, we found that manipulation of specific candidate SMAD target genes supress HSN developmental defects in *sma-3* mutant animals, suggesting the mechanism through which TGF-β signalling regulates neuronal guidance.

# Neuron-glia interaction: remodelling axonal attachment following axonal injury

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Distinct cell types adhere to each other to form functional systems in an organism. In vertebrates, ensheathing glia attach to axons forming a functional unit that responds to injury. In *C. elegans*, the posterior lateral mechanosensory (PLM) neurons have an intimate relationship with the epidermis. During development, the PLM axon becomes ensheathed by the epidermis and is mechanically coupled to this tissue via specialized attachment structures. The transmembrane protein LET-805/Myotactin is a component of these attachment structures, and is proposed to be required for correct attachment of the epidermis to the mechanosensory neuron axon as well as for the attachment of the epidermis to the body wall muscles. We visualized the localization of LET-805, and attachment sites, using a CRISPR/Cas9 engineered C-terminal wrmScarlet tag in a wild-type background, together with a PLM neuron-specific cytosolic GFP marker. To characterize the role of neuronal attachment in axonal maintenance and repair after axonal injury, we axotomized the PLM neuron and visualized LET-805::wrmScarlet before, during, and after injury. In uninjured animals at the L4 stage, LET-805::wrmScarlet localized to periodic puncta over the PLM axon. After injury, LET-805::wrmScarlet was slowly lost in regions corresponding to the disconnected distal fragment of PLM, often occurring after the loss of the cytosolic neuronal marker. On the proximal site, LET-805::wrmScarlet did not localize to the regrowing axon for at least 48 hours, after which it reassembled into puncta following the path traced by the regrowing axon. Taken together, our data suggests that following injury the attachment of the PLM neuron to its surrounding tissue is maintained and is not highly dynamic. We propose that the regrowing axon induces re-attachment to the epidermis. We are currently testing whether modulation of axonal attachment impacts axonal degeneration or regeneration, and how injuries on PLM axon are detected by the epidermis.

# Decoding transcriptional control of germline development in *C. elegans*

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The *C. elegans* hermaphroditic germline efficiently generates approximately 300 progeny over 3 days. Such rapid progeny production is driven by molecular mechanisms controlling stem cell maintenance, proliferation, cell fate specification, sperm/oocyte development and maturation. However, little is known of the function of transcription factors in germline development. Our initial work used RNA sequencing to identify 91 transcription factors that are expressed in the germline. The majority of these transcription factors have not previously been implicated in germline development. Therefore, deciphering their role(s) in the germline may lead to interesting new findings and more comprehensive understanding of germline development, stem cell maintenance and differentiation. Using RNA-mediated interference, confocal imaging and automatic germline nuclei number analysis, we performed a screen to investigate the roles of these transcription factors in germline development. We performed RNAi by commencing knockdown from either the L1 or L4 larval stage and analysed young adults. This enabled us to distinguish between genes acting in germline development (L1-L4) or germline function (adult). Subsequently, we used the germline-specific RNAi strain *rrf-1*(*pk1417*) and somatic-specific RNAi strain *ppw-1*(*pk1425*) to dissect the broad focus-of-action for each transcription factor. In our ongoing screen, 60 out of 87 transcription factors are found to regulate germline development when knocked down from the L1 stage, 36 out of 50 transcription factors regulate adult germline when knockdown from the late L4 stage, and 7 out of 21 transcription factors function cell autonomously in the adult germline.

# Neural coding of a nociceptive response in *Caenorhabditis elegans*

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How brain encodes diverse behaviours is a central question in neuroscience. When animals receive noxious stimuli, they initiate programed motor behaviours to avoid or escape from the noxious cues. Although many sensory neurons have been mapped to be involved in nociception in different species, it remains unclear how nociceptive inputs are integrated in the neural network under different conditions, such as brief stimuli versus prolonged stimuli. To address this question, we use *C. elegans* as a model. Despite with a small nervous system, *C. elegans* is able to perform complex behavours including nociceptive responses. By employing optogenetics, quantitative behavioural analysis and molecular genetics. We found that brief stimulation elites a reliable avoidance motor response. In contrast, prolonged stimulation triggers a reliable response followed by a stochastic motor response. We further found that this neural coding difference involves a switching at the interneuron level. These findings suggest that neural network is not a hardware. It is highly plastic to encode appropriate behaviours under different conditions. Discoveries from this project advance our understanding of neural network functions and is expected to give insights into neural coding in other species.

# TIG-2 modulates age-related mobility loss and lifespan in *C. elegans*

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Ageing, as a time-dependent physiological functional decline, is driven by multiple factors, and is accompanied by an increased risk of many chronic diseases, such as cardiovascular disorders, diabetes, cancer, and some neurodegeneration diseases. Among all age-related functional decline, locomotion impairment is one of the most prominent outcomes. Although slower movement with age could result from multi types of impairment in different tissues, previous research highlights the age-dependent changes at neuromuscular junctions (NMJs) play a key role in mobility loss. Given its short lifespan and tractable genetic tools, nematode *C. elegans* offers an ideal model for ageing study, especially at genetic and cellular levels. Previous study has demonstrated that age-related decline in neurotransmission at NMJs directly induces slow locomotion in older *C. elegans*. However, the underlying biological principals of age-related functional deterioration at NMJs is still unclear. Here, we show that a genetic mutation in *tig-2*, a retrograde BMP signaling of TGF-β family not only slows age-related mobility loss, but also extends lifespan in *C. elegans*. Surprisingly, *tig-2* mutant exhibit comparable presynaptic components and physiological function of body wall muscle cells as wild type worms, suggesting an unknown physiological function of TIG-2 in *C. elegans*.

# Neuronal control of cell non-autonomous stress responses through ETS-5-mediated signalling

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Cellular stress responses are essential molecular mechanisms that maintain homeostasis when an organism is faced with stress. Stress responses can be communicated to distal tissues – enabling global reactions to local challenges, which increases the organism's chance of survival. The nervous system is critical for coordinating the stress response, yet it remains largely unknown what mechanisms neurons use to regulate stress responses in distal tissues. It is clear that neurodegenerative disease, where neurons suffer from severe proteotoxic stress, leads to a systemic stress response, alteration in sleep and widespread metabolic changes. However, it is unclear how a healthy, functioning nervous system modulates cell non-autonomous stress responses to enable an organism to cope with environmental and nutritional challenges. We have previously shown that the ETS-5 transcription factor functions cell non-autonomously through the BAG and ASG sensory neurons to control intestinal fat storage. The increased intestinal fat storage in the absence of ETS-5 function induces a sleep-like state known as quiescence. As sleep and metabolic alterations are a hallmark of the systemic stress responses seen in neurodegenerative disease, the combination of increased fat storage and quiescence in an *ets-5* mutant implicates ETS-5 as a potential stress mediator. Our subsequent analysis has revealed that ETS-5 indeed mediates a systemic stress response. We will present our analyses of stress markers and relevant transcriptional networks that have enabled us to begin characterising the stress response pathway that ETS-5 governs.

# ATFS-1 protects mitochondrial DNA from damage

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Mitochondria are ubiquitous eukaryotic organelles that generate the majority of the cell's energy. They house their own genome (mtDNA), RNA, and protein synthesising systems, which code and coordinate the assembly of key enzymatic components of oxidative phosphorylation. Mutations in the mtDNA underlie a multitude of inherited mitochondrial diseases, a hallmark of which is their extreme variability in clinical severity and age of onset. Environment and nuclear-encoded factors may contribute to heterogeneity by influencing the expressivity of mtDNA mutations. Through the development of a spatiotemporally inducible system to invoke mtDNA double-strand breaks in *C. elegans*, we uncovered ATFS-1/Atf5, a key mediator of the mitochondrial unfolded protein response, as a potent modifier of mtDNA damage. ATFS-1 displayed dichotomous functions, which were dependent upon its subcellular activities. Nuclear-restricted ATFS-1 exacerbated the pathogenicity of mtDNA damage, whereas mitochondria-restricted ATFS strongly suppressed mtDNA damage in multiple cell types, including muscle and intestinal cells. We propose a new mitochondrial role for ATFS-1, which through its bZip domain, acts to protect somatic mtDNA from endogenous damage that accumulates over the animal's lifetime. We found that this role preserved mtDNA health during ageing, thereby promoting the functional longevity of energetically demanding cells and buffering their activities against exogenous threats to mtDNA integrity.

# Manipulating the NAD/Sirtuin pathway with neutraceuticals

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Mitochondrial dysregulation is an underlying cause of ageing, contributing to damaging levels of reactive oxygen species and declining energy production that can result in cell loss and tissue damage. A key pathway regulating mitochondrial biogenesis is the activation of sirtuin deacetylases (eg. SIRT1) by NAD+ (nicotinamide adenine dinucleotide), leading to increased transcription of mitochondria-related genes. This pathway is accessible to nutritional intervention through nutraceuticals (nutrition-based medications) including nicotinamide riboside (NR), a NAD+ precursor, and the blueberry-derived polyphenol pterostilbene (PT), a putative SIRT1 activator similar to Resveratrol. Individual exposure of NR and other SIRT1 activators have shown positive effects on life and healthspan in *C. elegans*, *D. melanogaster* and rodent trials. However, it has not been tested whether boosting NAD+ in combination with SIRT1 activation has a therapeutic advantage over NAD+ alone. We take advantage of the short-lived *C. elegans* model organism to determine if NR:PT combinations improve mitochondrial and/or muscle health more than either alone. A preliminary dosage response testing 125, 500 and 2000 µM NR on wild-type N2 *C. elegans* revealed a negligible effect of the higher doses on lifespan but a slight benefit to healthspan. PT, however, decreased the median lifespan of N2 *C. elegans* even at a low (6 µM) concentration. This toxicity has not been noted in rodents or humans. Continuing with the lifespan assays we compare 125 µM NR, 25 µM PT and two NR:PT combinations: 1) 125 µM NR: 25 µM PT (5:1) and 2) 125 µM NR: 6 µM PT (20:1). We also monitor activity levels on the lifespan plates with the wMicrotracker ARENA to identify the optimum timepoints to assess mitochondrial and muscle health. These results will guide future work to characterise nutraceuticals and their potential role in mitigating mitochondrial dysfunction.

# Biooorthogonal RNA labelling in *Caenorhabditis elegans*

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Small RNAs have been implicated as a major mechanism of epigenetic inheritance, but the identity and interactions of these involved RNA molecules has previously been hard to elucidate. A key to studying these heritable RNA molecules is the ability to selectively isolate RNA from particular cell types and time points of interest. Recently a method of bioorthogonally tagging nascent RNA molecules has been developed which uses the bacterial enzyme uracil phosphoribosyl transferase (UPRT) to insert a chemically tagged uracil analogue, 5-ethynyluracil (5EU) into RNA, allowing for temporal and cell-type control of RNA labelling. This method also allows for bioconjugation via click-chemistry and can be used for biotinylation and streptavidin extraction of the specific RNA of interest. This method was developed in immortalised cell lines and has so far only been adapted to mice and *Drosophila melanogaster*, but the technique shows great promise as a means to search for heritable RNA molecules within our *Caenorhabditis elegans* model of transgenerational epigenetic inheritance. Transgenic *C. elegans* strains that express UPRT under the control of targeted promoters have been created. These strains will be used to optimise the 5EU labelling method to the *C. elegans* model and then to isolate and identify RNA molecules involved in the TEI mechanism.

# How can we rescue the worms of Alzheimer's? And what can they tell us?

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Alzheimer's disease is the most prevalent form of dementia and has become a huge burden on the health sector. To date, the treatment options are still limited to alleviating the symptoms and slowing progression of neurodegeneration. One of the histopathological features of the Alzheimer's disease is the deposition of amyloid-β (Aβ) peptides around the hippocampal and cortical tissues. Investigators have focused on determining the origin of these peptides, the neurotoxicity of different types of aggregates and possible clearance pathways. On the latter point, an ATP-binding Cassette protein, P-glycoprotein (P-gp), has attracted attention for its ability to extrude Aβ peptides across the BBB to the systemic circulation. Our hypothesis is that improving the clearance of Aβ peptides through P-gp will relieve the symptoms of Alzheimer's disease. To achieve this, we will use Alzheimer's models from *C. elegans* that exhibit codon-optimised human Aβ1-42 peptide expression driven by a pan-neuronal promoter. *C. elegans* has a significantly simplified nervous system, which enables the nematodes to respond to inflicted stimuli, thereby enabling phenotypic investigation. We have shown that compared to wild-type *C. elegans*, the strains bearing neuronal amyloid-β1-42 peptide expression behave differently to experimental stimuli. For example, they have difficulty in detecting or locating food, and are unable to tell the presence of unwanted compounds. Our next objective is to introduce human P-gp expression in the Alzheimer's nematode model and compare their behaviour to worms without exogenous P-gp expression. Using this *C. elegans* P-gp redeeming Alzheimer's model, it will be possible to experimentally test whether manipulating P-gp expression or activity can achieve any benefit from preventing or slowing down the progress of the Alzheimer's disease.

# GCNA has a conserved role in regulating germ-cell genome stability by resolving DNA-protein crosslinks

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Genomic replication during meiosis requires stringent recognition and repair mechanisms to ensure that error-free genetic material is passed to the next generation. GCNA-1 is an evolutionarily conserved protein across eukarya that functions in reproductive genomic maintenance. While Spartan/DVC-1 and Wss-1 are involved in clearing DNA protein crosslinks (DPCs) from germ-cell chromosomes involving topoisomerase 1 (Top1), little is known about the resolution of topoisomerase 2 (Top2) DPCs. In this study, we show that while GCNA-1 does not interact with Top1, it does co-localise with Top2 on mitotic condensed chromosomes in the absence of Spartan, indicating a role for GCNA-1 in Top2 DPC repair in germline nuclei. We also found that *gcna-1* mutants in *C. elegans* incubated in the presence of Top2 poison undergo low fidelity DNA damage repair, leading to mortal germlines. These phenotypes are also seen in *Gcna*-mutant mice, indicating an inability to process DPCs, and suggesting that the reproductive role of GCNA is highly conserved. All together this strongly indicates that GCNA-1 has a highly conserved replicative role in genomic stability, more specifically in clearing Top2 DPCs.

# Ivermectin & fertility in nematodes

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Ivermectin (an antinematode drug) is the treatment of choice for onchocerciasis, a human disease caused by the nematode *Onchocerca volvulus*. Treatment with ivermectin results in the death of the immature larvae of *O. volvulus* (found in the skin and eyes) and a temporary loss of adult female fertility. Multiple rounds of treatment result in a cumulative loss of fertility such that old females exposed to > 8-10 rounds of treatment are often sterile. Failures of ivermectin treatment are appearing in populations subjected to 15+ years of treatment. Surprisingly, comparison between the embryograms of two populations of *O. volvulus* that differ in the long term, cumulative effects of ivermectin suggest that the non-response phenotype may be due to a population-level failure of the cumulative loss of female fertility over multiple rounds of treatment, i.e. a fertility-related phenotype. Furthermore, whole genome association studies of the non-response phenotype suggest it is most likely a quantitative trait that does not appear to involve genes directly linked to the molecular target of ivermectin (a glutamate gated chloride channel). Assessing candidates from these genome data in *O. volvulus* is, however, challenging due to the poor genome annotation and absence of any functional genomic tools. Investigation of the effects of ivermectin on *C. elegans* has shown that it acts similarly: kills early stage larvae and alters adult egg laying behaviour. The long-term effects of repeated ivermectin treatment cannot be assessed easily in *C. elegans* due to the short duration of hermaphrodite fertility, but comparisons between wild isolates suggest that hermaphrodite fertility varies widely between isolates. We will present data describing the *O. volvulus* phenotype, and preliminary data investigating parallels of this in *C. elegans* with a view to using genome wide QTL analysis in *C. elegans* to identify likely candidates from the *O. volvulus* GWAS.

# Mitochondrial DNA damage induces a premature ageing phenotype in neurons

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Neuronal function heavily relies on mitochondria for several aspects, such as providers of ATP and calcium ion reservoir. Therefore, mitochondrial health and distribution across the cell and along neuronal processes are essential for neuronal function. Disruptions can cause neurodegenerative diseases affecting both the central and peripheral nervous system, or aggravate them. A major component of mitochondrial health is the integrity of the mitochondrial genome (mtDNA). Damage to the mtDNA is frequent due to its exposure to reactive oxygen species, high replication rate and scarce repair mechanisms, so that mtDNA mutations accumulate during ageing. We have established a model in the nematode *C. elegans* that allows the investigation of the consequences of isolated intrinsic mtDNA damage in individual neurons, without also affecting the nuclear genome or mitochondrial metabolism. Intrinsic mtDNA damage massively impacts neuronal functionality and causes morphological changes even at damage levels below the threshold of inducing functional deficits. Contrary to other neurological conditions, intrinsic mtDNA damage does not result in an adjustment of mitochondrial distribution along the main neuronal process. This suggests that affected neurons are unable to determine mtDNA integrity or reallocate damaged mitochondria; facilitating the premature transition to a functional and morphological phenotype resembling that of aged neurons.

# *WormJam*—an international open research community effort for *C. elegans* systems biology and metabolic modelling

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Despite considerable efforts, information on *Caenorhabditis elegans* metabolism, its genes, proteins and metabolites is currently scattered across different databases. The *WormJam* (short for Worm Jamboree) research consortium is a world-wide community of researchers who aim to rectify this situation by constructing and curating a high-quality consensus genome-scale metabolic model (GSM) of this important biomedical model organism. The resulting *WormJam* GSM is currently one of the best curated metabolic models for *C. elegans*. *WormJam* collaborates with WormBase (www.wormbase.org), the premier repository for *C. elegans* genes, proteins, phenotypes, and related information. We have recently started to also contribute metabolite information to this repository. The *WormJam* model uses ChEBI as primary information and deposition source for metabolite structures, and together with the *WormJam* community we have started towards full coverage of the *C. elegans* GSM with metabolite structures. In addition, we are working on the integration of RheaDB to allow comparison of metabolic reactions between other organisms and *C. elegans*. Furthermore, we are collaborating with MetaboLights to link metabolomics raw and reference data. The second key focus of the *WormJam* consortium is improving the structure of the metabolic network reconstruction in order to improve the *in silico* modelling capabilities of the model. These improvements result from both general curation of the knowledgebase and targeted curation of problematic metabolic processes. We are aiming to further develop the *WormJam* model and connected database as a comprehensive knowledge base that will lay the foundation for future investigations and for bringing this important model organism to the forefront of metabolism research. This presentation will cover the aims of *WormJam*, the current work being undertaken, and the proposed developments to the model over the next few years.

# CRISPR-RNP based methods for genome editing in *Caenorhabditis elegans*

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Since the advent of CRISPR, the ability to generate targeted genome modifications for analysing gene function has rapidly evolved. CRISPR genome editing using purified single-guide RNA and Cas9 protein as preassembled ribonucleoprotein complexes (RNPs) enhances the rate of insertion whilst minimising off-target effects, when compared to delivery via a plasmid. We describe our RNP optimisation process and strategies for a number of specific genome modifications *Caenorhabditis elegans* (*C. elegans*). The generation of mutants was achieved by direct injection of pre-assembled RNPs and single-stranded repair (ssODN) templates, using a co-CRISPR strategy and restriction enzyme site to facilitate detection of genome editing events. Our results demonstrate an effective, consistent and convenient approach to generate heritable genetic alterations in *C. elegans*.

# Role of the SRX-97 GPCR in modulating *C. elegans* behavior

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The G protein (heterotrimeric guanine nucleotide–binding protein)–coupled receptors in the olfactory system function to sense the surroundings and respond to various odorants. The olfactory receptor encoding genes in *C. elegans* are more in number when compared to the mammalian genome suggesting complexity in combinations of receptor– odorant relationships. Recent studies have shown that the same odorant could act on different receptors in different neurons to induce attractive or repulsive responses. The ASH neuron is known to be responsible for responding to high concentrations of volatile odorants. I will present our work on the characterization of a new GPCR, SRX-97. We find that the *srx-97* promoter shows expression specifically in the head ASH and tail PHB chemosensory neurons of *C. elegans*. The SRX-97 protein localizes to the ciliary ends of the ASH neurons. CRISPR-based deletion mutants the *srx-97* gene suggest that it is involved in the recognition of high concentrations of benzaldehyde. This was further confirmed through the rescue experiments using endogenous and neuron specific promoters. Our work gives insight into concentration dependent receptor function in the olfactory system and the molecules that could help worms to navigate their surroundings.

# GerontomiRs—conserved microRNAs as mediators of longevity

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In 2050, the number of elderly individuals aged 60 years and above is expected to double and account for 22% of the world population. Evidence suggests that microRNAs (miRNAs), small non-protein-coding regulatory RNAs, are important determinants of lifespan. However, the genetic regulation of these tiny RNAs and its relationship with ageing is a field that still requires further research. The small animal nematode (*C. elegans*) is an excellent *in vivo* model for the genetic study of ageing, due to its short lifespan and genetic similarities with humans. In this study, the functional role of miRNA in *C. elegans* was investigated and results indicate that genetically lacking *mir-60* animals have a significantly extended lifespan (by almost 60%) relative to wild type. The analysis of global mRNA and protein expression through microarray and proteomics approaches revealed that the number of expressed proteins (various categories) are well matched with microarray data. The *zip-10* gene, which encodes a bZIP transcription factor functioning in the innate immunity, serves as a key player in the adaptive response to oxidative stress. Proteomics study has identified a number of heat shock and glutathione redox cycle proteins that are differentially expressed in *mir-60* mutant animals and up regulation of these proteins is linked with oxidative stress resistance. This study also examines other two miRNAs such *miR-83* and *miR-245*, which are human homologs to *miR-29* and *miR-13* respectively. *C. elegans* animals lacking both miRNAs had a dramatically increased lifespan. Prediction targets screening of *miR-83* and *miR-245* suggested that 45 and 17 genes for each miRNA are predicted as conserved targets between *C. elegans* and humans. Through suppression screening studies using RNAi, we have identified a few genes involved in oxidative stress regulation of *mir-60*, *mir-83* and *mir-245* mutants. Finally, we concluded that oxidative stress management is an important determinant of longevity, and miRNAs, particularly above three miRNAs, facilitates adaptive responses against chronic oxidative stress by ensuring the maintenance of cellular homeostasis.

# Small RNA responses to mitochondrial dysfunction in *Caenorhabditis elegans*

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Mitochondria are a remarkable hub of metabolic activity, generating the majority of the cell's energy through oxidative phosphorylation (OXPHOS). Ancestral vestiges of their bacterial origin, mitochondria retain a small circular genome (mtDNA) that encodes the key genes necessary for the coordinated assembly of the OXPHOS protein machinery. This genome is susceptible to damage and mutation, the consequences of which can often be devastating to the individual. However, cells can sense and respond to mitochondrial perturbations in a manner that allows adaptation and functional survival of the cell. Recent work indicates that epigenetic mechanisms involving small non-coding RNA (ncRNA) pathways act as important sensors of environmental changes and can alter metabolism by influencing posttranscriptional gene regulation. However, it is unknown whether direct damage to the mtDNA elicits this particular epigenetic pathway. Such a mechanism may be critical in adapting the cell to an altered mtDNA landscape during disease and ageing. Using small RNA sequencing, we uncovered a specific and potent ncRNA response to tissue-specific mtDNA damage in *C. elegans*. Interestingly, some of these small ncRNAs that are induced by mtDNA damage can counteract tissue-specific defects caused by mtDNA damage when artificially over-expressed.

# Toxic protein aggregate induces axonal swelling in *C. elegans* nervous system

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Toxic protein aggregates are widely observed in different neurodegenerative diseases. To study if protein aggregates trigger a general cellular mechanism in neurons, and how neurons dispose the toxic proteins before neuronal death, we over-expressed DsRed, an aggregation-prone fluorescent protein with low to medium cytotoxicity, in *C. elegans* motor neurons. By employing quantitative behavioural analysis, optogenetics, confocal imaging and molecular genetics, we found that *C. elegans* motor function is partially impaired by the over-expression of DsRed. We further found that in those motor neurons, a broad axonopathy phenotype is observed, including axonal swelling. The size and number of axonal swellings were developmentally regulated. We further found mutants that suppresses the axonal swelling phenotype through a forward genetic screening.

# Ferrous-glutathione coupling mediates ferroptosis and frailty during ageing

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All eukaryotes require iron. Replication, detoxification, and ferroptosis, a cancer-protective form of regulated cell death, all depend on iron metabolism. Ferrous iron accumulates over adult lifetime in the *Caenorhabditis elegans* model of ageing. Here we explore how glutathione depletion is coupled to ferrous iron elevation during normal ageing, where both occur in late life to prime cells for ferroptosis. We demonstrate that blocking ferroptosis, either by inhibition of lipid peroxidation or by limiting iron retention, mitigates age-related cell death and markedly increases lifespan and healthspan in *C. elegans*. Temporal scaling of lifespan is not evident when ferroptosis is inhibited, consistent with this cell death process acting at specific life phases to induce organismal frailty, rather than changing the rate of ageing. Because excess age-related iron elevation in somatic tissue, particularly in brain, is thought to contribute to degenerative disease, our data indicate that post-developmental interventions to limit ferroptosis may promote healthy ageing.

# The role of *set-9* and *set-26* in transgenerational epigenetic inheritance

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In recent years, it has become apparent that gene expression patterns can be transmitted between generations with no change to the DNA sequence in a phenomenon called transgenerational epigenetic inheritance (TEI). This occurs in a range of species, including *Mus musculus*, *Drosophila melanogaster*, and *Caenorhabditis elegans* and has implications for several biochemical and evolutionary processes. Our study utilises *C. elegans* in an RNA interference (RNAi) assay to assess the inheritance of gene silencing, involving silencing of a GFP transgene by exposure of parent animals to an RNAi trigger and assessing following generations for inheritance of this silencing in the absence of this trigger. Previous work has distinguished three parts to this TEI process, including initiation of gene silencing, the establishment of a heritable signal, and the maintenance of this silencing within the progeny. Our study examined the highly homologous predicted histone methyltransferases *set-9* and *set-26*, to determine in which stage of TEI they act, implicating *set-9* in the establishment of a silencing signal and *set-26* in both the establishment of a silencing signal and its maintenance in subsequent generations. Information regarding the structure and function of SET-9 and SET-26 was elucidated through open source bioinformatics tools to understand their differential roles in TEI. Our analysis showed that both proteins lacked the residues currently understood as necessary for methyltransferase activity. Current research involves validating these predictions to more closely analyse the function of SET-9 and SET-26 and will incorporate experiments to determine whether nuclear localisation and chromatin structure play a role in the mechanisms of TEI. In addition, we are in the process of optimizing single-worm proteomics in the context of epigenetic inheritance for use as a tool to better understand the dynamics of the proteome in relation to TEI.

# Decoding the role of the NFY transcription factor in brain development

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The establishment of correct brain architecture during development is an exceptionally complex process requiring precisely controlled cell specification, migration, axon outgrowth and guidance. These events are controlled by multiple transcription factors, conserved guidance systems and environmental cues. The *Caenorhabditis elegans* nervous system is an excellent model to study brain development due to its relative simplicity and conserved nature of development. My PhD project aims to identify molecular mechanisms that drive brain development. Using an unbiased genetic screen, we identified a Nuclear Factor Y transcriptional complex (NFY) that controls the development of a pair of glutamatergic interneurons. The NFY family is one of the most abundant and conserved transcription factors in eukaryotes and is involved in the regulation genes associated with several developmental steps. The NFY trimeric complex comprises the conserved NFY-A, -B and -C subunits that regulate gene expression through binding specific motifs in promoter regions. Our preliminary data show that the NFY complex regulates neuronal fate and axon guidance of specific neurons. Using single-cell resolution analysis, transcriptomics and ChIP sequencing I will decipher the molecular mechanism(s) through which NFYs control neuronal development.

# Modelling X-linked Charcot-Marie-Tooth (CMTX6) disease *in vivo* using *C. elegans*

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Although mutations in more than 85 genes are known to cause Charcot-Marie-Tooth (CMT) neuropathy, the molecular and cellular mechanisms that underlie the pathogenesis of CMT still needs to be understood. Animal models closely reflecting pathogenic *in vivo* events in patients are crucial for investigating mechanisms of axonal degeneration and the development of drug therapies. The p.R158H mutation in the pyruvate dehydrogenase kinase 3 (*PDK3*) gene causes an X linked form of CMT (CMTX6). Our group has previously shown that the p.R158H mutation in PDK3 results in reduced ATP and pyruvate levels and increased lactate levels. However, the exact molecular mechanisms that leads to CMT pathogenesis as a result of mutation in *PDK3* gene is still unclear. To address this, we have generated a *C. elegans* model of CMTX6 overexpressing human wild type (*PDK3WT*) and mutant PDK3 (*PDK3R158H*), both of which demonstrates axonal degeneration. We have recently utilized the CRISPR/cas9 system to knock-in the p.R158H mutation into the worm ortholog of PDK3, *pdhk-2R159H*. Using behaviour studies in the PDK3 overexpression and knock-in mutants, we demonstrate that synaptic transmission is affected in our CMTX6 animal models. Furthermore, we carried out locomotion assays to calculate the thrashing rate and average speed of the engineered PDK3 mutants. Defective synaptic transmission may lead to loss of signal at the neuromuscular junction resulting in muscle atrophy and neurodegeneration. In addition, we have characterised the effect of PDK3 mutations on the morphology of cholinergic excitatory and GABAergic inhibitory neurons. Further investigation of PDK3 associated synaptic transmission loss will help identify genes and pathways impacted by the mutation that can be targeted for drug development and therapy in CMTX6.

# Development of an Amyotrophic Lateral Sclerosis disease model in *Caenorhabditis elegans*

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Toxic protein-mediated neurodegenerative diseases such as Amyotrophic lateral sclerosis (ALS), Parkinson disease (PD), Huntington disease (HD) and Alzheimer's disease (AD) affect massive people's life. Although huge research effects have been devoted to dissect neural mechanisms of ALS, it is still not clear of how neurons are degenerated and how to prevent neural death using drugs. Previous research suggests that the missense mutation on superoxide dismutase (SOD1) gene is the possible reason that induce the neurodegenerative disease ALS. In order to study the molecular mechanisms of how aggregated proteins including SOD1 mutations lead to neural dysfunction and death, we constructed the different SOD1 point mutations and injected the plasmids into *C. elegans*. We then quantified the inclusion bodies in soma and swellings formed in the neurites in *C. elegans* GABAergic motor neurons. We found that, unexpectedly, some widely used fluorescent proteins are toxic and are prone to form aggregates *in vivo*, such as mApple and Kaede. In addition, we found that different toxic proteins cause different cellular phenotypes in motor neurons, some of them are easy to express in the axon and induce the axon swelling. Others are easy to express the toxicity in the cell bodies, with aggregated proteins in inclusion bodies. The four point mutations of SOD1 gene (A4V, G147P, N53I and G93A) cause different degrees of cellular toxicity, consistent with the results in mammalian system. Different toxicities lead to different number and size of swelling and inclusion bodies. These results suggest that *C. elegans* is good model to study toxic protein-mediated neurodegenerative diseases. In addition, the ALS disease model generated using *C. elegans* provides a possibility to uncover the molecular mechanisms underlying ALS using large scale genetic screening.

# Elucidating the role of H3K4 methylation in nervous system development

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Defective development of the nerve system can result in a number of human neurodevelopmental disorders. A critical step for correct brain development, architecture and function is the guidance of axonal processes through the extracellular environment. Axon guidance is a process tightly spatio-temporally regulated by dynamic changes of gene expression. Transcriptional regulation of a gene is, in part, controlled by the epigenome and more specifically the histone tail modifications. The histone tail modifications can alter chromatin structure, governing the accessibility of different factors to the gene body and hereby controlling gene activity. The methylation status of Lysine 4 (K4) on the tail of histone H3 is paramount for correct brain development as mutation in the genes encoding H3K4-specific methyltransferase and demethylases have been linked to neurodevelopmental disorders in human. Here we show how the majority of H3K4 methylation enzymes are important for axon guidance in *Caenorhabditis elegans*. Knock-in mutants of SET-2/SETD1A-B, a H3K4-methyltranferase, with mutations in the methyltransferase domain exhibited axon guidance defects, supporting the notion that H3K4 methylation is essential for axon guidance. Moreover, we performed RNA-seq in early embryos showing that the majority of genes controlling nervous system development are down regulated in *set-2* mutants compared to wild type. Interestingly, transient loss of SET-2 methyltransferase activity cause disturbance of the H3K4 methyl landscape in wild type animals in a transgenerational manner and this disturbance is accompanied by nervous system defects.

# Cell and argonaute-specific loading of miRNAs and isomiRs

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MicroRNAs (miRNAs) are a class of small, non-coding RNAs that help to regulate the transcriptome of eukaryotes during development and in response to environmental change. They achieve this by loading into argonaute effector proteins and binding mRNAs—resulting in their deadenylation and degradation, which in turn alters the level of proteins within cells. Due to their widespread effect, miRNAs have been implicated in an array of different phenotypes, but underpinning their exact roles is difficult. To systematically help uncover the roles of miRNAs, we have purified tissue-specific miRNAs and determined the cell-type-specific activities by coimmunoprecipitating tagged argonaute-miRNA complexes from three major somatic cell types in *C. elegans*. We found that of the 437 miRNAs known to *C. elegans*, 125 (28.7%) were bound specifically to argonautes located in intestinal, neuronal and muscle cells. Of these, 102 (82%) were bound exclusively to one tissue or another. Due to the increased resolution of our tissue- and argonaute-specific technique, we were also able to identify the presence of 37 novel miRNAs previously undetected in whole animal sequencing experiments. The presence of isomiRs, or lowly-expressed miRNA sequence variants were also detected, each with their own patterns of activity. Surprisingly, we were able to correlate the binding pattern of isomiRs with the frequency of different nucleotide transitions, however predicting the localisation of their parent miRNA remains an avenue for future work. Our results provide a broad atlas of miRNA activities across spatiotemporal dimensions of an animal and have provided insights into the complex regulatory processes that govern their activities and functions.

# The *C. elegans* TRIM protein NHL-2 regulates *let-7* miRNA pathways through the RNA binding domain

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TRIM-NHL proteins engage in few miRNA pathways (Loedige & Filipowicz, 2009) and has been shown to regulate cell proliferation and development. Interestingly, they impact the efficiency of the RISC both positively and negatively. The question is that if TRIM-NHL proteins function in the miRISC complex. Newly discovered in different context, with their intrinsic RNA binding activity, TRIM-NHL proteins could regulate RNA directly (Tocchini & Ciosk, 2015). NHL-2 is one of the five TRIM-NHL proteins in *C. elegans* that has been shown to modulate miRISC of two specific miRNAs, *let-7* and *lsy*-6. The TRIM-NHL family protein, NHL-2 in *C. elegans*, has an important effect on the miRNA and siRNAs pathways (Hammell et al., 2009; Davis et al., 2018). This family of proteins consists of following domains: RING, B-Box and Coiled-Coil domains in association with NHL motifs. Combination of domains enables TRIM-NHL proteins to control gene expression in various ways, such as the ubiquitination of protein targets via the RING domain (Borden, 2011), as well as regulating mRNA stability or translation via the NHL domain (Loedige et al., 2014). Small ncRNAs including micro-RNAs (miRNAs), small interfering RNAs (siRNAs) and piwi-interacting RNAs (piRNAs), are involved in regulating RNA function in the cytoplasm and nucleus. In the miRNA context. The C- terminal NHL domain has been shown bona-fide RNA binding protein with preference for poly u sequences in *C. elegans* (Davis et al., 2018). To characterize the role of NHL domain *in vivo* in *C. elegans* miRNA pathways, point mutations in NHL domain were designed to inhibit the function of the domains. NHL domain has been mutated in five amino acids. Using the transgenic worms carrying extra chromosomal arrays, we demonstrated that the NHL-2 localizes in P-bodies and P-bodies show increase in size and number when NHL-2 is mutant in RNA binding domain. Phenotypic analysis of adult cuticle formation shows that RNA binding domain of NHL-2 can be essential to regulate the heterochronic pathways through *let-7* miRNAs. Transcriptional deep sequencing and phenotypic analysis support the hypothesis that RNA binding domain of NHL-2 is important for translational repression of target mRNA in *let-7* family miRNA pathway in *C. elegans*.

# Previous nociceptive experience modulates behaviours in *C. elegans*

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Past experience is widely observed to modulate animal future behaviour in different species. To understand how animals adapt to harsh conditions based on their previous experience, we apply a prolonged noxious stimulation to *C. elegans* and investigate how it modulates animal behaviours. By employing approaches including quantitative behaviour analysis and molecular genetics, we found that prolonged treatment of high osmotic pressure induced a range of behavioural changes including locomotion, sensory-motor behaviour and the adjustment of body size. We also observed a sleeping-like status induced by the hypertonic stimulation. We speculate that the behavioural variations to previous experience can help organisms to cope with the unpredictable and stressful environment.

# Characterisation of novel genes involved in mitochondrial fusion

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Charcot-Marie-Tooth disease type 2A (CMT2A) is a peripheral neuropathy characterised by gradually progressive distal motor and sensory loss. The disease is caused by mutations in the gene encoding Mitofusin 2 (Mfn2), a protein essential for mitochondrial fusion. De-regulation of fusion results in impaired mitochondrial function that causes major disruptions in cellular health. Although mutations in *MFN2* are known to cause CMT2A, the molecular mechanisms underpinning Mfn2 dysfunction in CMT2A are largely unknown, and there is currently no cure or specific treatment for the disease. We hypothesise that understanding the mechanisms by which mutant Mfn2 causes axonal degeneration is important for the future development of treatments for CMT2A. This project aims to describe novel interactors of Mfn2 in order to further understand how the protein normally functions, and to identify potential therapeutic targets. Preliminary results have identified three novel interactors of the orthologous protein in *C. elegans* (FZO-1): TIAR-1, STI-1 and HSP-90. We will present data on how the loss of these molecules affects mitochondrial morphology, and generate double mutants between these genes and *fzo-1* to test if they can modulate defects associated with loss of *fzo-1* function. As the knockout of *fzo-1* is associated with overt locomotion and muscle structural defects, we will demonstrate how loss of the interactors affects these in single knockout mutants as well as in double knockout mutants with *fzo-1*. This will allow us to determine whether the proteins of interest are interacting with FZO-1 on a genetic level, and if so, whether they are functioning in the same or different pathways. By characterising these novel interactors of FZO-1/Mfn2, we will present novel insights into protein function and identify biological targets for the development of CMT2A therapeutics.

# Probing the proteostasis capacity of *C. elegans* upon aging

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A cell needs to maintain protein homeostasis (proteostasis) in order to keep proteins folded over space and time. To ensure proteome fidelity and to buffer proteotoxicity, proteostasis allows cells to rapidly respond to disturbances in the proteome by coordinating protein synthesis and folding, and mitigating protein misfolding by refolding, aggregation and degradation. Proteostasis is managed in a robust and coordinated fashion under the constant surveillance of a collective of proteins known as the proteostasis network (PN). However, the performance of PN is compromised during normal aging, and acute and chronic stress, resulting in the misfolding of proteins and aggregation. Currently, there is limited capacity to quantitatively measure proteostasis buffering capacity (health of proteostasis). In order to probe proteostasis changes and to measure the latent proteostasis buffering capacity *in vivo*, we will use a Forster-Resonance Energy Transfer (FRET)-based biosensor to sample aggregation states of a 'bait' protein that engages with quality control system (proteostasis network). Upon engagement with quality control proteins, a higher FRET will result. Utilizing information from these FRET changes, we will build a mathematical model to quantify and define the proteostasis buffering capacity under different contexts such as aging, and stress. This has been successfully shown to report the health of proteostasis in human cells and hence we would like to establish this biosensor system in intact *C. elegans*, targeting specific cells and tissues. The outcome of this experiment will allow for an improved understanding on how protein quality control systems keep the proteome folded in time and space, and how dysregulation of this systems contributes to the process of aging and age-dependent diseases.

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