ACeS 2017

The inaugural Australian *C. elegans* Symposium

October 25–27

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 AUSTRALIA | QBI
Queensland Brain Institute

Australian *C. elegans* Symposium

Queensland Brain Institute, The University of Queensland

Day 1: Wednesday 25th October	
11:00am–2:00pm	Registration, LEVEL 3 RECEPTION
2:00–2:15pm	Welcome, LEVEL 7 AUDITORIUM
2:15–4:45pm	**Session 1: Sensory function and behaviour** Chairs **ROGER POCOCK** and **SEAN COAKLEY**
2:15pm	**Keynote lecture: Modeling complex behaviors—fear in nematodes?** A/Prof Sreekanth Chalasani, SALK INSTITUTE FOR BIOLOGICAL STUDIES, LA JOLLA, CA, USA
3:00pm	**Mitochondrial dynamics are essential for normal behaviour in *Caenorhabditis elegans*** Joe Byrne, BIOMEDICINE DISCOVERY INSTITUTE, MONASH UNIVERSITY, MELBOURNE, AUSTRALIA
3:15pm	**Behavioural consequences of neuronal cell-cell fusion** Rosina Giordano-Santini, CJCADR, QBI, THE UNIVERSITY OF QUEENSLAND, BRISBANE, AUSTRALIA
3:30–4:00pm	Afternoon tea, LEVEL 7 TERRACE
4:00pm	**Invited talk: Exploring the architecture and working mechanisms of neural circuit motifs in *C. elegans*** Zhaoyu Li, UNIVERSITY OF MICHIGAN, ANN ARBOR, USA
4:30pm	**Using *C. elegans* forward and reverse genetics to identify new compounds with anthelmintic activity** Mark Mathew, DEPT. ZOOLOGY AND MSL, UNIVERSITY OF BRITISH COLUMBIA, VANCOUVER, CANADA
4:45–7:00pm	Symposium BBQ, LEVEL 7 TERRACE

Day 2: Thursday 26th October	
9:00am–12:15pm	**Session 2: Synapse formation and function** Chairs **GAWAIN MCCOLL** and **ARNAUD AHIER**
9:00am	**Keynote lecture: What is a mutant? Deep phenotyping with microfluidics and high-throughput quantitative microscopy** Prof Hang Lu, GEORGIA INSTITUTE OF TECHNOLOGY, ATLANTA, USA
9:45am	**Spontaneous vesicle fusion is differentially regulated at cholinergic and GABAergic synapses of the *C. elegans* neuromuscular junction** Zhitao Hu, CJCADR, QBI, THE UNIVERSITY OF QUEENSLAND, BRISBANE, AUSTRALIA
10:00am	**Tau induced excitotoxicity promotes local apoptotic events at the synapse** Joseph Benetatos, CJCADR, QBI, THE UNIVERSITY OF QUEENSLAND, BRISBANE, AUSTRALIA
10:15am	**Glua1 subunit ubiquitination mediates amyloid-β-induced loss of surface AMPA receptors** Sumasri Guntupalli, CJCADR, QBI, THE UNIVERSITY OF QUEENSLAND, BRISBANE, AUSTRALIA
10:30–11:00am	Morning tea, LEVEL 7 TERRACE Poster session
11:00am	**Invited talk: Super resolving *C. elegans*** Fred Meunier, CJCADR, QBI, THE UNIVERSITY OF QUEENSLAND, BRISBANE, AUSTRALIA
11:30am	**The calcium binding protein Copine-6 mediates AMPA receptor exocytosis to the postsynaptic membrane** Se Eun Jang, CJCADR, QBI, THE UNIVERSITY OF QUEENSLAND, BRISBANE, AUSTRALIA
11:45am	**C-terminal binding protein-1 (CTBP-1) regulates dorsal SMD axon development** Tessa Sherry, SCHOOL OF LIFE & ENVIRONMENTAL SCIENCES, THE UNIVERSITY OF SYDNEY, AUSTRALIA
12:00noon	**Sorting Nexin 27 regulates the exocytosis of NMDA receptors** Tong Wang, CJCADR, QBI, THE UNIVERSITY OF QUEENSLAND, BRISBANE, AUSTRALIA
12:15–1:15pm	Lunch, QBI RECEPTION LAWN, FRONT OF BUILDING
1:15–3:30pm	Poster session, COFFEE AND WATER PROVIDED
3:30–5:15pm	**Session 3: Mechanisms of development and regeneration** Chairs **HANNAH NICHOLAS** and **AVA HANDLEY**
3:30pm	**Chromatin modifiers SET-32 and SET-25 establish a transgenerational silencing signal** Alyson Ashe, SCHOOL OF LIFE & ENVIRONMENTAL SCIENCES, THE UNIVERSITY OF SYDNEY, AUSTRALIA
3:45pm	***atz-1* is a novel meiotic co-factor and is required for germline chromosomal stability in *C. elegans*** Gregory Davis, SCHOOL OF BIOMEDICAL SCIENCES, FEDERATION UNIVERSITY, CHURCHILL, AUSTRALIA
4:00pm	**The TRIM-NHL protein NHL-2 is a novel co-factor of the CSR-1 and WAGO 22G-RNA pathways** Peter Boag, BIOMEDICINE DISCOVERY INSTITUTE, MONASH UNIVERSITY, MELBOURNE, AUSTRALIA.
4:15pm	**A syndecan-TRP channel axis controls stem cell development in *C. elegans*** Sandeep Gopal, BIOMEDICINE DISCOVERY INSTITUTE, MONASH UNIVERSITY, MELBOURNE, AUSTRALIA
4:30pm	**A non-canonical TGF-β pathway drives neuronal guidance** Oguzhan Baltaci, BIOMEDICINE DISCOVERY INSTITUTE, MONASH UNIVERSITY, MELBOURNE, AUSTRALIA
4:45pm	**A novel mutation in Rac GTPase reveals domain-specific functions in nervous system development** June Shuer Deng, BIOMEDICINE DISCOVERY INSTITUTE, MONASH UNIVERSITY, MELBOURNE, AUSTRALIA
5:00pm	**The role of membrane phospholipids in axonal regeneration in *Caenorhabditis elegans*** Jean-Sebastien Teoh, BIOMEDICINE DISCOVERY INSTITUTE, MONASH UNIVERSITY, MELBOURNE, AUSTRALIA
5:15–7:00pm	Cocktail Party, LEVEL 7 TERRACE

Day 3: Friday 27th October	
9:00am–12:20pm	**Session 4: Ageing and degeneration** Chairs **MARINA KENNERSON** and **SIMRAN CHANDHOK**
9:00am	**Mechanisms and impact of impaired iron metabolism during ageing** Gawain McColl, THE FLOREY INSTITUTE OF MENTAL HEALTH, MELBOURNE, AUSTRALIA
9:15am	**Developmental and Ageing-related roles of Homeodomain-Interacting Protein Kinase (HPK-1) in *C. elegans*** Mallory Wood, SCHOOL OF LIFE & ENVIRONMENTAL SCIENCES, UNIVERSITY OF SYDNEY, AUSTRALIA
9:30am	**Developing a *C. elegans* model system to screen for novel gene mutations causing inherited peripheral neuropathy** Megan Brewer, ANZAC RESEARCH INSTITUTE, CONCORD, AUSTRALIA
9:45am	**Acetylation of a-tubulin is essential for maintaining synaptic integrity in *C. elegans* neurons** Michelle Wong, BIOMEDICINE DISCOVERY INSTITUTE, MONASH UNIVERSITY, MELBOURNE, AUSTRALIA
10:00am	**The epidermis protects sensory axons from degeneration** Sean Coakley, CJCADR, QBI, THE UNIVERSITY OF QUEENSLAND, BRISBANE, AUSTRALIA
10:15–10:45am	Morning tea, LEVEL 7 TERRACE Poster session
10:45am	**Disease-associated tau impairs mitochondrial autophagy by inhibiting Parkin translocation to mitochondria** Nadia Cummins, CJCADR, QBI, THE UNIVERSITY OF QUEENSLAND, BRISBANE, AUSTRALIA
11:00am	**Affinity purification of cell-type-specific mitochondria from whole animals reveals subtle patterns of mosaicism** Arnaud Ahier, CJCADR, QBI, THE UNIVERSITY OF QUEENSLAND, BRISBANE, AUSTRALIA
11:15am	**Uncovering mechanisms that modify the expressivity of mitochondrial genome damage** Chuan-Yang Dai, CJCADR, QBI, THE UNIVERSITY OF QUEENSLAND, BRISBANE, AUSTRALIA
11:30am	**Fluorescent Intra-body Localisation Microscopy (FILM): A novel method for tracking single intracellular endogenous and GFP-Tagged proteins *in vitro* and *in vivo*** Rachel Gormal, CJCADR, QBI, THE UNIVERSITY OF QUEENSLAND, BRISBANE, AUSTRALIA
11:45am	**Invited talk: Development optogenetic actuators and fluorescent sensors for neuroscientific research** John Lin, UNIVERSITY OF TASMANIA, HOBART, AUSTRALIA
12:15pm	Prizes and concluding remarks

Modelling complex behaviour—fear in nematodes?

Sreekanth Chalasani.

Molecular Neurobiology Laboratory, The Salk Institute for Biological Studies, La Jolla, CA 92037.

Animals used specialized cells (sensory neurons) to detect relevant changes from their environment and use that information to modify their behaviours on multiple timescales. While studies in multiple models have revealed insights into how information is detected and processed, less is known about the underlying molecular machinery. We use the nematode *C. elegans* with its small, well-defined nervous system consisting of just 302 neurons along with powerful genetic tools, behavioural assays and imaging methods to provide mechanistic insights into information processing. Specifically, I will describe our efforts to model a complex "fear"-like behaviour in *C. elegans*. We show that *C. elegans* does exhibit some characteristics of this behaviour, which is attenuated by a human anti-anxiety drug. Additionally, I will also discuss our latest results using ultrasound as a non-invasive tool to control neurons, Sonogenetics.

Mitochondrial dynamics are essential for normal behaviour in *Caenorhabditis elegans*

Joe Byrne, Ming Soh, Gursimran Chandhok, Nethmi Yapa, and Brent Neumann.

Neuroscience Program, Monash Biomedicine Discovery Institute and Department of Anatomy and Developmental Biology, Monash University, Melbourne VIC 3800, Australia.

Mitochondrial fission/fusion processes allow mitochondria to maintain their dynamic nature, which is important for maintaining cellular bioenergetics. In humans, Mitofusin 1 and 2 (MFN1/2) control fusion of the outer mitochondrial membrane, while the dynamin-like GTPase, OPA1, is essential for fusion of the inner mitochondrial membrane. Mitochondrial fission is mediated by DRP1, a Dynamin-1-like protein. Disruption of these proteins can therefore have major consequences for mitochondrial health, and thus influence larger processes such as behaviour and neuronal circuitry. Despite this link, the role of mitochondrial dynamics in maintaining organismal health remains unclear.

To study the functional and behavioral changes that occur in the absence of mitochondrial dynamics proteins, we have targeted the *C. elegans* orthologs of the fusion and fission machinery: FZO-1 (MFN2), EAT-3 (OPA1), and DRP-1 (DRP1). Using an extensive set of assays, we have found that disruption of mitochondrial dynamics causes a striking and progressive decline in animal behaviour. *Mfn2/fzo-1* mutant animals show defects in locomotion, swimming behavior, and endurance, as well as significantly reduced muscle strength. Furthermore, neuronal function is also impaired, with reductions in response to light touch and pharyngeal pumping indicating that mitochondrial dynamics are crucial for maintaining neuronal activity. We have identified similar behavioural deficits in *drp-1* and *eat-3* mutant strains. In addition, the morphology and location of mitochondria within a subset of neurons was examined, showing significant differences in size and shape, confirming disruptions in mitochondrial dynamics.

Our results highlight the crucial role of mitochondrial dynamics in maintaining normal animal behaviour. As these proteins are linked to neuropathies such as Charcot-Marie-Tooth disease (CMT), our study provides important insights into the molecular mechanisms linking mitochondrial fusion/fission defects to disease.

Behavioural consequences of neuronal cell-cell fusion

Rosina Giordano-Santini, Eva Kaulich and Massimo A. Hilliard.

Clem Jones Centre for Ageing Dementia Research, Queensland Brain Institute, The University of Queensland, Brisbane, Australia.

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) in the head of the animal, mediate attraction to specific odors, 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 analysing the chemosensory response, we found that animals with fused AWCs neurons are still able to mediate attraction to benzaldehyde, 2-butanone, and 2,3-pentanedione, which confirms the robustness of their response. On the contrary, preliminary data show that fusion between AWC neurons and the Amphid Wing "B" neurons (AWBs), which mediate avoidance to specific odours, suppresses the attraction response mediated by the AWCs. These data suggest that cell-cell fusion between neurons of the same class does not alter neuronal response, whereas fusion between different neuronal classes can impair the animal behavioural output. We are currently investigating if this defect in chemosensation is due to a change in neuronal identity, or to a "shortcut" between the neuronal circuit mediating attraction and the one mediating repulsion. To our knowledge, this is the first study that shows how neuronal fusion affects the function of the nervous system *in vivo*. We propose neuronal cell-cell fusion as a synthetic biology approach to study neuronal circuits and how changes in neuronal connectivity modify behaviour.

Exploring the architecture and working mechanisms of neural circuit motifs in *C. elegans*

Zhaoyu Li[1,2] and Shawn Xu[1].

[1] *Life Sciences Institute, University of Michigan, Ann Arbor, USA.*
[2] *Queensland Brain Institute, The University of Queensland, Australia.*

Neural networks are fundamental to animal behaviour. However, how neural networks spatially and temporally regulate behaviour is still poorly understood. As the only organism with a fully mapped connectome, *C. elegans* provides an ideal platform to address this question. In the *C. elegans* connectome, a striking feature is it's highly represented circuit motifs with distinct architecture. Using locomotory behaviour as a readout and employing multifaceted approaches including optogenetics, calcium imaging, laser ablation and quantitative behavioural analysis, we found that diverse circuit motifs are involved in regulating motor behaviours, and these motifs recruit different molecules to process distinctly spatial versus temporal motor information. The architecture and working mechanisms of neural circuit motifs revealed in *C. elegans* will provide important insights into how neural networks may function in the mammalian brain.

Using *C. elegans* forward and reverse genetics to identify new compounds with anthelmintic activity

Mark D. Mathew[1,2], Neal D. Mathew[1,2], Angela Miller[1], Mike Simpson[1], Vinci Au[1], Stephanie Garland[1], Marie Gestin[3], Mark L. Edgley[1], Stephane Flibotte[1,2], Aruna Balgi[4], Jennifer Chiang[2], Guri Giaever[2], Pamela Dean[5], Audrey Tung[6], Michel Roberge[4], Calvin Roskelley[5], Tom Forge[6], Corey Nislow[2], and Donald Moerman[1].

[1] *Department of Zoology and Michael Smith Laboratories, University of British Columbia, Vancouver, British Columbia, Canada.*
[2] *Department of Pharmaceutical Sciences, University of British Columbia, Vancouver, British Columbia, Canada.*
[3] *Universite de Poitiers, Poitiers, France.*
[4] *Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, British Columbia, Canada.*
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[6] *Summerland Research and Development Centre, Agriculture and Agri-Food Canada, Summerland, British Columbia, Canada.*

The lack of new anthelmintic agents is of growing concern because there are over two billion people infected and many billions of dollars of lost crops annually. This can be attributed to nematode resistance to anthelmintic drugs is increasing worldwide and many effective nematicides pose environmental hazards. Additionally, compounds with anthelmintic activity are considered rare. We address these problems by developing a high throughput screening platform for anthelmintic drug discovery using the nematode *Caenorhabditis elegans* as a surrogate for infectious nematodes. This method offers the possibility of identifying new anthelmintics in a sensitive, cost-effective and timely manner. We have identified 14 new potential anthelmintics by screening more than 26,000 compounds from the Chembridge and Maybridge chemical libraries. Using phylogenetic profiling we identified a subset of the 14 compounds as potential anthelmintics based on the relative sensitivity of *C. elegans* when compared to yeast and mammalian cells in culture. We showed that a subset of these compounds might employ mechanisms distinct from currently used anthelmintics. Genetic studies of D19 indicated its target mitochondrial complex II. We then used structural modelling to elucidate the resistance mechanism and nematode specificity versus mammalian cells. Most critically, in field studies D19 was found to be effective against the parasitic nematode, *Meloidogyne hapla.* Accordingly, an approach that can screen large compound collections rapidly is required. *C. elegans* as a surrogate parasite offers the ability to screen compounds rapidly and, equally importantly, with specificity, thus reducing the potential toxicity of these compounds to the host and the environment. We believe this approach will help to replenish the pipeline of potential nematicides.

What is a mutant? Deep phenotyping with microfluidics and high-throughput quantitative microscopy

Hang Lu.

Love Family Professor of Chemical & Biomolecular Engineering and Deputy Director of the Bioengineering Interdisciplinary Graduate Program, Georgia Institute of Technology.

Defining phenotypes is central to understanding functions of genes. Traditionally one thinks of large changes in an observable to facilitate the study, i.e. obvious defects allow us to conclude that the genes involved must be central to normal functions. This approach is fruitful to identify essential genes in many contexts, but increasingly large single-genic changes are no longer the focus of problems, especially those relevant to human diseases, e.g. psychiatric or developmental disorders. To study multigenic and complex phenomena, new methods to greatly enhance our ability to measure small changes in phenotypes, potentially in noisy conditions, are needed. Additionally, what constitutes a defect and how to define a mutant should be re-examined. We are interested in developing and using a set of automation, microfluidics, and image-based data mining technologies to address these questions in quantitative biology. I will show how we combine the power of experimental tools and computational tools to study problems in synapse development and behaviour in *C. elegans*. The power of these engineered systems lies in that the throughput that can be achieved by using automation and microfluidics is 100-1,000 times that of conventional methods; furthermore, we can obtain information unattainable by conventional tools and "invisible" to human vision to discover new genes and gene functions.

Spontaneous vesicle fusion is differentially regulated at cholinergic and GABAergic synapses of the *C. elegans* neuromuscular junction

Haowen Liu, Lei Li, and **Zhitao Hu**.

Clem Jones Centre for Ageing Dementia Research, Queensland Brain Institute, The University of Queensland, Brisbane, Australia .

The locomotion of *C. elegans* is balanced by excitatory and inhibitory neurotransmitter release at neuromuscular junctions. However, the molecular mechanisms that maintain the balance of synaptic transmission remain enigmatic. Here we investigated the function of voltage-gated Ca^{2+} channels in triggering spontaneous release at cholinergic and GABAergic synapses. Recordings of the miniature excitatory/inhibitory postsynaptic currents (mEPSCs and mIPSCs) showed that UNC-2/CaV2 and EGL-19/CaV1 channels are the two major triggers for spontaneous release. Notably, however, Ca^{2+}-independent spontaneous release was observed at GABAergic but not cholinergic synapses. Functional screening led to the identification of hypomorphic *unc-64*/Syntaxin-1A and *snb-1*/VAMP2 mutants in which mEPSCs are severely impaired, whereas mIPSCs remain unaltered, indicating differential regulation of those currents at cholinergic and GABAergic synapses. Moreover, Ca^{2+}-independent spontaneous GABA release was nearly abolished in the hypomorphic *unc-64* and *snb-1* mutants, suggesting distinct mechanisms for Ca^{2+}-dependent and Ca^{2+}-independent spontaneous release.

Tau induced excitotoxicity promotes local apoptotic events at the synapse

Joseph Benetatos, Liviu G Bodea, and Jürgen Götz.

Clem Jones Centre for Ageing Dementia Research, Queensland Brain Institute, The University of Queensland, Brisbane, Australia.

Aberrant accumulation of the mainly axonal microtubule associated protein tau in the somatodendritic compartment is a critical event promoting NMDAR mediated excitotoxicity in Alzheimer's disease pathogenesis. We sought to determine if PTEN, an NDMAR activity driven regulator of synaptic plasticity was altered in synapses after tau accumulation. Using the rTg4510 mouse model which overexpresses human tau harbouring the frontotemporal dementia (FTD) mutation P301L, we found increased synaptosomal activity of the lipid phosphatase PTEN, previously implicated in regulating long-term synaptic depression. The increase of PTEN activity in the synapses of mice was recorded as early as 2 months of age and was maintained at both 6 and 12 months, while the total brain lysate measurements of PTEN activation revealed an increased activity only at the later time point. Interestingly, PTEN activity in synaptosomes occurred prior to the cleavage of caspase 3 and exposure of phosphatidylserine, both well described molecular hallmarks of apoptosis. These apoptotic events occurred first specifically at synapses, as demonstrated by western blot and flow cytometry analysis of purified synaptosomes and total brain lysates. These results indicate a synapse-specific type of apoptosis that is induced by tau mediated excitotoxicity.

Glua1 subunit ubiquitination mediates amyloid-β-induced loss of surface AMPA receptors

Sumasri Guntupalli[1], Se Eun Jang[1], Tianyi Zhu[1], Richard L. Huganir[2], Jocelyn Widagdo[1], and Victor Anggono[1].

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[2] *Department of Neuroscience and Kavli Neuroscience Discovery Institute, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.*

The accumulation of soluble amyloid-β (Aβ) peptides produces profound neuronal changes in the brain during the pathogenesis of Alzheimer's disease. Excessive levels of Aβ disrupt excitatory synaptic transmission by promoting the removal of synaptic AMPA receptors (AMPARs), dendritic spine loss, and synaptic depression. Recently, activity-dependent ubiquitination of the GluA1 subunit has been shown to regulate the intracellular sorting of AMPARs toward late endosomes for degradation. However, whether this ubiquitin signaling pathway mediates Aβ-induced loss of surface AMPARs is unknown. In this study, we demonstrate that acute exposure of cultured neurons to soluble Aβ oligomers induces AMPAR ubiquitination concomitant with the removal of AMPARs from the plasma membrane. Importantly, expression of the GluA1 ubiquitin-deficient mutants inhibited the adverse effects of Aβ on the surface expression of AMPARs in neurons. Furthermore, we revealed the cross-talk between GluA1 ubiquitination and phosphorylation, in particular phosphorylation at Ser-845, which is crucial for AMPAR recycling and is known to be dephosphorylated in the presence of Aβ. Our data showed that the GluA1 ubiquitin-deficient mutant enhances GluA1 phosphorylation on Ser-845. Conversely, the GluA1 S845D phosphomimetic mutant reduced binding with Nedd4-1 and hence the ubiquitination of AMPARs. Importantly, the GluA1 S845D mutant also prevented Aβ-induced removal of surface AMPARs. Taken together, these findings provide the first demonstration of the dynamic cross-modulation of GluA1 ubiquitination and phosphorylation, a process that is perturbed by Aβ, in regulating the membrane sorting decision that ultimately determines the number of AMPARs on the cell surface.

Super-resolving *C. elegans*

Rachel S. Gormal[1], Adekunle D. Bademosi[1], Merja Joensuu[1], Ramon Martínez Mármol[1], Jean Giacomotto[2], Sean Coakley[1], Pranesh Padmanabhan[2], Ravikiran Kasula[1], Harriet P. Lo[3], Srikanth Budnar[3], James Rae[3], Charles Ferguson[3], Brett M. Collins[3], Alpha Yap[3], Geoffrey J. Goodhill[2,4], Massimo A. Hilliard[1], Rob G. Parton[3], **Fred A. Meunier**[1].

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[2] *The University of Queensland, Queensland Brain Institute, Brisbane, Queensland, 4072, Australia.*
[3] *The University of Queensland, Institute for Molecular Bioscience, Brisbane, Queensland, 4072, Australia.*
[4] *The University of Queensland, School of Mathematics and Physics, Brisbane, Queensland 4072, Australia.*

Super-resolution techniques are gaining momentum. By breaking the Abbe law of diffraction, super-resolution microscopy techniques provide unprecedented details of biological structures and processes. Access to nanoscale imaging in live and fixed cells is paramount to gain a better understanding of the dynamic molecular mechanism underpinning essential cellular functions such as exocytosis. In this talk I will provide a brief overview on our recent super-resolution technique development and results. In particular, I will discuss our recent published work on imaging subdiffractional synaptic vesicles in live hippocampal nerve terminals. We implemented a novel pulse-chase technique based on the **s**ub**d**iffractional **t**racking of **i**nternalized **m**olecules (sdTIM). This technique allowed us to image anti-green fluorescent protein Atto647N-tagged nanobodies trapped in SVs from live hippocampal nerve terminals expressing vesicle-associated membrane protein 2 (VAMP2)–pHluorin (Joensuu *et al.*, *JCB* 2016; Joensuu *et al.*, *Nature Protocols*, in press).

I will also describe unpublished data on the development of Fluorescent intra-body Localization Microscopy (FiLM). This technique enables endogenous or GFP-tagged proteins to be tracked by super-resolution microscopy by co-expressing single chain antibody in cells as intra-nanobodies tagged with a photoconvertible probe. FiLM has allowed us to image various GFP-tagged and endogenous proteins with unprecedented high resolution in live and fixed cells. More interestingly, this technique is also amenable to imaging proteins in organisms such as *C. elegans* and zebrafish providing an easy access to the nanoscale.

The calcium binding protein Copine-6 mediates AMPA receptor exocytosis to the postsynaptic membrane

Se Eun Jang[1], Tong Wang[1], Mintu Chandra [2], Jocelyn Widagdo[1], Brett Collins[2], and Victor Anggono[1].

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[2] Institute for Molecular Bioscience, The University of Queensland, Brisbane, Queensland, Australia.

AMPA-type glutamate receptors (AMPARs) mediate the majority of fast excitatory neurotransmission in the mammalian central nervous system. Activity-dependent trafficking of AMPARs is a major determinant of synaptic plasticity, which has long been considered as a cellular correlate of learning and memory. During long-term potentiation (LTP), the influx of Ca^{2+} through NMDA receptors induces AMPAR insertion to the postsynaptic membrane. The mechanism that regulates Ca^{2+}-dependent recruitment of AMPARs remains unclear. Here we report a novel interaction between AMPARs and the Ca^{2+}- and lipid-binding protein, Copine-6, which plays a critical role in LTP, learning and memory. shRNA-mediated knockdown of Copine-6 significantly reduces the levels of surface and total AMPARs, but not of NMDA and transferrin receptors in cultured hippocampal neurons. Live-cell imaging analysis of pHluorin-GluA1 reveals a defect in the insertion AMPARs to the postsynaptic membrane in Copine-6 knockdown neurons. Importantly, we also demonstrate that Copine-6 is also required for activity-dependent exocytosis of AMPARs following glycine stimulation. Finally, we demonstrate that Copine-6 mutant that is defective in Ca^{2+}-binding ablates AMPAR insertion under basal conditions, as well as during glycine-induced synaptic potentiation. Altogether, our data suggest that Copine-6 in a postsynaptic Ca^{2+} sensor that mediates AMPAR forward trafficking in mammalian central neurons, a process that is critical for learning and memory consolidation.

C-terminal binding protein-1 (CTBP-1) regulates dorsal SMD axon development

Tessa J Sherry[1], Anna Reid[1,2], Amanda Chen[1] and Hannah Nicholas[1].

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[2] *Max Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin, 13125, Germany.*

C-terminal binding proteins (CtBPs) are transcriptional co-repressors which are conserved across many species, including *Caenorhabditis elegans*. *C. elegans* CTBP-1 is expressed in both the nervous system and hypodermis, and regulates several processes, including lifespan. We previously identified defective exploration behaviour and 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 the morphology of SMDD axons revealed that *ctbp-1* mutant animals display longer axons than wild-type animals, 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 CTBP-1b. Using mutations that affect one or both isoforms, we have observed differential roles for CTBP-1a and CTBP-1b in the regulation of SMDD axonal morphology and exploration behaviour.

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 SMDD 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. Putative target genes were identified from microarray datasets by misregulated expression in a *ctbp-1* mutant background. Loss of function of two of these putative target genes in a *ctbp-1* mutant background results in the amelioration of SMDD axon development. These target genes are associated with insulin signalling and lipid metabolism. These findings thus implicate novel factors in the development of SMDD axons.

Sorting Nexin 27 regulates the exocytosis of NMDA receptors

Tong Wang[1], Xuan-Ling Yong[1], Se-Eun Jang[1], Xiaojun Yu[1], Brett M. Collins[2], and Victor Anggono[1].

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Sorting nexin (SNX) is a family of cytoplasmic and membrane proteins commonly involved in the endocytosis and trafficking of surface receptors. SNX27 is the only sorting nexin to contain a postsynaptic density 95/discs large/zona occuldens (PDZ) domain, and is playing an important role in mediating PDZ-dependent endosomal sorting and recycling of cargo molecules to the plasma membrane. Mutations of *SNX27* gene is linked to intellectual disability, epilepsy and growth retardation. Mice lacking SNX27 display impairments in glutamatergic neurotransmission and deficits in learning and memory. Previous studies have attributed synaptic dysfunction in SNX27 knockout mice to impairment in the trafficking of AMPA-type glutamate receptors. However, our recent finding found no evidence for direct interaction between SNX27 with AMPA receptor subunits. Instead, we found that SNX27 PDZ domain directly interacts with subunits of NMDA receptors and that this interaction is regulated by the phosphorylation of NMDA receptor near the carboxy-terminal PDZ ligands. Here, we report that SNX27 regulates the forward trafficking of GluN2A subunit of NMDA receptors in cultured hippocampal neurons. Overexpression of SNX27 upregulates surface expression GluN2A under basal conditions. In contrast, loss of SNX27 function abolishes activity-dependent insertion of GluN2A to the plasma membrane. Our results suggest that SNX27 plays critical roles in synaptic plasticity by enhancing the surface insertion of NR2A subunit containing NMDA receptors during synaptic potentiation.

Chromatin modifiers SET-32 and SET-25 establish a transgenerational silencing signal

Rachel Woodhouse and **Alyson Ashe**.
School of Life and Environmental Sciences, University of Sydney.

Mounting evidence in a number of organisms suggests that some epigenetic modifications acquired by an individual during its lifetime can be inherited for multiple future generations. This phenomenon is termed transgenerational epigenetic inheritance, and may provide a mechanism for the inheritance of environmentally acquired traits. We are studying transgenerational epigenetic inheritance using the nematode *Caenorhabditis elegans*.

We have developed a system in which RNAi-induced silencing of a GFP transgene is robustly inherited for multiple generations in the absence of the initial RNAi trigger. We have shown that the histone methyltransferase SET-25 and the putative histone methyltransferase SET-32 are required for effective transmission of transgene silencing. Specifically, whilst *set-25* and *set-32* mutant animals exposed to RNAi display silencing of the GFP transgene, their unexposed offspring fail to inherit this silencing. Intriguingly however, the few animals which escape this failure and remain silenced then produce subsequent generations of silenced progeny. Furthermore, *set-25* and *set-32* mutants segregated from silenced *set-25*/+ and *set-32*/+ heterozygotes respectively remain fully silenced. Together, this data suggests that SET-25 and SET-32 are required for the establishment of a transgenerational silencing signal, but not for the long-term maintenance of this signal between subsequent generations. We thus propose a three-step model of transgenerational epigenetic inheritance consisting of Initiation, Establishment and Maintenance.

In order to further support our model we have performed small RNA sequencing and proteomic studies on both SET-25 and SET-32 mutants and will also present these results.

atz-1 is a novel meiotic co-factor and is required for germline chromosomal stability in *C. elegans*

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Meiosis occurs in all sexually reproducing species to produce haploid gametes from diploid cells. This process commences with the replication of homologous chromosomes which then recombine to exchange genetic information. Maintenance of genomic stability is achieved through the tight coordination of key meiotic events, including cohesion of sister chromatids, synapsis of homologous chromosomes, and genomic surveillance by DNA damage repair mechanisms. In this study, we describe a putative myosin heavy chain protein orthologous to human myosin 1, F28D1.2, which we named *atz-1* (*A*bnormal *T*ransition *Z*one). Deletion of *atz-1* results in embryonic lethality and a depletion of germ cells transitioning from mitosis to meiosis, accompanied by reduced expression of the meiotic cohesion co-factor, REC-8. Additionally, *atz-1* mutants display various levels of DNA damage repair, including disorganised and aggregated oocyte chromosomal bodies, enhanced germ cell apoptosis and elevated expression of the DNA damage repair protein, BRCA-1. Interestingly, *atz-1* mutants exhibit defects associated with chromosome pairing and are hypersensitive to mild inhibition of DNA damage repair, suggesting that DNA replication is impaired in *atz-1* mutants. Moreover, the *atz-1* mutant phenotype is germline specific and resupplying somatically expressed *atz-1* does not rescue the reproductive defects associated with *atz-1* mutants. Overall, our data suggests that *atz-1* is a novel co-factor of the meiotic program and influences early meiotic events to maintain germline chromosomal stability.

The TRIM-NHL protein NHL-2 is a novel co-factor of the CSR-1 and WAGO 22G-RNA pathways

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Proper regulation of germline gene expression is essential for fertility and maintaining species integrity. In the *C. elegans* germline, a diverse repertoire of regulatory pathways promotes the expression of endogenous germline genes and limits the expression of deleterious transcripts to maintain genome homeostasis. TRIM-NHL proteins have emerged as conserved node of gene regulation across tissues and species, interfacing with multiple facets of RNA and protein regulatory pathways to ensure proper gene expression. Initially studied for its role in modulating miRISC activity via *let-7* and *lsy-6* in the soma, here we show that the conserved TRIM-NHL protein, NHL-2, plays an essential role in the *C. elegans* germline, modulating germline chromatin and meiotic chromosome organization. We show that NHL-2 localizes to germ granules, and uncover another role for NHL-2 in small RNA biology: as a co-factor in the both positively and negatively acting germline small RNA pathways (22G-RNAs). Furthermore, we demonstrate that NHL-2 is a bona fide RNA binding protein and, along with RNA-seq data from *nhl-2(ok818)* mutants, these results point to a small RNA independent role for NHL-2 in regulating transcripts at the level of RNA stability. Collectively, our data implicate NHL-2 as an essential hub of gene regulatory activity in both the *C. elegans* germline and soma.

A syndecan-TRP channel axis controls stem cell development in *C. elegans*

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Proteoglycans are glycosylated proteins involved in multiple cellular functions, including cell adhesion, cancer and neuronal development. The proteoglycans encoded in the *C. elegans* genome include, a syndecan (*sdn-1*), a perlecan (*unc-52*) and two glypicans (*lon-2, gpn-1*). Our research focuses on the function of *sdn-1* in stem cell development in the *C. elegans* germline. In addition to developmental defects, loss of sdn-1 leads to reduced brood size, suggesting a function for SDN-1 in germline development. The germline houses the only stem cell population in *C. elegans* and our data suggest that *sdn-1* plays a role in stem cell proliferation and differentiation. Our analysis shows that *sdn-1* mutant hermaphrodites have a reduced number of cells in the mitotic region and increased germline apoptosis. Further, germline specific RNAseq and qPCR analysis shows that the *glp-1*/Notch gene is downregulated in the absence of *sdn-1*.

We also found a reduction in meiosis promoting genes *gld-1*, *-2*, *-3* and *-4*, whereas apoptosis related genes remained unchanged. Previously, we showed that syndecan regulates several of its functions including cell adhesion and neuronal migration through the control of transient receptor potential (TRP) channels. TRP channels are mechanically sensitive Ca^{2+} channels that directly interact with syndecan. We found the reduction of cells in the mitotic region and increase in apoptosis in *sdn-1* worms were restored in *sdn-1; trp-1* and *sdn-1; trp-2* animals. This suggests the Ca^{2+} regulation through syndecan is the underlying reason for the germline phenotype observed in sdn-1 mutant worms. To conclude, this study currently focuses on the role of Ca^{2+} in the *C. elegans* germline in regulating stem cell proliferation and apoptosis.

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 have discovered that SMA-6, a TGF-β receptor type I homologue in *Caenorhabditis elegans* is required for specific neuronal migration and axon guidance events of the hermaphrodite-specific neurons (HSNs). Conversely, DAF-4 which is the sole TGF-β receptor type II homologue is not required for these processes. In the canonical TGF-β pathway, the type I receptor requires phosphorylation by the type II receptor in order to function. Our findings therefore suggest that SMA-6 may act independently of DAF-4 in HSN development. Furthermore, we found that TIG-2, the human bone morphogenetic protein-7 (BMP7) homologue, acts upstream of SMA-6 to direct HSN guidance. Using co-immunoprecipitation experiments, we found that SMA-6 and TIG-2 interact without the presence of DAF-4 which provides insight into the SMA-6 independence. It has previously been reported that SMA-6 and DAF-4 are recycled to the cell membrane via different pathways. We have shown that the retromer protein RME-1, which specifically regulates SMA-6 trafficking to the cell membrane is required for HSN guidance. This provides an important clue on how SMA-6 may act independently in neuronal development. Dysregulation of the TGF-β pathway in humans is associated with neurological disorders such as epilepsy and schizophrenia. We therefore predict that our results will have wide-ranging implications in TGF-β pathway biology and reveal mechanisms important in development and disease.

A novel mutation in Rac GTPase reveals domain-specific functions in nervous system development

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Establishment of the nervous system requires complex regulation of extracellular guidance cues and intracellular signalling pathways. Extracellular guidance molecules important for neuronal development, such as netrin, Wnt, Slit and ephrin, as well as their cognate receptors, are deeply conserved. Ligand-receptor interactions trigger intracellular signalling cascades that ultimately regulates actin remodelling, which is important for the motility of migrating neurons and extending axons. Human Rac1 GTPase, an actin cytoskeleton regulator, is an important intracellular signalling component regulating nervous system development. *C. elegans* CED-10, MIG-2 and RAC-2 are orthologous to human Rac1. Previous studies suggested that the three *C. elegans rac* genes function redundantly. However, our recent study identified a *ced-10* missense mutation, affecting a conserved residue in the CED-10 Switch 1 region, that causes axon outgrowth defects independently of *mig-2* and *rac-2*. CED-10 interacts with a multitude of proteins to regulate neuronal and non-neuronal development. We used a combination of forward and reverse genetics to identify several established and novel genetic interactors that function with *ced-10* in growing axons. Furthermore, we are using overexpression studies in mammalian cells and *in vitro* GTPase activation assays to unravel mechanisms that underpin the specific function of the Switch 1 domain in Rac proteins.

The role of membrane phospholipids in axonal regeneration in *Caenorhabditis elegans*

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Axonal damage accounts for approximately 50% of traumatic brain injury admissions for which very limited treatment options currently exist. We have recently demonstrated that exposure of the phospholipid phosphatidylserine (PS) on the axonal membrane after nerve injury is essential for initiating a highly effective means of axon regeneration known as axonal fusion. During regenerative axon fusion, damaged axon segments can reconnect and fuse together using conserved components of the apoptotic pathway, thereby offering a simple, yet highly effective mechanism for repairing damage to the nervous system. In the nematode *Caenorhabditis elegans*, the symmetry of the axonal membrane is lost after axonal damage, exposing PS on the external surface of the damaged axon to serve as a reconnection, or 'save-me' signal. However, the molecular mechanisms that govern PS exposure and its contribution towards initiating axon fusion is poorly understood. Through candidate gene approach, we have identified a scramblase protein (SCRM-1) that is essential for effective axonal regeneration. Scramblases have previously been shown to mediate the exposure of PS to the outer leaflet of the plasma membrane of apoptotic cells to allow them to be recognized by engulfing phagocytes. To study the role of SCRM-1 in *C. elegans* neurons, we performed UV-laser surgery to transect the axons of individual sensory neurons and monitored their regenerative capacity after 24 hours. Our data suggests that *scrm-1* mutants show significantly defective axonal reconnection and fusion rates compared to wild-type worms. To further investigate the role of SCRM-1, we are currently exploring its cell autonomous role, its interaction with components of the apoptotic recognition pathway, and how it modulates the rate of PS exposure to facilitate axonal regrowth and repair. Collectively our data reveals the importance of SCRM-1 in axonal regeneration and fusion post injury.

Mechanisms and impact of impaired iron metabolism during ageing

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Iron is integral to essential biological processes including DNA replication, oxygen transport and neurotransmission. Disturbed iron homeostasis, such as the accumulation of reactive iron with age, can lead to oxidative stress and cellular damage. This may underlie the degenerative processes common to age related decline. Age is the single biggest risk factor for numerous serious diseases, ranging from metabolic disorders and cancers to neurodegeneration.

Ferritin is a highly conserved protein responsible for safe iron storage. A focus of my laboratory is to understand the relationship between iron metabolism and the fundamental biology of lifespan. We are developing new analytical methods to assess ferritin levels in aged tissues, iron load in ferritin, and post translational modifications that alter protein function, to better understand the biochemistry of ageing. I will present data from several studies, including X-ray based imaging and spectroscopic analyses, proteomic and absolute quantitation of ferritin and models of genetic intervention. In addition, I will describe insights from data mining of complementary *C. elegans* transcriptomics and proteomic studies. Finally, I will discuss how we hope to transfer our approaches to vertebrate systems, to understand the contribution of iron homeostasis to human ageing.

Developmental and ageing-related roles of Homeodomain-Interacting Protein Kinase (HPK-1) in *C. elegans*

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Proteins of the Homeodomain-Interacting Protein Kinase (HIPK) family regulate an array of processes in mammalian systems, such as the DNA damage response and cellular proliferation. Members of this protein family are serine/threonine kinases that are predominantly localised to the nucleus. *Caenorhabditis elegans* expresses a single HIPK protein, called HPK-1. The HPK-1 protein is expressed in many cell types in the nematode and, like its mammalian counterparts, is localised to nuclear speckles, suggesting that it may be involved in analogous cellular processes.

Previous studies have implicated HPK-1 in longevity control and we have recently suggested that this protein may be regulated in a stress-dependent manner as animals carrying a fosmid-based fluorescent reporter exhibit increased levels of HPK-1 following heat stress. To expand these observations, we have been investigating the role of HPK-1 in various processes, such as development, longevity, and in the response to stress, by conducting phenotypic analyses on a worm strain carrying a deletion mutation within the *hpk-1* gene. HPK-1 appears to be required for normal somatic and germline development, as animals lacking HPK-1 are reduced in size compared with their wildtype counterparts, and show a decrease in brood size. We have previously shown that HPK-1 is required for normal longevity, with loss of HPK-1 function leading to a faster decline of physiological processes that reflect premature ageing.

In order to investigate the mechanisms involved in these processes, a nuclear phosphoproteomic study is underway to identify potential HPK-1 phosphorylation targets. Putative nuclear targets such as transcription factors for developmental, ageing and stress response pathways have been identified using Isobaric tags for relative and absolute quantitation (iTRAQ) labelling and tandem mass spectrometry, and these are currently being tested for functional associations with HPK-1.

Developing a *C. elegans* model system to screen for novel gene mutations causing inherited peripheral neuropathy

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Inherited peripheral neuropathies (IPNs) cause progressive length-dependent axonal degeneration of the peripheral nerves. Clinical symptoms include muscle wasting and weakness starting in the hands and/or feet, sensory loss and gait abnormalities. Although over 80 genes have been identified as a cause of IPN, up to 50% of families remain unsolved. Whole exome sequencing (WES) has greatly facilitated the screening of IPN families for gene mutations. However, multiple variants of unknown significance (VOUS) are often identified in the unsolved small nuclear families. VOUS are rare, non-synonymous protein-coding variants that segregate with the disease. Identification of multiple VOUS hinders genetic diagnosis due to the absence of genetic power in the family to resolve the pathogenic status of these variants. Additional functional evidence is therefore required. We have developed a system where candidate gene variants, identified through WES studies, will be overexpressed in *C. elegans* to determine which variants cause neurotoxicity within the organism.

We are validating this model using the *PDK3* R158H mutation, which we identified in patients with X-linked inherited peripheral neuropathy (CMTX6). We have generated *C. elegans* strains overexpressing the human PDK3 R158H mutation specifically in the GABA motor neurons (*Punc-25::PDK3*^{R158H}). Visual assessment of the GABA motor neurons in adult day 1 *C. elegans* revealed twice as many animals exhibiting abnormal axon morphology when overexpressing R158H PDK3 compared to animals overexpressing wild-type PDK3. Additional assays are being performed to determine whether this observed difference is 1) maintained at older ages, 2) translates into locomotive defects, and 3) is observed in *C. elegans* overexpressing benign PDK3 polymorphisms.

Correct identification of the disease causative mutation is imperative for both providing accurate genetic counselling and developing effective treatments. We predict this model system will be an invaluable tool that complements the gene discovery research undertaken in our laboratory.

Acetylation of a-tubulin is essential for maintaining synaptic integrity in *C. elegans* neurons

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Proper neuronal function requires neurons to form appropriate synaptic connections with post-synaptic partners; connections that need to remain malleable and adaptable to dynamically changing environments. Correct stabilization of the synaptic cytoskeleton is crucial for this resilience. A major component of the synaptic cytoskeleton is formed by microtubules, which are assembled from repeating subunits of a-tubulin and b-tubulin. Microtubules are modified by various post-translation modifications, including the acetylation of a-tubulin. *C. elegans* overexpressing different forms of MEC-17, one of two acetyltransferase enzymes responsible for acetylating a-tubulin, were scored for neuronal defects at various stages of larval development and at the 3 day-old adult stage. Animals with overexpression of MEC-17 were observed to have intact synapses from the first to third larval stage of development. However, by the fourth larval stage, 80% of these animals lack the synapse and the synapse does not reappear in adult animals. These results indicate that that although synapses can form during development in the presence of MEC-17 overexpression, they are unstable and are poorly maintained throughout the life of the organism. Interestingly, animals that overexpress a mutant form of MEC-17 that lacks the ability to acetylate a-tubulin have comparatively more resilient synapses that are less likely to develop this defect. Overall, our data reveals an important role of MEC-17 in the maintenance of synaptic structure. Our results also suggest that MEC-17 mediates the stabilization of synapses through its tubulin acetylation function. Collectively, these results establish the role of tubulin acetylation in the stabilization of neuronal synapses.

The epidermis protects sensory axons from degeneration

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Maintenance of neuronal integrity is essential for the preservation of correct neuronal function. Sensory neurons and their axons are subject to continuous mechanical stress due to their location within the skin, muscles, and moveable joints. Despite the strong forces experienced, these neurons are able to maintain their structure and functional circuitry. The ability to resist strain has been shown to be a combination of intrinsic and extrinsic protection mechanisms, but the precise interplay between cell autonomous and cell-non-autonomous stress resistance is not known. Mutations in *C. elegans* ß-spectrin/*unc-70* cause spontaneous axonal breakage due to mechanical strain. Through an unbiased forward genetic screen using *unc-70* mutants as a sensitised background, we have identified a novel mutant allele of the conserved gene *tbc-10,* which results in enhanced axonal damage to the PLM mechanosensory neuron. TBC-10 is a Rab-GTPase-activating protein that we demonstrate localises to the membrane of the hypodermis surrounding the PLM axon and functions non-cell-autonomously within this tissue to exert an axonal protective effect via inactivation of the conserved small GTP-ase RAB-35. Inactivation of RAB-35 within the hypodermis, by either expression of a GDP-locked RAB-35 or a loss of function mutation, is sufficient to rescue the enhanced axonal breakage phenotype in *tbc-10* mutants. We show that in *C. elegans* the epidermis acts to protect the axons of mechanosensory neurons from spontaneous degeneration induced by disruption of the spectrin network, demonstrating a crucial role for non-neuronal support cells in maintaining an intact and functional nervous system.

Disease-associated tau impairs mitochondrial autophagy by inhibiting Parkin translocation to mitochondria

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Damaged mitochondria are degraded via mitochondrial autophagy, a process termed 'mitophagy'. This involves translocation of the ubiquitin ligase Parkin from the cytosol to mitochondria, initiation of the autophagic machinery and eventual degradation of mitochondria by lysosomes. Although mitochondrial damage is a key feature of Alzheimer's disease (AD), alterations in mitophagy are not well characterised. One of the two defining protein abnormalities in AD is the accumulation of hyperphosphorylated protein tau, which is known to impair mitochondrial function. Here, we explored how disease-relevant forms of tau affect steps of the mitophagy pathway in mammalian cells and *C. elegans*. In neuroblastoma cells, tau reduced Parkin translocation in response to mitochondrial depolarisation, demonstrating a defective mitophagy process. This was despite unaltered mitochondrial membrane potential between control and tau-expressing cells, indicating that the effect of tau on Parkin translocation occurred downstream of depolarisation. Interestingly, proximity ligation assays revealed tau-Parkin interactions, which were absent for mitochondrial Parkin, suggesting that pathological tau traps Parkin in the cytosol and therefore prevents its translocation to mitochondria. In support of this, truncated tau lacking a microtubule-binding domain, which is therefore mostly cytosolic, was also able to inhibit Parkin translocation.

To investigate mitophagy in an *in vivo* model, we used *C. elegans* expressing the fluorescent mitophagy biosensor mito-Rosella in neurons. Mito-Rosella worms were crossed to strains expressing either wild-type or mutant human tau. We found that tau impaired mitophagy in *C. elegans* neurons after mitochondrial depolarisation, extending our *in vitro* data. Currently, we are delivering mito-Rosella to the mouse brain, using stereotaxic injections of adeno-associated virus. Our findings reveal a new aspect to tau-dependent toxicity and illustrate a complimentary approach to using different model systems.

Affinity purification of cell-type-specific mitochondria from whole animals reveals subtle patterns of mosaicism

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Mitochondria are crucial organelles central to the conversion of dietary calories into stored chemical energy (ATP). They are also important in a myriad of other processes, including intracellular calcium signaling, apoptotic cell death, and innate immunity. Energy is generated via oxidative phosphorylation (OXPHOS), the key enzymatic components of which are encoded by the mitochondria's own genome (mtDNA). Inherited mtDNA mutations are at the origin of an ever-expanding group of metabolic disorders. Additionally, acquired mtDNA mutations have been proposed to contribute to the progressive nature of common age-related diseases, such as neurodegenerative disorders.

In this study, we developed an affinity purification approach in *C. elegans* to isolate mitochondria in a cell-specific manner with 96% purity. Purified mitochondria retain endogenous proteins and mtDNA and are able to maintain a mitochondrial membrane potential and respire, indicating that they are intact and functional. Moreover, we demonstrate that organelles can be purified to single-cell and single animal resolution. Using this approach, it is possible to accurately determine the burden of mtDNA mutations in each cell type of an animal. Pathogenic mtDNA mutations usually exist in a heteroplasmic state, whereby a mixture of wild type and mutant mtDNA molecules coexist within the same cell. The underlying genetic makeup of the mtDNA pool may give rise to non-systemic manifestations of human disease. Indeed, mosaic patterns of OXPHOS failure can be caused by a non-equal distribution of mtDNA mutations in different cells, which can result from either random segregation of mitochondrial genomes during mitosis or genetic drift due to the high rate and relaxed replication of mtDNA. To determine whether non-stochastic effects could also contribute to mtDNA mosaicism, we analysed the mtDNA of purified mitochondria from cohorts of >10,000 animals, thereby overcoming random inter-individual variation. In animals harbouring a mixture of mutant *(uaDf5*) and wild-type mtDNA, we found subtle, yet highly consistent cell specific differences in mtDNA heteroplasmy. As the *uaDf5* deletion removes genes coding four core subunits of the electron transport chain, our results suggest that certain cell types in the body are more prone to propagating deleterious mtDNA genomes than others. Here we will present this new method for cell-specific mitochondria isolation and discuss the cell types and potential mechanisms that predispose certain tissues to a deteriorating mtDNA landscape.

Uncovering mechanisms that modify the expressivity of mitochondrial genome damage

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Mitochondria are ubiquitous eukaryotic organelles that generate the majority of the cells ATP through oxidative phosphorylation (OXPHOS), providing energy for virtually every biological process. Mitochondria house their own DNA (mtDNA), RNA, and protein synthesising systems. The mtDNA encodes key protein components of the OXPHOS machinery, as well as tRNAs and rRNAs required for their production. Pathological mtDNA lesions are now known to underlie a multitude of inherited mitochondrial diseases that induce dysfunction in energetically demanding organs, such as muscle and the brain. Moreover, evidence now suggests that acquired mtDNA lesions may accumulate in ageing tissues, contributing to the progressive nature of common late-onset degenerative diseases such as Parkinson's and Alzheimer's disease. The onset and severity of mitochondrial disease phenotypes are highly variable between siblings harbouring the same mutation, and it is therefore apparent that yet to be identified environmental or genetic components may modify the expressivity of mtDNA mutations. Using a novel spatiotemporal controllable transgenic model of mtDNA damage in *C. elegans* muscle cells, we identified the conserved factor ATFS-1/Atf5, a key regulator of the unfolded protein response in mitochondria (UPR^{mt}) as a modifier of mtDNA damage expressivity. Surprisingly, we have found that ATFS-1 does not act through this protective pathway, which is achieved through ATFS-1 nuclear localisation, but rather acts via a second, as yet unidentified role within the mitochondria itself to protect against mtDNA damage. Overexpression of mitochondrial localised ATFS-1 in muscle cells harbouring mtDNA damage improves muscle function by 191.16%, whereas localisation and activation of the UPR^{mt} in the nucleus is detrimental. Hence, we have uncovered a nuclear encoded determinant of mtDNA damage expressivity, modulation of which can rescue tissue function from pathological mtDNA lesions. We are currently elucidating the mechanism of action of ATFS-1 in the mitochondria as well as determining whether ATFS-1 can protect other cell types, such as neurons, from mtDNA damage over the lifespan of the animal.

Fluorescent Intra-body Localization Microscopy (FILM): a novel method for tracking single intracellular endogenous and GFP-tagged proteins *in vitro* and *in vivo*

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By breaking the Abbe law of diffraction, super-resolution microscopy techniques provide unprecedented detail of biological structures and processes. However, the requirement for fluorescent photoconvertible tags has hampered progress and exposed PALM and spt-PALM techniques to over-expression artifacts, raising the need for developing novel tools to bypass these limitations. Herein, we describe the development of single chain expressed in cells as intra-nanobodies to perform single molecule imaging of any GFP-tagged or endogenous intracellular proteins. Configuration 1: Co-expression of a GFP binding nanobody tagged with a photoconvertible mEOS2 to image any GFP-tagged protein in cells. We co-expressed anti GFP-intra-nanobody-mEos with PH-PLCδ-GFP allowed super-resolution imaging of phosphatidylinositol(4,5)bisphosphate nanodomains in fixed and live neurosecretory PC12 cells. We found identical domain size and mobility when using PH-PLCδ-mEos2. Further, combining the intra-nanobody with an Apex tag allowed us to perform 3D electron microscopy on these nanodomains. We also visualized cell-cell junctions at nanoscale in genetically modified CACO2 cell line expressing GFP tagged E-cadherin at endogenous levels. Expressing the GFP intra-nanobody, within the nematode *C. elegans* PLM mechanosensory neurons, allowed us to visualize the fusogen protein EFF-1 at nanoscale *in vivo*. In addition, using zebrafish *D. rerio* expressing the GFP intra-nanobody, we were able to visualize Caveolin 3 within its muscle fibers. Configuration 2: Purpose-designed intra-nanobodies to probe the nanoscale organization of endogenous proteins. Two specific nanobodies developed against the endogenous β2 adrenoreceptor, Nb80 and Nb37, were used to track endogenous receptors either in their active or inactive states. Fluorescent intra-body Localization Microscopy (FiLM), therefore enables GFP-tagged constructs to be localized by super-resolution microscopy, avoiding cloning and reagent requirements for existing *in vitro* or *in vivo* models. This technique can also be extended to identify the diffusional signature and nanoscale organization of endogenous proteins by expressing selective purpose-designed intra-nanobodies.

Development optogenetic actuators and fluorescent sensors for neuroscientific research

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Optical approaches have been used extensively in the last few decades to study biological functions. These advances have often been coupled with the developments of new actuators and sensors to manipulate and detect cellular functions. I will describe our past and recent works in the development of protein-based optogenetic tools to manipulate membrane excitability, inhibit synaptic vesicular release, inhibit calcium mediated intracellular signaling, activate and inhibit G-protein coupled receptors pathways and activate tyrosine kinase. I will also outline our latest projects in *C. elegans* to develop novel approaches to control the release of different synaptic vesicles. I will also discuss our recent development of a near-infrared fluorescent protein based on biliverdin binding phycobilisome and our current attempts to generate novel fluorescent sensors based on this new fluorescent protein.

Identification of CED-10/Rac1 interactors required for PVQ development

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A spontaneous missense mutation in CED-10, named *rp100*, was isolated in our laboratory. *ced-10* encodes a GTPase, orthologous to human Rac1, which belongs to Rho family of small GTPases. Modulated by guanine-nucleotide-exchange factors (GEFs) and GTPase activating proteins (GAPs), CED-10/Rac1 interacts with downstream effectors to regulate cell corpse engulfment, axon guidance and outgrowth. Unlike canonical *ced-10* mutants, *rp100* causes severe defects in PVQ axon development, but does not affect apoptotic cell corpse engulfment. The *rp100* genetic lesion causes a substitution in the Switch I region of CED-10, which is responsible for CED-10's interaction with GEFs and downstream effectors.

To elucidate the effect of the *ced-10(rp100)* mutation on its protein interactome, we used yeast two-hybrid screening to identify interactors of mutant CED-10. Of the 17 high-ranking interacting proteins, we unexpectedly identified two CDC-42 pathway components and two deacetylase-complex components. Subsequent genetic mutant analysis shows that these interactors play important roles in the PVQ developmental defects caused by the *rp100* mutation. Further analysis on these genetic and protein interactions will be presented.

Identifying and characterizing novel genetic modulators of mitochondrial dynamics

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The ability of mitochondria to undergo dynamic state of fusion and fission is critical for cellular functions. Mitochondrial dynamics is a complex process regulated by several intracellular proteins. Mitofusin (MFN2) is one such protein that regulates mitochondrial fusion and mutations in Mfn2 are causal for one of the most common forms of Charcot-Marie-Tooth disease, CMT2A. Despite the clear link between MFN2 and disease, its biological role in neuronal survival remains elusive. Therefore, we aim to identify the basic mechanism of how MFN2 leads to neurological disease and identify pathways that could prevent disease progression. Ease of genetic resources and 95.9% sequence similarity of human MFN2 with the *C. elegans* ortholog (FZO-1) provide compelling reasons for developing *C. elegans* as a model to study the role of MFN2 in neuropathology associated with disease. Initial preliminary results using several surrogate phenotypes for neuronal (thrashing, body bends, and touch reflex) and muscle (endurance, burrow assay, and pharyngeal pumping) health highlights the important role of FZO-1/MFN2 in regulating organismal health. The overt movement defects we identified in *fzo-1* mutants were exploited for forward genetic mutagenesis screening using a WMicrotracker instrument to identify both suppressors and enhancers of *fzo-1*. Whole genome sequencing will be used to identify causative mutations, and candidate genes will be studied independently and in combination with *fzo-1* for their effects on animal movement, mitochondrial dynamics, and neuronal morphology. As a complementary approach, direct interactors of FZO-1 will be identified using a classical Yeast Two-Hybrid screen. The interactors will be confirmed using a reporter tagged system. Our ongoing genetic and biochemical characterizations of identified candidates will provide a comprehensive understanding of the normal function of FZO-1 and its binding partners. Our findings will significantly expand our knowledge of how impairment of FZO-1 can lead to neurological dysfunction and neurodegeneration.

Identification of novel molecular elements mediating neuronal response to reactive oxygen species

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Reactive oxygen species (ROS) include a number of molecules involved in many biochemical activities of cells, such as cell signaling and homeostasis. However, accumulation of ROS leads to oxidative stress that is recognized as a pathological factor in several neurodegenerative conditions. To date, the molecular mechanisms of neurodegeneration upon ROS accumulation are poorly understood. KillerRed is a genetically encoded red fluorescent molecule that is able to generate ROS upon illumination with green light, in a temporally and spatially controlled manner. Here we use the nematode *C. elegans* expressing KillerRed selectively in the six mechanosensory neurons, as an experimental model system to investigate the cellular response to ROS damage *in vivo*. We developed a new multi-illumination platform (named iBox) that allows forward genetic screening and candidate gene approaches to be easily performed. Using a candidate gene approach, we provide evidence that the superoxide dismutase SOD-5, the catalase CTL-3 and the peroxidase PRDX-6, are involved in the ROS response. Furthermore, using a forward genetic screen, we identified two novel mutants, *vd60* and *vd44*, presenting significantly increased neuronal damage after KillerRed activation. Full genome sequencing and genetic mapping revealed that these mutations identify two independent and novel loci. The results obtained from our studies have the potential to reveal the neuronal-specific components that regulate the response to ROS-induced damage and untangle some of the pathological mechanisms that threaten our nervous system.

The role of the glutamate cysteine ligase modifier subunit in regulating glutathione homeostasis in aged *Caenorhabditis elegans*

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A consistent underlying index of ageing is a decline in the cellular levels of reduced glutathione (GSH) and/or a pro-oxidising shift in the reduced/oxidised glutathione (GSH/GSSG) redox couple. In mammals, cellular GSH is the most abundant low-molecular weight thiol (1-10 mM), with the GSH/GSSG ratio being a key indicator of the redox environment and cellular health. The age-related decline of the cell's ability to synthesise GSH by the *de novo* synthesis pathway has been implicated to contribute to the perturbations in GSH homeostasis. Intracellular *de novo* GSH synthesis involves two ATPdependent enzyme catalysed reactions; the rate-limiting and first reaction is mediated by glutamate cysteine ligase (GCL), which condenses glutamate and cysteine, to form γglutamylcysteine (γ-GC). The second reaction involves the addition of glycine to γGC by glutathione synthetase (GS) to form GSH. The production of γ-GC can become a rate-limiting step for GSH synthesis due to either a limitation in cysteine availability or a decrease in GCL activity. In the mammalian system, GCL is a heterodimeric holoenzyme consisting of the catalytic (GCLC) and modifier (GCLM) subunits. Though the GCLC subunit contains all the cofactor and substrate-binding sites, holoenzyme formation with the GCLM subunit increases the catalytic efficiency of the enzyme. In *Caenorhabditis elegans* the *gcs-1* gene encodes the catalytic subunit, while the lesser studied *Gclm* gene is also present. The only known function of the GCLM subunit in mammals is to form the holoenzyme complex with the GCLC subunit as a seemingly ancillary role in the regulation of *de novo* GSH synthesis. Our current work is investigating the role of the *Gclm* gene in the decline of GSH homeostasis in the ageing worm.

A fluorescence-based assay to study ectopic neuronal fusion in *Caenorhabditis elegans*

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Cell-cell fusion is essential to the proper development of many vertebrate and invertebrate tissues, with its misregulation involved in the pathogenesis of numerous diseases, including the progression of cancer. Although the precise mechanisms by which cells fuse during development is poorly understood, trans-membrane proteins called 'fusogens' are known key mediators of this event. Indeed, expression of fusogens in any tissue type is sufficient to induce fusion between adjacent cells. Importantly, in the *C. elegans* nervous system, several neurons are positioned in close proximity to one another and broadly express the fusogen EFF-1; yet neuronal fusion is rarely observed and only occurs in specific circumstances. Thus, we hypothesize that neurons possess key regulatory mechanisms that prevent ectopic fusion from occurring, in order to preserve the cellular individuality essential to their functioning. Here, we report a novel strategy to effectively detect and study ectopic fusion events between two adjacent neurons, PLM and PLN. We utilized a fluorescence based approach, whereby partial GFP polypeptides were selectively expressed in either PLM or PLN neurons. Individually, these polypeptides are non-fluorescent and would only reconstitute into a fluorescent molecule when present simultaneously in the same cytoplasm, as would occur in a neuronal fusion event. We are currently testing whether GFP reconstitutes into a functional fluorophore when the two fragments are brought together in the same cytoplasm, after fusion of the two neurons of interest. These strains would provide a critical starting material for identifying and studying, with a forward genetic screen, the molecular regulators of neuronal fusion.

Neuronal control of intestinal metabolism through ETS-5-mediated neuropeptide signalling

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In *C. elegans*, intestinal fat storage is closely linked to feeding behaviour. Excess stored fat promotes sedentary behaviour, and can induce a sleep-like state known as quiescence. Depleted fat stores promote roaming behaviour, where the nematode extensively searches for more food. The nervous system is crucial for modulating these metabolism-driven behavioural changes. Importantly, the nervous system can also directly regulate intestinal fat storage and metabolism—through neural-signalling mechanisms that are yet to be fully understood.

We previously discovered that the transcription factor ETS-5 acts within the BAG/ASG CO_2 sensing neurons to control *C. elegans* intestinal fat levels and exploratory behaviour. Precisely how ETS-5 acts is yet to be elucidated; however we have identified that neuropeptide secretion from the BAG/ASG neurons is an important component of the pathway. To identify the specific neuropeptides that may be involved in communicating between the BAG/ASG neurons and the intestine, we have now screened neuropeptide mutants for defective exploratory behaviour. In this poster we will present the results of this screen and further analysis of candidate neuropeptides.

Juozaityte et al. (2017) The ETS-5 transcription factor regulates activity states in *Caenorhabditis elegans* by controlling satiety. *PNAS*

Discovering novel regulators of axonal repair

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Neurons of the central nervous system (CNS) have a limited ability to regenerate after injury, accounting for the incurable nature of injuries to the CNS. Successful axonal repair is a multifactorial process that involves regulators of intrinsic growth potential of neurons, a permissive extracellular environment, and the ability of regenerating axons to reconnect with their original target tissue. In several invertebrate species, a specific repair mechanism - known as axonal fusion - is characterized by the regrowing axon recognizing and fusing with its own separated axon fragment to reestablish the original axonal tract. Our aim is to identify and study molecules that regulate axonal regeneration with a specific focus on axonal fusion. Using a laser-induced injury model and *C. elegans* mechanosensory neurons as an experimental system, we tested candidate genes for a role in axonal repair, selecting genes based on their potential to regulate membrane remodeling and fusion. The preliminary results from this screen will be presented. In addition, we are currently developing a semi-automated analysis platform to precisely and objectively characterize the different steps of axonal repair, including axonal regrowth, guidance, reconnection and fusion following injury. By furthering our understanding of the molecular and cellular mechanisms underlying axonal repair, we hope to build a strong foundation for the design of effective therapies for neuronal injuries.

Regulation of lifespan by a potentially secreted novel gene

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Transposable elements (TE), often described as "jumping genes" or "selfish DNA", can re-insert into new genomic loci to decrease genomic stability and cause poor host fitness, giving rise to the term of "selfish DNA". TEs are largely categorised into two classes depending on their intermediate phase. RNA transposons utilise multiple self-encoded genes to reverse transcribe from their RNA intermediate back into DNA, resembling retrovirus, and are previously studied for their ability to regulate biological ageing. In contrast, DNA transposons, encodes an enzyme called transposase that excises the transposon from the genome, and allows it to be re-inserted back into the genome. Unlike RNA transposons, DNA transposons are not known to impact the ageing process. Here, we have discovered that a previously undescribed, putative type II DNA transposon regulates lifespan in *C. elegans*. RNAi silencing of the uncharacterised gene C09B7.2, which has predicted transposase protein-coding region, extends lifespan by 40% without impacting on reproduction or mobility. This transposon appears to be active during early stage of life, with silencing from early in life leading to the greatest extensions in lifespan. Interestingly, C09B7.2 has a predicted N-terminal signal peptide, which is indicative of secretion into the extracellular space.

These data indicate that type II DNA transposons are important regulators of lifespan, with silencing of a single member of this family extending lifespan 40%. The presence of a signal peptide would suggest that DNA transposons can be secreted, and may act in a non-cell autonomous manner to regulate whole body ageing. In this way, genome instability in small population of cells could dictate whole body ageing, throughout an organism.

Investigation of small RNA responses to direct and tissue-specific mitochondrial DNA damage 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 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 post-transcriptional gene regulation. One class of ncRNA, the ~21 nucleotides long, single-stranded microRNAs (miRNAs) bind to partly complementary messenger RNA (mRNA) sequences and inhibit their expression in a wide range of important biological processes. 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.

By using an enzymatic approach of tissue-specific mtDNA damage, we created *C. elegans* strains which exhibit mitochondrial dysfunction in either muscle cells, neurons or intestinal cells. We have performed next generation sequencing of small ncRNAs in these backgrounds to identify those molecules that sense and respond to mtDNA damage. We have also developed tools that will allow us to identify small ncRNAs that act as intercellular signals to communicate mtDNA damage across tissue types.

SNT-1 functions as the Ca^{2+} sensor for spontaneous and evoked neurotransmitter release in *C. elegans*

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Synaptotagmin-1 (Syt1) binds Ca^{2+} through its tandem C2 domains (C2A and C2B) and triggers Ca^{2+}-dependent neurotransmitter release. Here we show that *snt-1*, the homologue of mammalian Syt1, functions as the Ca^{2+} sensor for both spontaneous and evoked release at the *C. elegans* neuromuscular junction. Blocking Ca^{2+}-binding in double C2 domains of SNT-1 massively impairs spontaneous release, whereas blocking Ca^{2+}-binding in single C2 domain has no effect, indicating that the two C2 domains binding Ca^{2+} are functionally redundant for spontaneous release. Analysis of the miniature excitatory postsynaptic currents (mEPSCs) shows that the mEPSC amplitude increases in the absence of SNT-1, suggesting a role of SNT-1 in regulating the size of synaptic vesicles. Stimulus-evoked release is significantly reduced in *snt-1* mutants, with prolonged release latency as well as faster rise and decay kinetics. Unlike spontaneous release, evoked release is triggered by Ca^{2+} binding solely to the C2B domain. Moreover, we showed that SNT-1 plays distinct roles in different subpopulations of synaptic vesicles depending on their coupling to calcium entry.

RAB-5 regulates EFF-1 localisation and function in *C. elegans* neurons

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Achieving repair of the nervous system is an enduring clinical problem, and discovering new mechanisms of nerve regeneration is crucial for developing new therapies. Following a transection injury to the axon, the *C. elegans* mechanosensory neurons can undergo spontaneous repair via fusion of the two separated axonal fragments. This regenerative axonal fusion is mediated by EFF-1, a nematode fusogen that acts as the final effector to fuse the axonal membranes. Identifying the molecular regulators of EFF-1 in neurons is critical to further understand this novel regeneration process. Here, we show that neuronal EFF-1 is regulated by the endocytic GTPase RAB-5. We find that perturbing RAB-5 activity increases the capacity of the neuron to undergo EFF-1-mediated regenerative axonal fusion. This change in EFF-1 function is associated with increased localisation of EFF-1 on the membrane, and secretion of extracellular EFF-1-containing vesicles. Conversely, enhanced RAB-5 activity leads to accumulation of EFF-1 in enlarged endosomes. These changes appear specific to RAB-5 activity, as a suite of other RAB molecules and endocytic regulators had no effect on EFF-1 localisation. This study is the first to identify a neuronal regulator of EFF-1, which may prove crucial to future strategies for translating axonal fusion into a clinical setting.

SNT-1 functions as the Ca^{2+} sensor for spontaneous and evoked neurotransmitter release at *C. elegans* neuromuscular junction

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Synaptotagmin-1 (Syt1) binds Ca^{2+} through its tandem C2 domains (C2A and C2B) and triggers Ca^{2+}-dependent neurotransmitter release at many synapses. Here we show that *snt-1*, the homologue of mammalian Syt1, functions as the Ca^{2+} sensor for both spontaneous and evoked release at *C. elegans* neuromuscular junction (NMJ). It has been reported that that Syt1 has two distinct roles in regulation of spontaneous release, triggering spontaneous fusion by the C2 domains binding Ca^{2+}, and clamping a second Ca^{2+} sensor which accounts for the increased spontaneous release in Syt1 knockout neurons. In contrast, the SNT-1 in *C. elegans* acts as the Ca^{2+} sensor triggering spontaneous release but lacks the clamping function. Blocking Ca^{2+}-binding ability in both C2 domains of SNT-1 massively impairs spontaneous release, whereas blocking Ca^{2+}-binding in single C2 domain has no effect, indicating that the two C2 domains binding Ca^{2+} are functionally redundant in triggering spontaneous release. The elevation of extracellular Ca^{2+} do not cause enhancement of spontaneous release in *snt-1* mutants, indicating that the SNT-1 is the only Ca^{2+} sensor for spontaneous release. The evoked neurotransmitter release is significantly reduced in *snt-1* mutants, with prolonged stimulus-secretion delay and, however, faster rise time and decay. Analysis of the evoked excitatory postsynaptic currents (evoked EPSCs) in transgenic animals carrying mutations of the Ca^{2+} binding sites in SNT-1 reveal that the evoked release is mainly triggered by Ca^{2+} binding to the C2B domain but not the C2A domain. By examining the roles of SNT-1 for distinct subpopulations of synaptic vesicles (SVs), we show that the SNT-1 determines the release of the distal SVs, whereas controls the response time of the proximal SVs to the stimulus. Taken together, our results demonstrate that the C2 domains of SNT-1 binding Ca^{2+} is essential to trigger the neurotransmitter release at worm NMJ.

Decoding conserved mechanisms that control neuronal development

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The establishment of correct brain architecture during development is an exceptionally complex process requiring precisely controlled cell migration, axon outgrowth and guidance. These events are controlled by conserved molecular guidance systems such as netrin, cadherin, Rac GTPases and Slit-Robo. 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 conserved molecular mechanisms that drive brain development. To achieve this aim, I study the development of neurons at single-cell resolution using the *in vivo C. elegans* model. I have already developed a new and unique neuronal reporter tool to enable me to simultaneously study multiple phases of neuron development (specification, axon outgrowth, axon guidance and synapse development). Using this tool, I will conduct forward and reverse genetic experiments, combined with state-of-the-art genomics, to identify molecules and signalling pathways that are important for the brain to develop and function.

The role of Claudins at the *C. elegans* neuromuscular junction

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The family of Claudin proteins are a class of four transmembrane proteins that are known to be required for maintaining the integrity of tight junctions in vertebrates. The Claudin superfamily of proteins are also known to be required for regulating channel activity, intercellular signal transduction and cell morphology. We are interested in finding the role of the Claudin homologs in the development and function of Neuromuscular synapses in *C.elegans.* In order to study the role of Claudins at the *C. elegans* Neuromuscular junction (NMJ), we performed an aldicarb-based screen with the available *claudin* mutants. We went on to characterise the role of one such Claudin like molecule, HPO-30, at the *C. elegans* NMJ. Mutants lacking *hpo-30* are resistant to both aldicarb and levamisole-based paralysis and show a marked reduction in the levamisole sensitive acetylcholine receptor (L-AChR), UNC-29 levels at the NMJ. These results were further corroborated by electrophysiological recordings of *hpo-30* mutants, which showed a large decrease in levamisole induced currents at the NMJ. Using electrophysiology and behavioural assays we show that HPO-30 functions in muscles and maintain the L-AChRs at the synapse. We will go on to discuss the possible mechanism of how HPO-30 function at the *C. elegans* NMJ.

Taken together, our results identify HPO-30 as the first Claudin-like molecule that is involved in maintaining post-synaptic receptor levels at the *C. elegans* NMJ.

Developing tools for Alzheimer's disease research

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Alzheimer's disease (AD), the most common form of dementia, is a complex neurodegenerative disorder leading to cognitive impairment and loss of associative learning. The pathological hallmarks of AD are the presence of neurofibrillary tangles composed of aggregates of hyperphosphorylated protein tau and senile plaques composed of aggregates of fibrillar amyloid β (Aβ) peptides, with Aβ1-42 peptide being the most abundant. The current hypothesis is that Aβ peptides 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. This project utilises *Caenorhabditis elegans* to study the *in vivo* toxicity of Aβ1-42 peptide and several variants. We have generated a panel of transgenic *C. elegans* expressing human Aβ peptides under the control of two pan-neuronal promoters, *snb-1* and *rgef-1*. Molecular characterization of these strains revealed variation in the copy number of Aβ minigene, and expression of Aβ peptides was confirmed by dot immunoblots. Preliminary phenotypic data suggest that these strains have slightly delayed development, shortened median life span and defects in chemotaxis. Current work focuses on investigating 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.

Drug screening to improve behavioural defects of a *C. elegans* model of CMT2A

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Charcot-Marie-Tooth disease (CMT) is characterised by progressive motor and sensory neuropathy, resulting in muscle weakness and mobility impairments. The most common axonal form of CMT, CMT2A, is caused by mutations in the Mitofusin 2 (Mfn2) protein, which is a large GTPase critical for optimal mitochondrial functioning. Despite mutations of *Mfn2* first being described as causative for CMT2A more than a decade ago, we still lack a precise understanding of how *Mfn2* mutations cause the disease, and thus have a complete lack of effective therapeutics. We aim to model CMT2A in *C. elegans* by targeting the ortholog of *Mfn2* in this species (*fzo-1*) in order to better understand the disease pathophysiology and to discover novel drugs that can modulate the disease. Using CRISPR-Cas9 methods, we have generated a novel allele of *fzo-1* with the entire genomic region deleted (*cjn020*), and used this allele to assess the consequences of *fzo-1* loss-of-function on animal behaviour, with a focus on locomotion. Notably, the absence of FZO-1 resulted in a significant reduction in the rate of locomotion compared to wild-type (WT) across all age groups tested, as demonstrated in both thrash and body bending assays. These observations mirror the mobility impairments endured by CMT2A patients. Finally, we have exploited the locomotor dysfunction of *fzo-1* mutants as a platform for high-throughput screening of small compounds, in order to discover novel modulators that can reverse the movement defects in *fzo-1* mutant animals. Our results help to define the normal biological role of Mfn-2/FZO-1, how this relates to CMT2A, and will identify effective modulators of the disease phenotypes.

Neuronal proteomic changes during ageing in *C. elegans*

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Neuronal ageing in *C. elegans* is characterised by morphological changes to the neurons themselves (such as axon blebbling, cell-body branching and neurite sprouting). Synaptic deterioration also accompanies neuronal ageing, characterised in part by a decrease in the number of vesicles at the neuronal synapse. Although these and other age-related changes in neurons have been documented over the past several years, the mechanisms behind their occurrence are still elusive. Identifying the proteomic changes within *C. elegans* neurons during ageing could help elucidate these mechanisms.

To study the neuronal proteome, we are using a technique called bio-orthogonal non-canonical amino acid tagging (BONCAT) to selectively label and subsequently purify neuronal proteins. We have acquired from the Sternberg lab (Yuet et al., 2015) an engineered strain of *C. elegans* that has a mutant phenylalanyl-tRNA synthetase, driven by the *rab-3* promoter for neuron-specific expression (*rab-3*::Thr-412Gly-*Ce*PheRS). This mutant tRNA synthetase preferentially substitutes the non-canonical amino acid p-azido-L-phenylalanine (Azf) for phenylalanine during protein synthesis.

After cultivating worms in the presence of Azf, the Azf-labelled neuronal proteins are tagged with biotin using a strain-promoted azide-alkyne click chemistry reaction (SPAAC). Biotin-tagged proteins can then be extracted and purified from total worm lysate using a streptavidin pulldown. The purified biotinylated Azf-labelled protein are then prepared for proteomic quantification using a single-pot sample-phase-enhanced sample preparation (SP3) technique. Nanoflow reverse-phase liquid chromatography coupled online to tandem mass spectrometry (nanoLC-MS/MS) is used to identify and quantify peptides in the sample.

To obtain a snapshot of neuronal proteomic changes during ageing, neuronal proteins from worm populations at days 1, 5 and 10 of adulthood will be analysed. We will report on progress towards establishing protocols for neuronal protein labelling, purification and quantification by mass spectrometry and on our analysis of the neuronal proteome during ageing.

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Discovering novel molecules that regulate axonal maintenance

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The maintenance of neuronal circuitry is critical for the correct function of a nervous system. Failure to maintain the integrity of the axon, the longest and most susceptible compartment of a neuron, results in axonal degeneration, which is a characteristic of both nerve injury and neurodegenerative disease. The genetic and cellular mechanisms that maintain axon integrity and prevent axonal degeneration remain largely unknown. Using the powerful genetic tools available in *C. elegans*, and focusing on a specific subset of sensory neurons, we have isolated a novel mutant strain with enhanced axonal maintenance defects. Using classical genetic mapping, combined with deep sequencing, we have identified the mutated gene that causes defects in axonal maintenance. This conserved molecule is expressed predominantly in muscles and not by the mechanosensory neurons of the animal, revealing that this molecule likely functions non cell-autonomously to regulate axonal maintenance. Here we will present the characterization of this conserved molecule, and its previously unknown functional role in maintaining the integrity of the axonal compartment post-developmentally.

Characterising the chromatin modifiers SET-25 and SET-32

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Mounting evidence in a number of organisms suggests that some epigenetic modifications acquired by an individual during its lifetime can be inherited for multiple future generations. This phenomenon is termed transgenerational epigenetic inheritance, and may provide a mechanism for the inheritance of environmentally acquired traits. We are studying transgenerational epigenetic inheritance using the nematode *Caenorhabditis elegans*.

We have developed a system in which RNAi-induced silencing of a GFP transgene is robustly inherited for multiple generations in the absence of the initial RNAi trigger. We have shown that the histone methyltransferase SET-25 and the putative histone methyltransferase SET-32 are required for effective transmission of transgene silencing. To further investigate the functions of these proteins, we used CRIPSR/Cas9 to tag the endogenous *set-25* and *set-32* loci with mCherry. This allows expression of tagged protein under complete endogenous control. We observed SET-25 expression in the distal germline, and will report SET-32 expression patterns.

SET-32 is an uncharacterized protein, and so we are also investigating the broader functions of SET-32. We show that *set-32* mutant hermaphrodites have reduced fertility, producing fewer live offspring, fewer total eggs and more unfertilized eggs in comparison to wild-type animals. This reduced fertility is rescued by providing mutant hermaphrodites with wild-type sperm, indicating a male germline defect. Closer investigation reveals that *set-32* mutant sperm are defective in crawling; they fail to crawl back into the spermatheca after being swept into the uterus by the passage of oocytes. Furthermore, *set-32* mutant animals display extended lifespan independent of reduced fertility. The contribution of chromatin modifiers to lifespan regulation is not well understood, and this result provides an interesting addition to current knowledge.

Characterizing the roles of Hedgehog-related genes in neuronal development

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C-terminal binding proteins (CtBPs) are transcriptional co-repressors involved in many biological processes, including neuronal development. Their loss results in embryonic lethality or gross neurodevelopmental defects in mice and *Drosophila.* In *Caenorhabditis elegans*, reduction of *ctbp-1* function disrupts development of the dorsal SMD (SMDD) axon, with the axon leaving the dorso-lateral path along which it normally extends.

Analysis of two sets of microarray data that compared transcript enrichment between young adult wild-type and two *ctbp-1* mutant strains revealed that 48 genes were consistently upregulated by more than 2-fold in the *ctbp-1* mutant strains. As CTBP-1 is expressed in both the nervous system and hypodermis, 12 candidate direct target genes expressed in either tissue were selected from amongst the 48 genes. Further examination for possible mechanistic links to axon guidance revealed that 4 were Hedgehog-related genes—*wrt-6, wrt-10, grl-5* and *grl-16*. The roles of these Hedgehog-related genes are relatively unexplored, but similar factors in other model organisms have been implicated in neuronal development and more recently in *C. elegans* by Riveiro *et al.* (2017), who demonstrated that *wrt-8* and *grl-16* influence PVQ axon migration.

This project investigates how CTBP-1 regulates SMDD development by characterizing the expression of the 4 Hedgehog-related genes and examining if their over-expression could be responsible for the misguided SMDD axon phenotype displayed by *ctbp-1* mutants. Previously, Aspöck et al. (1999) and Hao et a. (2006) reported on the expression profiles of *wrt-6, wrt-10* and *grl-5*, but did not detect expression of *grl-16* from a reporter. We have successfully generated transcriptional reporter lines for the 4 Hedgehog-related genes and have begun characterizing where these genes are expressed in wild-type and whether that expression is influenced by loss of *ctbp-1* function. We will additionally report our progress towards determining the effect of over-expression of the Hedgehog-related genes on SMDD development.

Computational identification of neuron-specific cis-regulatory elements in *Caenorhabditis elegans*

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Gene transcription is controlled by transcriptional factors (TFs) interacting to DNA cis-regulatory elements. This occurs mainly in the promoter region where distinct compositions of TFs can promote specific patterns of gene transcription in different cells and tissues. However, a comprehensive understanding of the regulatory code underlying cell-specific control of gene expression remains a great challenge. *C. elegans* is a valuable model system to investigate transcriptional networks associated with specific cell-types, such as differentiated neurons. We propose a systems-based approach to discover and validate specific DNA motifs present in promoter regions that dictate cell-specific expression in selected neurons. We performed DNA motif discovery analysis on 19 high-confidence genes expressed in the I5 pharyngeal interneuron. Our computational analysis identified 35 DNA motifs significantly enriched in the promoter region of these genes. In addition, this platform also identified five genes with a specific combination of DNA motifs that may be associated with neuron-specific transcription. We are validating these predictions using GFP reporter transgenic animals. If successful, our integrated computational and experimental approach could be used for other cell-specific gene expression analysis, and provide a general framework to systematically test combinations of DNA motifs that might confer cell-specific control of gene expression.

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