12e/th Conférence canadienne de biologie du développement
Canadian Developmental Biology Conference

Monday & Tuesday Sessions:

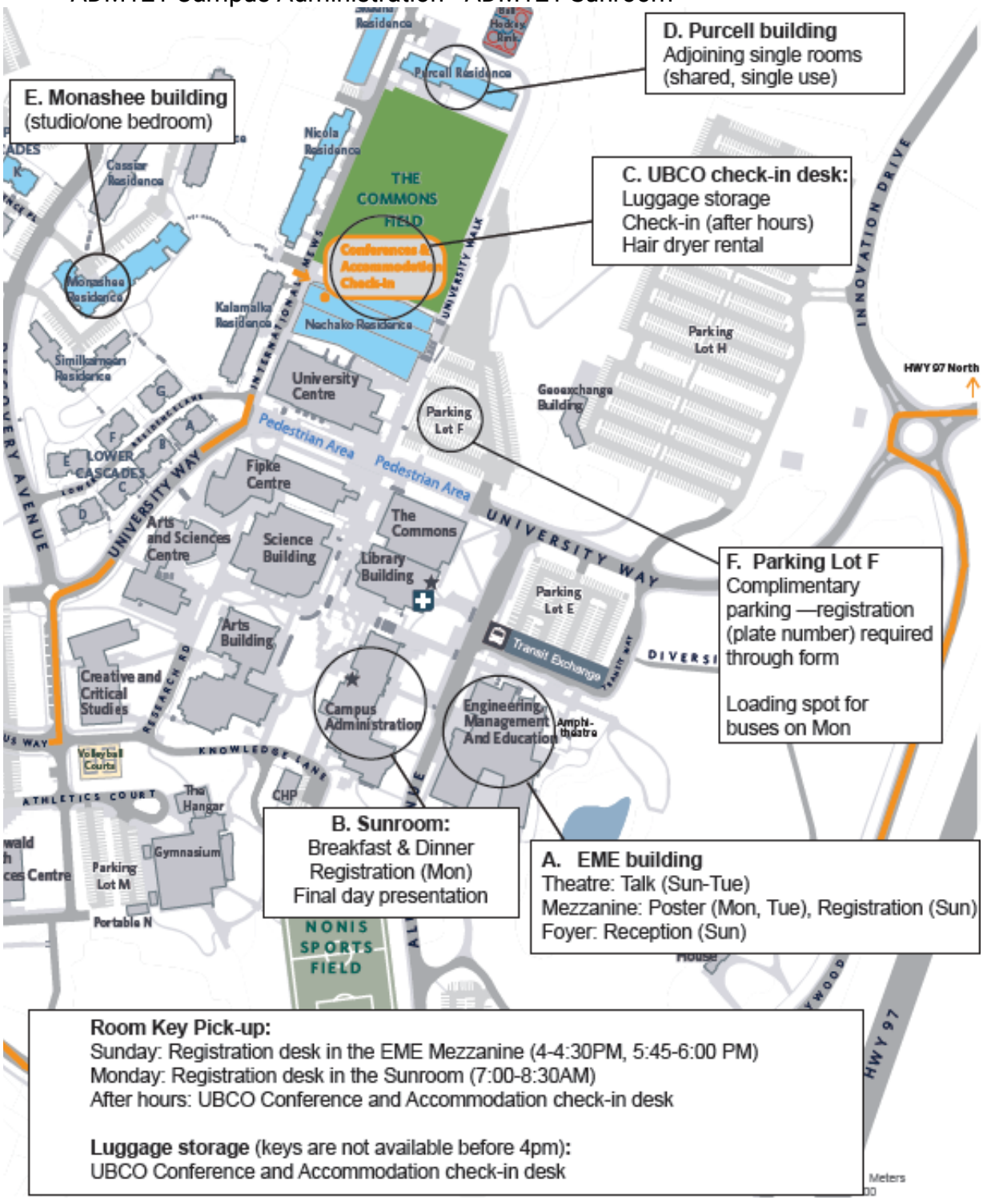
EME 0050 Engineering, Management and Education Building EME-Floor B1

Wednesday AM Sessions:

ADM121 Campus Administration - ADM121 Sunroom

Food:

ADM121 Campus Administration - ADM121 Sunroom

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SUNDAY, MAY 25

2:00 – 4:30 pm Registration -EME building – Mezzanine

Workshop - EME 0050

3:00 – 4:00 pm '*Putting GenAI to Work: A Workshop for Trainees and Faculty"* by **Stanley Bardal**

Introduction and welcome - EME 0050

4:30 – 4:40 pm **Fred Berry / Sarah Childs / Brian Eames / Jennifer Hocking / Peng Huang / Sarah Hughes / Andrew Simmonds / Kenji Sugioka / Guy Tanentzapf**

4:40 – 4:45 pm Guy Tanentzapf Canadian SDB Representative, University of British Columbia *"Information from SDB"*

KEYNOTE LECTURE - EME 0050

4:45 – 5:45 pm **John Wallingford**
Professor, Department of Molecular Biosciences, University of Texas at Austin
"Form and function in the ciliary proteome"

5:45 – 7:00 pm Buffet Dinner (Sunroom)

EDUCATION SESSION

7:00 – 8:30 pm **Stanley Bardal** Associate Professor, Department of Anatomy, Physiology, and Pharmacology, University of Saskatchewan *"GenAI as a Tool in Academia: Promise, Pitfalls, and (Current) Limitations"*

8:30 – 11:30 pm **OPENING RECEPTION** – EME Foyer

MONDAY, MAY 26

7:00 – 8:30 am Breakfast -Sunroom

7:30 – 8:30 am Registration - EME building – Mezzanine

SESSION 1 **STEM CELLS AND REGENERATION -** EME 0050
Session Chair, Li-Fang (Jack) Chu

8:30 – 9:00 am **Li-Fang (Jack) Chu,** Assistant Professor, Department of Comparative Biology & Experimental Medicine, University of Calgary *"Species-specific developmental clock models derived from pluripotent stem cells"*

9:00 – 9:15 am **Prajakta Bodkhe** Graduate Student, University of Calgary*"Cytokine stem cell priming promotes gut homeostasis under conditions of hypoxia-reoxygenation in Drosophila"*

9:15 – 9:30 am **Anushka Sharma** Graduate Student, University of Calgary **"***Investigating the role of CUP-2 (Derlin) protein in Niche positioning in C. elegans"*

9:30 – 10:15 am Break

10:15 – 10:45 am **Madeline Hayes** Assistant Professor, Department of Molecular Genetics, University of Toronto *"Developmental cell state transitions as drivers of pediatric solid tumor metastasis"*

10:45 – 11:00 am **Mara Whitford** Graduate Student, McGill University *"Why don't we get more cancer? Epithelial tissue architecture acts as a barrier to cancer initiation"*

11:00 – 11:30 am **Qiumin Tan** Associate Professor, Department of Cell Biology, University of Alberta *"The birth and death of hippocampal neurons"*

11:30 – 11:45 am **Maryam Khandani** Graduate Student, University of Alberta *"Determining the role of Moesin protein palmitoylation"*

11:45 – 12:00 pm **SIXTY-SECOND SCIENCE**
#26 -Debapriya Roy, #36 -Madyson Syvenky, #44 -Sydney Ko, #48 -Lucas Chuk, #56 -Ahelee Noor, #60 -Haocun Ye, #66 -Lily Buckles-Whittle, #70 -Sean Carter, #76 -James Stewart, #80 -Carly Sullivan

12:00 pm Pick up Boxed Lunch – EME Foyer

12:00 – 3:30 pm **FREE TIME (busses to Kelowna waterfront, Knox Hill Park)**

3:30 – 5:30 pm **POSTER SESSION 1 (EVEN NUMBER) - E**ME building – Foyer

5:30 – 6:30 pm Buffet Dinner - ADM121 Sunroom

SESSION 2 **GENE EXPRESSION AND DEVELOPMENT -** EME 0050
Session Chair, Deniz Top

6:45 – 7:15 pm **Deniz Top** Assistant Professor, Department of Cell Biology, University of Alberta *"Coordination of distinct transcription programs between different neurons regulate coherent behavior"*

7:15 – 7:30 pm **Soeren Huettner** Postdoctoral Fellow, McGill University *"CTCF is essential for limb muscle development by preventing ectopic gene expression in myogenic progenitors"*

7:30 – 7:45 pm **Craig Jacobs** Postdoctoral Fellow, University of Calgary "*A non-linear response to Hedgehog signalling provides a mechanism for novel directions of shape change during development"*

7:45 – 8:00 pm **Kevin Wei** Assistant Professor, Department of Zoology, University of British Columbia *"Evolved embryonic suppression of recently expanded retroelements through heterochromatin nucleation"*

8:00 – 8:30 pm **Pierre Mattar** Associate Professor, Department of Cellular and Molecular Medicine, University of Ottawa *"Chromatin remodelling complexes link developmental timing to neurodevelopmental disorders"*

TUESDAY, MAY 27

7:00 – 8:30 am Breakfast - ADM121 Sunroom

7:30 – 8:30 am Registration - EME building – Mezzanine

SESSION 3 **GROWTH, DIFFERENTIATION, AND PATTERNING -** EME 0050
Session Chair, Jessica Rosin

8:30 – 9:00 am **Jessica Rosin** Assistant Professor, Department of Oral Biological and Medical Sciences, University of British Columbia *"New insights into immune regulation of craniofacial morphogenesis"*

9:00 – 9:15 am **Menghao Lu** Graduate Student, University of British Columbia *"The functions of CaMKII and PKC in Wnt-dependent neurite pruning in C. elegans"*

9:15 – 9:45 am **Heather Bruce** Assistant Professor, Department of Zoology, University of British Columbia *"How the old becomes new: tracking morphological and gene network evolution over half a billion years"*

9:45 – 10:30 am Break

10:30 – 11:00 am **Muhammed Simsek** Assistant Professor, Department of Biology, McMaster University *"Signalling dynamics required for sequential segmentation of vertebrate embryos"*

11:00 – 11:15 am **Shreyosi Bose** Graduate Student, University of Alberta *"Regulation of PP1 phosphatase in C. elegans sperm development and post-fertilization signaling"*

11:15 – 11:45 am **Eric Hall** Assistant Professor, Department of Human Anatomy and Cell Science, University of Manitoba *"Uncovering cytoneme regulated signaling events in spinal cord development"*

11:45 – 12:00 pm **SIXTY-SECOND SCIENCE**
#29 -Zain Patel, #37 -Serhiy Havrylov, #39 -Nguyen Cai, #41 -Mizuki Kurashina, #43 -Nooshin Shekari, #53 -Marziyeh Hassanzadeh, #71 -Nuwanthika Wathuliyadde, #75 -Henry Luo, #77 -Xinyi (Stephanie) Xie

12:00 – 1:00 pm Lunch (Sunroom)

1:00 – 1:30 pm **Technical presentation from Leica -** EME 0050

1:00 – 3:00 pm **POSTER SESSION 2 (ODD NUMBER) - E**ME building – Foyer

SESSION 4	**CONTROL OF SIZE, SHAPE, AND MORPHOGENESIS -** EME 0050 Session Chair, Heather Szabo-Rogers
3:15 – 3:45 pm	**Heather Szabo-Rogers** Assistant Professor, Department of Anatomy, Physiology, and Pharmacology, University of Saskatchewan *"Insights from Robinow Syndrome reveal crosstalk between HH and Wnt/PCP pathways"*
3:45 – 4:15 pm	**Arif Ashraf** Assistant Professor, Department of Botany, University of British Columbia *"Function of nuclear envelope proteins beyond the nuclear envelope"*
4:15 – 4:30 pm	**Maria Sharkova** Graduate Student, University of Alberta *"Unveiling the Photoreceptor Outer Segment Cage Formed by Calyceal Processes, Müller Glia, and the Retinal Pigment Epithelium"*
4:30 – 5:00 pm	Break
5:00 – 5:15 pm	**Gonca Erdemci-Tandogan** Assistant Professor, Department of Physics and Astronomy, University of Western Ontario *"Cell divisions challenge tissue boundaries and sharpen them through tissue fluidity"*
5:15 – 5:30 pm	**Min Zhu** Postdoctoral Fellow, Hospital for Sick Children, University of Toronto *"Tissue stiffness mapping by light sheet elastography"*
5:30 – 6:00 pm	**Jessica Feldman** Associate Professor, Department of Biology, Stanford University *"Mechanisms of epithelial polarization and connectivity"*
6:00 – 6:15 pm	**YuXuan (Rain) Xiong** Graduate Student, University of British Columbia *"Twisting Cytokinesis: Cell Adhesion and Cortical Flow Underlie Chiral Morphogenesis in Caenorhabditis elegans Embryos"*
6:15 – 6:30 pm	**Andreas Dauter** Graduate Student, University of Calgary "*A Model of the Middle: How do cell behaviours shape the embryonic face?"*
7:00 – late	**Wine Tasting hosted by Gone West Wine Club** - ADM121 Sunroom (includes canapes and non-alcoholic drink options)

WEDNESDAY, MAY 28

7:00 – 8:30 am Breakfast (Sunroom)

SESSION 5 **DEVELOPMENTAL MODELS OF DISEASE -** EME 0050
Session Chair, Brittany Carr

8:30 – 9:00 am **Brittany Carr** Assistant Professor, Department of Ophthalmology, University of Alberta "*The role of prominin-1 in photoreceptor development and disease*"

9:00 – 9:15 am **Andy Cheng** Graduate Student, University of Alberta ***"****Lipid coordination between peroxisomes, peroxins, and lipid droplets"*

9:15 – 9:45 am **Jeff Rasmussen** Associate Professor, Department of Biology, University of Washington *"Building and maintaining the touch system: Insights from zebrafish skin"*

9:45 – 10:30 am Break

10:30 – 10:45 am **Gurpreet Moroak** Research Associate, Simon Fraser University *"Tumor growth in Drosophila larval epithelial tissue induces distant organ wasting through fat body metabolic dysregulation"*

10:45 – 11:00 am **Gabriel Bossé** Assistant Professor, Département de psychiatrie et de neurosciences, Université Laval *"Investigating the neurodevelopmental impact of polydrug exposure using zebrafish"*

11:00 – 11:30 am **Jiami Guo** Assistant Professor, Department of Cell Biology and Anatomy, University of Calgary *"Neuronal primary cilia modulate synaptic development and function"*

11:45 – 12:00 pm **CLOSING REMARKS**

12:00 – 1:30 pm Lunch and Trainee Award Presentations

1:30 pm Departure

[2] Samir Merabet *samir.merabet@ens-lyon.fr*

Hox proteins repress autophagy by anchoring autophagy-related-genes in the Lamin-C rich environment of the nuclear periphery

Samir Merabet[1, 2, 3]

[1]IGFL, [2]ENSL, [3]CNRS

Gene regulation is not occurring randomly within the nucleus, but is under the control of nuclear matrix and chromatin-remodelling proteins that participate to the genome-wide compartmentalization of transcriptionally active and inactive regions. Surprisingly, how the specific regulatory activity of transcription factors (TFs) could be influenced by the spatial localization within the nucleus has rarely been considered. As a consequence, how nuclear matrix components could participate to gene-specific regulatory complexes with TFs remains poorly understood. Here, we show that the Hox protein Ultrabithorax (Ubx) represses autophagy in the Drosophila larval fat body by localizing autophagy related (atg) genes at the nuclear periphery. This activity depends on its DNA-binding and protein-protein interactions with the nuclear membrane-associated protein Lamin-C. Accordingly, Hox proteins are not able to repress autophagy in the absence of Lamin-C. In addition, the absence of Hox proteins is sufficient to induce autophagy and correlates with a more distant positioning of atg loci from the nuclear membrane. Interestingly, Lamin-C is also required for stabilizing Hox proteins in fat body nuclei. Overall, our results demonstrate that Hox proteins are able to localize their target genes at specific places of the nucleus for their specific transcriptional regulation during development.

[3] Heather Bruce *heather.bruce@ubc.ca*

Cryptic persistence of truncated abdominal legs in insects enabled diverse outgrowths with novel functions

Heather Bruce[1], Nipam Patel[2], [3]

[1]University of British Columbia, [2]Marine Biological Laboratory, [3]University of Chicago

An iconic feature of insects is the apparent lack of legs on the abdomen, which is believed to be due to the repression of the leg-patterning gene Distalless (Dll) by abdominal Hox genes. However, in contrast to these molecular observations, it is not widely appreciated that the embryos of most insect groups do in fact form paired protrusions on most abdominal segments that appear to be homologous to the thoracic legs. However, these degenerate before hatching to form the abdominal body wall. To resolve this discordance between molecular and morphological observations, the expression patterns of pannier and araucan, genes known to distinguish proximal leg segments in all arthropods, are examined in embryos of the flour beetle Tribolium castaneum. In Tribolium embryos, all pregenital abdominal segments develop leg-like paired protrusions, and the stripes of pannier and araucan expression that delineate the proximal leg segments of the thorax are also expressed in the same configuration around these abdominal protrusions. This suggests that insect abdominal legs are homologous to only the proximal portion of the thoracic legs, which in insect adults forms the lateral body wall (lateral tergum and pleura). These cryptic, truncated abdominal legs - likely inherited from their crustacean ancestors - appear to be an important wellspring for new functions in insects, such as caterpillar prolegs, gills, and structures for camouflage and aposematic warning.

[4] Gabriel Bossé gabriel.bosse@cervo.ulaval.ca

Investigating the neurodevelopmental impact of polydrug exposure using zebrafish

Lise Hermant[1], Gabriel Bossé[1]

[1]Université Laval

Every year in Canada, approximately 15,000 infants are affected by neonatal drug exposure. Substance use during pregnancy has been linked to a variety of adverse outcomes for infants, including congenital disabilities, neurodevelopmental delays, and long-term challenges such as learning difficulties. Currently, there is no known treatment for these detrimental effects. The situation is further complicated by the fact that many newborns are often exposed to multiple substances in utero. The impact of polysubstance exposure on neurodevelopment and neural circuit connectivity is not yet well understood in either animal models or human subjects.Zebrafish offers a promising alternative for enhancing our understanding of the biological effects of neonatal drug exposure. Taking advantage of zebrafish's scalability, we are exposing embryos to some of the most consumed substances: nicotine, alcohol, opioids, and all possible combinations. We are using a multidisciplinary approach combining behavioural studies, transcriptomic and functional imaging to investigate the neurodevelopmental impact of such exposure. We hypothesize that we will observe drug-combination-specific molecular and physiological signatures.Our preliminary data indicates that exposure to these substances during embryonic development leads to behavioural changes. These alterations affect sensorimotor responses, sleep cycles, and sociability. We are using the calcium reporter GCaMP6 to perform whole-brain imaging to measure neuronal activity in live animals. Our analysis has shown that the combination of nicotine and ethanol alters brain activity. Additionally, an initial characterization of gene expression in exposed larvae has identified drug-specific differentially expressed genes, including neurodevelopmental factors, regulators of neurotransmitter functions, and circadian rhythm genes.Our study will play a key role in enhancing our understanding of neonatal drug exposure by directly comparing the neurobiological impact of both single and polydrug at the molecular level. This has critical clinical considerations since future treatment options must be adapted based on the distinct neurobiological effects of drug combinations.

[5] Adda-Lee Graham-Paquin adda-lee.graham-paquin@mail.mcgill.ca

Efferocytosis: a role in Modulating Epithelial Cell Plasticity

Adda-Lee Graham-Paquin[1], [2]

[1]McGill University, [2]Goodman Cancer Institute

The regeneration of adult tissues after injury, or the replacement of cells in tissues with high turnover rates, is well understood in tissues with distinct stem cell populations. However, in the adult prostate, despite its ability to regenerate through cycles of castration-induced regression and regrowth, no identifiable stem cell population has been observed. It has been shown that differentiated epithelial cells behave as facultative progenitors to contribute to repopulation during regeneration.We hypothesize that apoptotic cells present during castration-mediated regression contribute to their acquired progenitor fateThrough histological analysis, we identify non-professional phagocytosis of apoptotic cells to be a predominant route of cell clearance in the regressing prostate. By quantifying changes tissue morphology as well as cell type heterogeneity throughout the process I plan to characterize the process of prostate regression more in depth.We propose that similar to professional phagocytes, such as macrophages, epithelial cells alter their gene transcription in response to their contact with dying cells. We also intend to explore the changes in metabolic programs in these cells. To determine the role of cell clearance in acquired tissue regeneration potential, we blocked phosphatidyl serine (PtdSer), an “eat-me” signal present on apoptotic cells, during prostate regression. I generated a mouse model in which I can induce expression of a dominant negative form of the potent PtdSer binding protein MFGE8, MFGE8-D89E in K8-expressing luminal cells (K8CreERT2; MFGE8-D89E) to block engulfment in vivo. We will assess the influence on regeneration potential of luminal cells through RNA sequencing, surgical regeneration, and immunofluorescent analysis of progenitor markers.Through this study, we hope to gain a better understanding of the role of phagocytosis by epithelial cells apart from cell clearance and to determine the contributions of apoptotic cell signaling to epithelial cell plasticity.

[6] Min Zhu min.zhu@sickkids.ca

Tissue stiffness mapping by light sheet elastography

Min Zhu[1], Kaiwen Zhang[1, 2], Evan Thomas[1], Ran Xu[1, 2], Brian Ciruna[1, 2], Yu Sun[2], Sevan Hopyan[1, 2]

[1]The Hospital for Sick Children, [2]University of Toronto

Tissue stiffness plays a crucial role in regulating morphogenesis. The ability to measure and monitor the dynamic progression of tissue stiffness is important for generating and testing mechanistic hypotheses. Methods to measure tissue properties in vivo have been emerging but present challenges with spatial and temporal resolution especially in 3D, by their reliance on highly specialised equipment, and/or due to their invasive nature. Here, introduce light sheet elastography, a noninvasive method that couples low frequency shear waves with light sheet fluorescence microscopy by adapting commercially available instruments. With this method, we achieved in toto stiffness mapping of organ-stage mouse and zebrafish embryos at cellular resolution. Versatility of the method enabled time-lapse stiffness mapping during tissue remodelling and of the beating embryonic heart. This method expands the spectrum of tools available to biologists and presents new opportunities for uncovering the mechanical basis of morphogenesis. This work was funded by the Canada First Research Excellence Fund/Medicine by Design (MbDGQ-2021-04 to Sevan Hopyan/Yu Sun and MPDF-2020-04 to Min Zhu), and the Canadian Institutes of Health Research (168992) to Sevan Hopyan/Yu Sun. Yu Sun also acknowledges support from the Canada Research Chairs program.

[7] Brian Ciruna ciruna@sickkids.ca

Oxidative stress induces intervertebral ECM remodelling, elevated tissue stiffness and idiopathic-like scoliosis

Patrick Pumputis[1], Ran Xu[1], Josh Gopaul[2], Arash Panahifar[3], Vida Erfani[1], Jenica Van Gennip[1,4], B. Frank Eames[5], Nikan Fakhari[6], Jérôme Baranger[2], David Lebel[7], Olivier Villemain[6], Brian Ciruna[1]

[1]Developmental & Stem Cell Biology Program, The Hospital for Sick Children; Department of Molecular Genetics, University of Toronto., [2]Translational Medicine Program, The Hospital for Sick Children, [3]BioMedical Imaging and Therapy Beamline, Canadian Light Source; Department of Medical Imaging, University of Saskatchewan, [4]Developmental & Stem Cell Biology Program, The Hospital for Sick Children, [5]Department of Anatomy, Physiology, and Pharmacology, University of Saskatchewan, [6]Translational Medicine Program, The Hospital for Sick Children; Department of Medical Biophysics, University of Toronto, [7]Division of Orthopaedic Surgery, The Hospital for Sick Children

Adolescent idiopathic scoliosis (AIS) is a paediatric disorder characterized by rotational spinal deformity, which develops in the absence of obvious congenital or physiological defects. 4% of children will develop AIS, and 1 in 10 patients (predominantly female) will experience severe curve progression. Patient exome/genome sequencing and mouse functional studies have associated genetic variants in musculoskeletal collagen and cartilaginous extracellular matrix (ECM) defects with a fraction of AIS cases. However, GWAS meta-analyses estimate that >95% of AIS heritability remains unknown. To date, the biology of AIS remains poorly understood, there are no prognostic biomarkers, and treatment options are limited to restrictive bracing and invasive corrective surgery

Using zebrafish models of AIS, we have discovered that oxidative stress and pro-inflammatory signals in the spinal cord, which develop because of cerebrospinal fluid homeostasis defects, are necessary and sufficient to drive spine curvature. Indeed, antioxidant and immunomodulating drugs can efficiently block scoliosis onset and severe curve progression in fish models. Although this provides proof-of-principle that AIS might be managed therapeutically, uncertainties regarding downstream mechanism and their link to human disease pose a barrier to clinical translation. Here, we demonstrate that neuroinflammation-associated reduction-oxidation (redox) imbalance induces cell stress, collagen remodelling defects and physical deformations within intervertebral segments of the developing spine. Using shear wave elastography (SWE), we show that zebrafish scoliotic spines are consequently stiffer than healthy controls - a property also reported for intervertebral discs in human AIS patients. Remarkably, both elevated spine stiffness and intervertebral ECM phenotypes are detectable prior to scoliosis onset in zebrafish models, suggesting a causal role in AIS, and can be suppressed by antioxidant treatment. Together, our studies implicate oxidative stress-induced intervertebral deformations in the pathogenesis of AIS and identify elevated spine stiffness and redox imbalance as plausible first-in-kind prognostic biomarkers and therapeutic targets.

[8] Deniz Top *dtop@ualberta.ca*

PKA instructs Drosophila Clock protein in regulating circadian transcription, mediating transcriptional synchrony in the circadian neuronal network

Minjeong Shin[1], Isabella Hajdu[1], Myah Verghese[1], Peter Johnstone[1], Deniz Top[1]

[1]Department of Cell Biology, University of Alberta

Circadian rhythms are behavioural and physiological responses to rhythmic environmental changes, such as photoperiod and temperature. Such behavioural rhythms are regulated by a transcription/translation negative feedback loop called the circadian clock, which regulates the daily expression of several hundred genes. In Drosophila, the circadian clocks are found in ~240 neurons that are arranged into distinct clusters across the brain, suggesting a need for communication across the clusters to maintain coherent behavioural rhythms. Neurotransmitters connect of these clusters, often acting through G-protein coupled receptors (GPCRs), forming a neuronal network. Among the functions of GPCRs is regulation of cytosolic cAMP levels that serve as a secondary signal to relay extracellular instructions to internal cellular machinery. PKA is a kinase that responds to cAMP and activates a wide variety of proteins through phosphorylation. We have found that PKA phosphorylates CLK protein at a single residue to repress function of the transcriptional activator complex of the circadian clock. This site is conserved across various species of insects, fish and mammals. When PKA is knocked down in distinct circadian neuronal clusters, both the local circadian clocks and overall behavioural rhythms change differently. Thus, PKA is a signaling molecule that responds to various instructions in distinct circadian neurons to regulate the circadian clock, maintaining both circadian clock synchrony and ensuring coherent development of behavioural rhythms.

[9] Thea Do thi.do.huynhlan@gmail.com

Heavy metal (Lead) alters auxin-mediated root gravitropic responses in maize and Arabidopsis

Thea Do[1], Olivia Hazelwood[1], Grace Delpit[2], Erin Sparks[3], Norman Best[4], Mohammad Arif Ashraf[1]

[1]University of British Columbia, [2]Howard University, [3]University of Delaware, [4]University of Missouri

Environmental stresses are caused by either fluctuations in the environment or pollutants contributed by human civilization. Heavy metal contamination is one example of the latter cause. Heavy metal contamination is of significant concern as it is retained in the environment, gets integrated into the food system, and eventually causes heath complications for humans and plants. Plants contaminated with heavy metals are the major route for these toxins to find their way into the food system. However, the plant responses to heavy metals such as lead (Pb) are poorly understood. This study shows that Pb regulates the phytohormone auxin and alters auxin-mediated root gravitropic responses through a conserved mechanism in the model plant Arabidopsis thaliana and crop plant *Zea mays*.

[10] Olivia Hazelwood olivia.hazelwood@botany.ubc.ca

Cell cycle follows "pause and play" mechanism in environmental stress recovery

Olivia Hazelwood[1], Vicky Hollenbeck[2], Dustin Herb[2], Joseph Gallagher[2], Arif Ashraf[1]

[1]Department of Botany, University of British Columbia, [2]Forage Seed and Cereal Research Unit, United States Department of Agriculture

Across the tree of life, organisms interact with the surrounding environment during growth and development. The alteration of organismal growth due to environmental stress is orchestrated at the cellular level and manifested at the organ level. Organismal growth relies on a combination of cell division, expansion, and differentiation. In a natural environment, a period of environmental stress is followed and preceded by favorable growth conditions. We tested cell cycle regulation during a control, stress, and recovery period for salt, osmotic, cold, and heat stresses using dicot *Arabidopsis thaliana* and monocots *Brachypodium distachyon*, *Lolium multiflorum* (annual ryegrass), and *L. perenne* (perennial ryegrass). We identified a conserved "pause and play" mechanism of the cell cycle during environmental stress and stress recovery across a wide range of plant systems.

[11] Bryan Crawford bryanc@unb.ca

Mechanical stretch detected by Piezo1 in the epidermis of growing zebrafish embryos triggers matrix remodelling through post-translational activation of Mmp2 mediated by Erk and Mmp14.

Jillian Hickey[1], Bryan Crawford[1]

[1]Department of Biology, University of New Brunswick

The emergence of tissue architecture during embryonic morphogenesis, wound healing and regeneration depend on the dynamic remodelling of the extracellular matrix (ECM). The best-known effectors of ECM remodelling are the matrix metalloproteinases (MMPs), all of which are translated as in-active pro-enzymes that undergo post-translational activation, typically via the proteolytic removal of an auto-inhibitory N-terminal pro-peptide. Using the Epitope-Mediated MMP Activation (EMMA) assay, we observe that matrix metalloproteinase 2 (Mmp2) is post-translationally activated in a curios 'patch-work' like pattern in the epidermis of zebrafish embryos. Here we show that this pattern is modulated by the mechanosensitive Piezo1 stretch receptor; activation and inhibition of Piezo1 signalling cause increases and decreases, respectively, in the extent of activated patches of Mmp2 in the epidermis. Furthermore, activation and inhibition of Piezo1 signalling increases and decreases expression of mmp14b, a well-known activator of proMMPs, and both Mmp2 activation and mmp14b expression are reduced by inhibition of Erk. Finally, both pharmacologically reduced ECM integrity and mechanical confinement of embryos result in perturbations of this patchy pattern of Mmp2 activation as predicted by our model. Thus, we demonstrate a novel mechanism linking the forces experienced by cells in tissues undergoing mechanical deformation to the biochemical mechanisms by which they remodel their extracellular matrix, and propose Erk as a central integration hub for mechanical signalling in developing tissues.

[12] Eric Hall eric.hall@umanitoba.ca

Uncovering cytoneme regulated signaling events in spinal cord development

Morgan Hiebert[1], Eric Hall[1]

[1]University of Manitoba

During development, morphogens and signaling ligands instruct cell fate across long distances, but visualizing the movement of morphogen transport in situ has been challenging. We've developed novel histology approaches allowing for the preservation and visualization of long specialized signaling filopodia called cytonemes. Using these methods, we've shown cytonemes transport morphogens like Sonic Hedgehog (SHH) across the developing mouse neural tube. Disrupting cytoneme formation by knocking out the filopodial motor protein Myosin 10 (MYO10) impairs signaling activity of SHH, WNT, and Notch pathways, resulting in severe neurodevelopmental defects in mice. However, whether cytonemes facilitate morphogen transport in later stages of neurodevelopment to further regulate developmental trajectories is unknown. We currently lack the capacity to preserve and study cytonemes in later stages of development and adult tissues. To address this question, we are building new imaging and tissue preservation approaches, combined with single-nuclei RNAseq to study cytonemes in later stages of spinal cord development. Transcriptomic analysis of e18.5 and adult mice spinal cords reveals continued ubiquitous decreased SHH, WNT, and Notch activity in MYO10-/- mice, suggesting cytonemes mediated signaling is required throughout development and tissue homeostasis. Single-nuclei transcript analysis of spinal cords combined with tissue imaging, is revealing specific glial and neuronal population that require cytonemes to facilitate morphogen delivery for accurate development throughout spinal cord formation.

[13] Bradley Hoskin bhoskin@bccrc.ca

Exploring hepatomesenchymal cell plasticity and cell states in liver development and regeneration

Bradley Hoskin[1, 2], Rebecca Cullum[2], Kwangjin Park[1, 2], Pamela Hoodless[1, 2]

[1]University of British Columbia, [2]BC Cancer Research Centre

The liver carries out hundreds of vital functions that are directly tied to its complex organization, which is disrupted in liver diseases. Understanding how cell fates and patterning are determined to properly organize the liver during development is crucial to effectively treat liver diseases. In mouse development, endodermal-derived hepatic progenitors, known as hepatoblasts, migrate as epithelial cords and invade the surrounding mesodermal-derived septum transversum mesenchyme to form a liver bud. Hepatoblasts and mesenchymal cells then intermix with endothelial cells, proliferate, and differentiate, organizing into a functional liver. We recently discovered hepatomesenchymal cells (HMCs), a novel hepatic cell-state found in the fetal liver that co-expresses hepatic and mesenchymal gene signatures. HMCs were found in the early mouse liver bud at embryonic day (E)10.5; however, their terminal fates and roles within the liver remain unclear. Using immunofluorescence, we identified hepatic cells that also express mesenchymal markers throughout the rest of development, indicating that HMCs and their progeny persist past E10.5. Additionally, hepatocytes that also co-express hepatic and mesenchymal markers have been found in the adult mouse liver, and are implicated to play a pivotal role in liver regeneration and hepatocyte repopulation following injury. Therefore, we hypothesize that HMCs have a role in liver development and contribute to a subpopulation of hepatocytes that act as the main hepatocyte progenitor in adult liver regeneration following injury. We will investigate the precise role, terminal fates, and plasticity of HMCs using fluorescent lineage tracing models and RNA sequencing. Understanding the origin and differentiation of HMCs through development and into the mature liver will provide insights into treating liver diseases. Funding is by the Canadian Institutes of Health Research and the National Sciences and Engineering Research Council of Canada.

[14] Gonca Erdemci-Tandogan *gerdemci@uwo.ca*

Cell divisions challenge tissue boundaries and sharpen them through tissue fluidity

Gonca Erdemci-Tandogan[1], Veronica Castle[2, 3], Merdeka Miles[1], Rodrigo Fernandez-Gonzalez[2, 3, 4, 5]

[1]Western University, Department of Physics and Astronomy, [2]University of Toronto, Department of Cell and Systems Biology, [3]University of Toronto, Translational Biology and Engineering Program, [4]University of Toronto, Institute of Biomedical Engineering, [5]The Hospital for Sick Children, Developmental and Stem Cell Biology Program

Tissue boundaries are essential for embryonic patterning, tumour suppression, and directional cell behaviour. They are associated with actomyosin cables that generate tension, preventing cell mixing between tissues. However, the mechanisms of actomyosin cable maintenance at boundaries remain unclear. We investigated the maintenance mechanisms of the mesectoderm-ectoderm (ME) boundary in Drosophila embryos, where ectoderm cells divide near the boundary while mesectoderm cells are internalized. Through computational modelling and in vivo experiments, we discovered a dual role for ectoderm cell divisions: they both challenge boundary integrity and enhance boundary refinement. By suppressing ectoderm divisions, we demonstrated that the ME boundary actively resists division-induced perturbations. Our model predicted that divisions facilitate boundary refinement by reducing tension and increasing tissue fluidity in the ectoderm. Laser ablation and cell tracking confirmed that divisions decrease junctional tension and enhance cell mobility. Our results suggest that cell divisions facilitate cellular rearrangements that increase tissue fluidity to refine tissue boundaries.

[15] Amir Sabeti amir.sabeti@usask.ca

Neuroprotection against HIF-1-induced neurodevelopmental defects relies on HIF-1c, a non-transactivating HIF-1 isoform in *C. elegans*

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As a first line of defence, metazoans respond to hypoxic stress through cell-autonomous stabilization of HIF-1 (Hypoxia-Inducible Factor), a conserved transcription factor that mediates adaptation to low oxygen environments. While HIF-1 is essential for viability under hypoxia, persistent HIF-1 activation during development leads to miswiring and connectivity defects in the nervous system. How neurons correctly balance the pro-survival requirements for HIF-1 activity while avoiding the associated HIF-1-induced neurodevelopmental defects is not understood. Here we present evidence that *C. elegans* neurons mobilize an internal promoter inside the hif-1 locus to generate a transcriptionally inactive HIF-1 isoform (HIF-1c) that lacks the DNA binding domain but retains its ability to migrate to the nucleus and associate with HIF-1's heterodimeric partner, AHA-1/HIF-1b through the PAS-B domain. hif-1c expression is first detectable during early neurogenesis when zygotic hif-1 transcription also begins and is subsequently observed across the nervous system during larval and adult stages. Specifically depleting worms of HIF-1c by deleting the internal promoter [hif-1(syb8674)] leads to widespread defects in axon migration that parallels a rise in HIF-1-dependent transcription in these mutants. Together with preliminary evidence that suggests HIF-1c plays a role in antagonizing baseline HIF-1 activity in normoxia, we propose a model in which neurons, a particularly hypoxia-sensitive cell type, evolved a protection mechanism that relies on expressing a dominant negative HIF-1 isoform to fine tune the transcriptional outputs of HIF-1 thereby escaping disruptions during wiring of the nervous system. This research has broad implications for understanding the role of hypoxia and HIF-1 deregulation in neurodevelopmental disorders characterized by neuronal connectivity defects.

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Ultraconservation of developmental timing mechanisms during retinal neurogenesis

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Cell type specification is thought to mainly be coordinated by combinatorial 'codes' of transcription factors. Across evolution, the expression patterns of transcription factor codes are often conserved in the same cell 'orthotypes'. However, while these codes are often conserved, the enhancers that transcription factors regulate are poorly conserved. How are combinatorial codes of transcription factors maintained if their genomic targets are constantly changing over evolutionary time? Here, we describe an exception to this pattern. We find that the zinc finger transcription factor Casz1 - which has deep functional orthology in regulating neurogenesis from insects to vertebrates - occupies a deeply conserved enhancer landscape in murine retinal progenitor cells. Casz1 recruits the NuRD nucleosome remodelling complex to these elements. Analysis of mutants for the NuRD protein Chd4 reveals that NuRD remodels neighbouring regulatory elements, leading to major shifts in cell fate specification. Interestingly, in other cellular contexts, we find that Casz1 does not occupy deeply conserved enhancers. These latter observations suggest a model where the ancestral Casz1 gene was originally deployed in neural progenitors, where it regulated enhancers under similarly strong selection pressure. More recently, Casz1 would have been redeployed in novel contexts, where it 'joined' the combinatorial codes of disparate cell types to regulate ensembles of pre-existing enhancers that are under much lower selection pressure. This model suggests that some transcription factor codes may depend upon a deeply conserved regulome, which can only be observed in the orthotypic context.

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Tauopathy, Seizures, and Neuroinflammation in Zebrafish Larvae Post-TBI

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This project aims to elucidate the mechanisms through which Traumatic Brain Injury (TBI) affects inflammation, potentially contributing to heightened seizure susceptibility and dementia pathology. Previous studies in our laboratory have demonstrated, using zebrafish as a model, causal relationships between TBI, increased seizure incidence, and pathological aggregation of tau brain proteins—a biomarker reflecting end-stage features observed in human dementias such as Alzheimer's disease. Crucially, we have shown that treatment of fish post-TBI with anti-epileptic drugs results in a significant reduction in tau aggregation. However, the contributions of inflammation, a well-established consequence of brain injury, remain contentious. I hypothesize that heightened inflammation drives the increased rate and intensity of seizures following TBI in zebrafish larvae, which, in turn, exacerbates dementia pathology. To manipulate brain inflammation during post-TBI seizures, I am focusing on Toll-like receptor 4 (TLR4), a pivotal component of the innate immune system initiating pro-inflammatory pathways. I have developed a zebrafish model with a reduced inflammatory response by inducing loss-of-function mutations in TLR4 zebrafish homologs. I have preliminary data showing success in reducing inflammation in this model. In parallel, inflammation will be pharmacologically manipulated using NSAIDs administered immediately after injury and at regular intervals. In these models, I will compare seizure frequency, intensity, and tau aggregation between larvae with intact inflammatory responses and those with attenuated inflammation. Failure to reject our hypothesis (i.e., if reduced inflammation post-TBI demonstrates favorable effects on injury consequences) would represent a significant advance in understanding the interplay between TBI, inflammation, seizures, and dementia. Such findings could inform therapeutic strategies targeting inflammation to mitigate long-term neurodegenerative outcomes. Conversely, rejecting the hypothesis would provide valuable insights into the mechanisms underlying TBI-induced pathologies.

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GM1 brain ganglioside as a prophylactic treatment for dementia and seizures after brain injury

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Dementia represents a serious disease burden in the aging population globally. It encompasses the rapid and severe loss of memory and cognitive abilities. Traumatic brain injury (TBI) represents an important modifiable risk factor for dementia. The investigation of treatments and therapies to prevent the onset of dementia after TBI is crucial. One of the main consequences of TBI is early post-traumatic seizures (PTS). Previous work in the Allison lab has determined that early PTS are a known druggable mechanistic link between TBI and dementia. We utilize a blast-like model of TBI in larval zebrafish to examine the neurological and behavioral changes related to dementia after head injury. Preventing seizures with anti-epileptic drugs (AEDs) reduces the abnormal aggregation of a neuronal protein called tau. The aggregation of tau is seen in various forms of neurodegenerative dementias like Alzheimer's Disease (AD) and Chronic traumatic encephalopathy (CTE). Although prevention of seizures with AEDs is effective at reducing dementia, these drugs have been shown to elicit some negative side effects in patients. This is why alternative therapies for preventing dementia after head injury are essential. GM1 brain ganglioside is a glycolipid molecule found in the brain, with roles in neuronal development and signaling. Monosialotetrahexosylganglioside (GM1) is one of a variety of brain gangliosides with neuroprotective roles in other protein misfolding neurodegenerative diseases. GM1 represents an exciting novel therapeutic that prevents TBI-induced seizures and dementia. GM1 brain ganglioside successfully reduces seizures after TBI by half. GM1 also halves the amount of tau aggregation and cell death after TBI. Overall, GM1 is an exciting novel therapeutic for dementia after TBI in this pre-clinical model. Future work to discern if GM1 modulates its neuroprotective roles through inflammation should be explored. As well as, examining if GM1 and other anticonvulsants can work synergistically to exert their dementia-preventing effects.

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Why don't we get more cancer? Epithelial tissue architecture acts as a barrier to cancer initiation.

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Although 40% of people will be diagnosed with cancer in their lifetime, given the trillions of cells that each person possesses, tumour formation is relatively rare. Similarly, up to 80% of cells in a phenotypically normal adult epithelial tissue can possess oncogenic mutations, suggesting that mutations alone are insufficient for tumour development. A hallmark feature of carcinoma development is progressive loss of epithelial tissue architecture, whereby epithelial structures, like ducts or alveoli, lose organization and become multilayered. It remains poorly understood how tissue disorganization occurs during carcinoma initiation, and more intriguingly, what protective mechanisms epithelial tissues possess to prevent cancer initiation upon oncogene expression. To address this, I have cultured 3D epithelial organoids expressing doxycycline-inducible oncogenes frequently expressed across diverse cancer types, and performed live cell imaging to observe cell division orientation. In control organoids, misplaced divisions are efficiently corrected to maintain a single layered epithelium, suggesting a protective mechanism to maintain organization. Conversely, expression of KRASG12V or ERBB2 reduced the efficiency of correcting misplaced divisions. This, combined with randomization of division orientation upon KRASG12V expression, significantly increased tissue multilayering. While ERBB2 expression increased proliferation, it caused in minimal tissue disorganization. KRASG12V, which efficiently promoted tissue disorganization, had minimal effects on proliferation, but did impair tight junction integrity. Transient disruption of tight junctions in ERBB2 organoids promoted epithelial disorganization similar to that seen with KRASG12V expression. These findings suggest that proliferation alone is insufficient to promote disorganization and highlights the protective nature of epithelial tissue architecture. To further support this, oncogene expression prior to the establishment of lumen and tight junctions increased the severity of tissue disorganization. These results demonstrate that losing normal tissue architectural features, such as tight junctions, through oncogene expression, creates a permissive environment for tissue disorganization during tumour initiation.

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Functional conservation of Shox2-expressing interneurons regulating physiological rhythms in the brainstem and spinal cord

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Many rhythmic physiological processes and behaviours fundamental to vertebrate survival, including respiration and locomotion, are driven by the activity of neural circuits located in the central nervous system. These circuits are called central pattern generators (CPGs) and are typically composed of interconnected groups of excitatory and inhibitory interneurons, which give rise to oscillating activities. In the lumbar spinal cord, V2d and a subset of V2a interneurons expressing the SHOX homeobox 2 (Shox2) gene function in stabilizing the activity of the locomotor CPG, which propagates rhythmic activity through motor neurons projecting to the hindlimbs. In the medulla, V2a interneurons in the medial reticular formation appear to contribute to stabilizing the activity of the respiratory CPG through projections to the rhythmogenic kernel controlling the inspiration phase of breathing, the pre-Bötzinger complex. Here, we demonstrated that just as for the lumbar spinal cord, Shox2-expressing V2d and a subset of V2a interneurons are present in the neonatal mouse brainstem. When we conditionally inactivated Shox2 in the interneurons of the medial reticular formation using CRE/loxP recombination, neonatal mutants suffered from a more variable breathing rhythm, lower inspiratory amplitude, and greater apnea duration than control littermates. On a C57BL/6 genetic background, which is less tolerant to breathing irregularities, 100% neonatal lethality was observed in the mutants 14-24 hours after birth, while on an outbred CD-1 genetic background, 44% of mutants survived beyond neonatal stages and into adulthood. This research further demonstrates the functional conservation of specific neuronal subtypes between discrete CPG circuits in the brainstem and spinal cord and shows how genetic mutations may contribute to respiratory dysregulation resulting in death in neonatal populations. This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC).

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Lipid coordination between peroxisomes, peroxins, and lipid droplets

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The coordination and communication between spatially separated organelles are essential for cellular homeostasis. Peroxisomes are small, ubiquitous organelles responsible for the breakdown of lipids, detoxification of reactive radical species, and signaling of immune cells. The dependency of cells on peroxisome activity is demonstrated by the assortment of genetic disorders that arise from Pex gene mutations, resulting in either impaired peroxisomal enzyme function or defective peroxisomal biogenesis. Zellweger Spectrum Disorders (ZSD) are the most severe form of these disorders, rapidly progressive in children with a high mortality rate. As the central hub of lipid metabolism, peroxisomes are intimately involved with lipid droplets (LDs), which are the main cellular storage sites of excess fatty acids and cholesterol. Recently, we showed that peroxisomal proteins Pex13 and Pex14 localize to lipid droplets and affect lipolytic enzyme activity. By combining the use of nanobody tagging, photoconvertible GFP, and transitID, we aim to investigate the orientation of Pex proteins at the LD surfaces, their localization after lipolysis, and how they affect the LD surface proteome. Additionally, we will also assess the effect of the absence of peroxisomes has on lipid liberation during lipolysis.

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Increased regenerative capacity of neuromast hair cells in zebrafish gmds mutants

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Background: Zebrafish have mechanosensory hair cells in their ears and lateral line neuromasts that are structurally and functionally similar to those found in human ears. Unlike humans, zebrafish can regenerate these hair cells after physical or chemically induced damage. Previous models have indicated that the Notch, FGF, and Wnt signalling pathways interact to regulate this regenerative process in support cells that are maintained in neuromasts. The GDP-mannose 4,6-dehydratase (gmds) gene, which encodes a biosynthetic enzyme required for the generation of GDP-fucose, is expressed in the neuromast support cells and regulates Notch signalling via fucosylation of EGF-like repeats on the Notch receptor extracellular domain. Moreover, gmds is down-regulated in fgf3 mutant neuromasts that have increased regenerative capacity. Accordingly, we hypothesized that gmds mutants may also display increased regeneration of neuromast hair cells. Results: We ablated neuromast hair cells with the aminoglycoside antibiotic neomycin and demonstrate that gmds mutants regenerate hair cells faster than their wild-type siblings. Homozygous gmds mutants have fully functional neuromasts present 48 hours post ablation, while wild-type siblings require 72 hours for the same process to occur. Regenerated neuromast hair cells stain with vital dyes (DASPEI, YO-PRO-1, FM-143) indicating that they are metabolically active and mechanosensory. Regenerated neuromasts in gmds mutants also contain an increased number of hair cells when compared to their wild-type siblings, which is at least partially dependent on Notch signalling. Conclusion: Our results demonstrate that while gmds mutant neuromasts are indistinguishable from wild-type siblings under homeostatic conditions, they are capable of regenerating faster and with excess hair cell number.

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Identification of regulators of optic cup morphogenesis

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The neural retina that mediates vision is covered by the single-cell layer thick retinal pigment epithelium (RPE), which maintains the health and function of the light-sensing photoreceptors. This organization emerges during optic cup morphogenesis, when the RPE and neural progenitors of the eye vesicles move to form bilateral optic cups. Zebrafish provide an excellent model to identify optic cup morphogenesis regulators as fluorescent transgenic lines are available to follow morphogenetic events in live embryos, and F0 CRISPR gene editing can be used to readily test potential candidates. To identify molecular players, we FAC-sorted eGFP+ RPE, neural retina and hypothalamic progenitors of 16-17 somite stage (ss) Tg(rx3:egfp) zebrafish, as optic cup morphogenesis begins. We performed single cell RNA sequencing on EGFP+ cells, downstream analysis with Seurat R, and revealed nine transcriptionally distinct clusters. Differential gene expression analysis indicated cluster-specific enrichment of tfec/bhlhe40, which directed our bioinformatic isolation of RPE progenitors. The discrete expression by these cells of mRNAs for both Nanos1, an RNA binding zinc finger protein, and the guidance molecule Netrin1a, drew our attention; nanos1 mRNA was expressed at 18 and 24 hours post fertilization (hpf) by RPE that bordered neural retinal cells, while netrin1a mRNA was expressed by 18 hpf RPE progenitors and by the RPE overlying the optic nerve head at 24 hpf. We used IDT's Alt-R CRISPR-Cas9 system and injected three RNP guides/gene immediately after fertilization, converting the majority of injected embryos directly into F0 biallelic knockouts; mRNAs were downregulated by nonsense-mediated mRNA decay. Our in-situ hybridization analysis of 24 hpf F0 mutants and controls identified defects in markers of eye patterning and RPE differentiation, arguing for key roles for these two genes in optic cup morphogenesis. Future work will use time lapse imaging to identify defects in the cell movements that underlie optic cup morphogenesis.

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The Regulatory Role of NID-1/Nidogen in Pioneer-Follower Axon Navigation in the Ventral Nerve Cord of *C. elegans*

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Pioneer axons form initial tracts in the developing nervous system, guiding follower axons to their targets. In C. elegans, the Ventral Nerve Cord (VNC) has two tracts: AVG neuron pioneers the right, while PVPR pioneers the left. The basement membrane (BM) protein, NID-1/Nidogen is essential for proper VNC axon navigation [Kim & Wadsworth, 2000]. nid-1 mutants show highly penetrant PVPR defects, including failure to pioneer the left VNC and ventral midline crossing. Mutations in several genes exacerbate AVG defects in nid-1 mutants, suggesting NID-1 interacts with multiple axon guidance pathways [Feresten AH, 2023]. These findings indicate that NID-1 plays a key role in establishing pioneer axon trajectories. NID-1 is a multimodular protein with three globular domains (G1/NIDO, G2, G3) linked by epidermal growth factor (EGF) repeats. Key BM components interact with these domains: Laminin (G3), Collagen IV (G2, G3), and Perlecan (G2). Imaging of NID-1 tagged with mNeonGreen revealed its presence in the BM surrounding the VNC, with enrichment near the right and left tracts. To determine the role of individual domains, we generated deletion mutants: nid-1(hd189), lacking G2, EGF, and G3 domains, and nid-1(hd188), lacking only G3 domain. Phenotypic analysis revealed that nid-1(hd189) caused severe PVPR and PVQL defects, with PVPR pioneering defects (80%) comparable to the null allele (83%) but more penetrant than nid-1(cg118) (58%), which lacks G1 and G2 domains. In contrast, nid-1(hd188) showed lower overall penetrance (64%) and reduced PVPR pioneering defects (19%). These findings suggest that the G2 and EGF domains are critical for PVPR axon navigation. We are now investigating whether AVG axon navigation also depends on these domains. We hypothesize that NID-1 regulates axon guidance either directly or via interactions with other proteins. To further clarify its function, we will examine known NID-1 binding partners for roles in pioneer axon navigation.

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A method to live-image lipid droplets in Drosophila tissues

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Lipid droplets are organelles composed of neutral lipids enclosed by an outer phospholipid monolayer, primarily involved in the storage of lipids. Under oxidative stress conditions, these organelles also protect lipids from oxidation, preventing accumulation of toxic lipid species. Various methods are currently used to detect lipid droplets, including the commonly used neutral lipid stains Nile Red, BODIPY, LipidTOX and AUTODOT; however, while possible to use these in live tissues, delivery to cells requires tissue dissection. Similarly, in-vivo expression of nano-antibodies to, or GFP fusions with lipid droplet surface proteins such as the perilipin Lsd-2 is possible, but altering the level or activity of these proteins affects organelle activity. LiveDrop was developed previously as an alternative way to visualize lipid droplets in live cells. LiveDrop is composed of the fusion between a fluorescent protein and the lipid droplet binding region of Glycerol-3-phosphate acyltransferase 4 (GPAT4), an enzyme which catalyzes the initial step in triglyceride synthesis. Since GPAT4 is delivered to the surface of lipid droplets beginning at their formation between the leaflets of the ER membrane, LiveDrop detects lipid droplets that may lack sufficient lipid accumulation required for detection by neutral lipid stains. Further, as the GPAT4 enzymatic domain is not part of LiveDrop, it does not affect lipid droplet formation or storage activity. Previously, LiveDrop GFP cells were generated in our lab by stable transfection of GPAT4 in Drosophila S2 cells (Ueda et al, 2022). Recently, we generated UAS LiveDrop GFP/RFP transgenic fly lines, which can be used to detect lipid droplets within specific cell types of interest using the GAL4/UAS system. Here, we compare LiveDrop to lipid droplet stains in various Drosophila organs, confirming the specificity of LiveDrop expression in various cells and tissue types, and how it can be used to probe lipid droplets in developing animals.

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Genetic Clonal Analysis of PAX3+ Progenitor Specification in the Somitic Mesoderm

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During embryonic development, cells progressively specialize and adopt different cell fates. The dermomyotome is a transient embryonic structure of the somite containing multipotent progenitors expressing the transcription factor PAX3 (PAX3-MPCs). In the developing embryo, these PAX3-MPCs give rise to all skeletal muscles of the trunk and limbs, dermis of the back, interscapular brown adipocytes, and contribute to a subset of endothelial and vascular smooth muscle cells. While brown fat, skeletal muscle and dorsal dermis are known to derive from a shared progenitor population, the exact clonal relationships among these and other emerging lineages have yet to be fully defined. Advances in microscopy and tissue clearing have allowed for unprecedented visualization of developmental processes. To achieve spatiotemporal visualization of PAX3-MPC-derived tissue development, we identified specific markers for these tissues. This was accomplished through tissue clearing and immunostaining of wild-type mouse embryos from embryonic day 10.5 (E10.5) to E13.5—key timepoints when these cell fate decisions occur. Then, using an innovative genetic approach based on Mosaic Analysis with Double Markers mouse model in combination with these specific markers, we will perform clonal analyses to determine the behavior and cell fate of PAX3-MPCs in vivo at the single-cell level. This strategy will allow us to elucidate the mechanisms governing the division of PAX3-MPCs and their commitment to different lineages in the developing embryo. We aim to elucidate the complex behavior of PAX3-MPCs and their contribution to the different cell lineages of the embryo. A better understanding of progenitor behavior during embryonic development is essential to improve the derivation of specific cell types from induced pluripotent stem cells for cell therapy strategies.

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Capicua regulates the programmed cell death of Cajal-Retzius cell in the postnatal hippocampus

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Programmed cell death is a crucial process during organ system development. As the nervous system develops, many neurons undergo programmed cell death to clear inappropriate synapses and to give room for mature neurons to develop. Cajal-Retzius (CR) cells are one such type of cell to undergo cell death during development. This process must be tightly regulated as inappropriate CR cell death has been linked to neurological disorders such as epilepsy. However, the mechanisms that regulate CR cell death and survival during brain development remain elusive. To explore the regulation of CR cell death during brain development, we generated a mouse model where capicua (Cic), a gene crucial for brain development, is selectively deleted from CR cells. Loss of Cic from CR cells impairs their programmed cell death, leading to their persistence in the adult mouse brain. However, abnormal CR cell persistence due to Cic loss did not affect hippocampal-dependent behaviors such as anxiety and memory, as well as seizure susceptibility, in both juvenile and adult mice.In investigating the regulation of CR cell death during development, we found that CR cell-specific loss of Cic results in a greater proportion of CR cells expressing the anti-apoptotic protein BCL2 in the mouse hippocampus. We performed single-cell RNA sequencing on CR cells from control and Cic knockout mice at postnatal day 14, the midpoint of CR cell death. Our analyses unveiled a previously-unknown role of CIC, wherein it represses Fgf1, a growth factor important in cell survival. We found that the selective overexpression of BCL2 or FGF1 in CR cells promotes their survival in the postnatal mouse hippocampus. Taken together, we uncover the mechanism by which CIC regulates CR cell death during development. Our study will broaden our knowledge of the regulation of programmed cell death during the early stages of brain development.

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Defining the development of larval zebrafish rods and short wavelength cones via transgenic nrl expression and lineage tracing

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The vertebrate retina contains two photoreceptor cell types, rods and cones. Conventional theories hold that these photoreceptor types develop from distinct “rod” or “cone” precursor cells. However, recent findings in our lab suggest that mouse and zebrafish short wavelength cones, which are homologous to human blue cones, share developmental mechanisms with rods specifically. Neural retina leucine zipper (nrl) is a key transcription factor that specifies rod developmental fate, as demonstrated by a complete absence of rods in larval zebrafish nrl mutants. Interestingly, nrl mutants also produce excess short wavelength (sws1) cones and transgenically expressing nrl in developing sws1 cones generates rod-like cells with both sws1 cone and rod markers. My project aims to further investigate the developmental relationship between these photoreceptor types. Firstly, I am performing microinjections of nrl constructs driven by different cone promoters. By immunostaining with antibodies targeting photoreceptor opsins and analyzing the retina with confocal microscopy, I can determine if only sws1 cones express rod-specific markers under the influence of nrl. Preliminary results suggest that nrl expression in sws1 cones, but not other cone subtypes, is sufficient to induce rod-like development, supporting our hypothesis of a developmental relationship between sws1 cones and rods. Next, I will determine if sws1 cones and rods develop from shared precursors via lineage tracing using a UBI:switch transgenic line and cre recombinase microinjection. This method fluorescently labels the precursor and its progeny cells, including photoreceptors. Using immunostaining and microscopy methods again, I will quantify the photoreceptor types present in these clonal cell groups. I expect that rods and sws1 cones will be present in the same groups, indicating that they share developmental precursor cells. Together, this research will provide new understandings of photoreceptor evolution and development, which is crucial to advancing treatments for photoreceptor diseases.

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Twisting Cytokinesis: Cell Adhesion and Cortical Flow Underlie Chiral Morphogenesis in *Caenorhabditis elegans* Embryos

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Chirality is one of the fundamental properties of living systems, wherein the external and internal body structures are asymmetric and cannot be superimposed on their mirror images. In animals, tissue- and organismal-scale chirality arise during morphogenesis, termed chiral morphogenesis. In organisms such as snails, frogs, and *Caenorhabditis elegans*, their chiral morphogenesis involves actin-based mechanisms during cytokinesis of early embryonic cell division. Although actin-based chiral morphogenesis has been reported in previous studies, the mechanisms by which cytokinesis establishes embryonic chirality remain elusive. Here, we found that cell adhesion and twisting cell cortex movement, termed chiral cortical flow, induce chiral morphogenesis in early *C. elegans* embryos. Embryonic chirality in *C. elegans* is specified during 4-cell stage cytokinesis, where division axes of ABa and ABp cells undergo clockwise tilting. To identify contributors to this division axis tilt, we performed an in vitro blastomere isolation assay. We found that attachment of an isolated ABa/p cell to adhesive substrate induced chiral cortical flow and clockwise cellular rotation. As previously reported, depletion of chiral cortical flow regulator CYK-1/Formin decreases cellular rotation, confirming that chiral flow is required for the division axis tilt. However, we also found that cellular rotation requires the attachment of both dividing cell halves to the adhesive surface. These results suggest that 1) adhesion is required for the amplification of intrinsically generated chiral cortical flow, 2) while chiral cortical flow is necessary, it is not sufficient to drive cellular rotation, and 3) a correct adhesion pattern, in addition to chiral flow, is required to induce cellular rotation. Using mathematical simulation, we confirmed that chiral cortical flow and adhesion are sufficient to generate cellular rotation and chiral cellular arrangements. Our study illuminates the novel interplay between cell adhesion and cytokinesis that regulates chiral morphogenesis, which is critical for shaping the embryonic body plan.

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Regulation of PP1 phosphatase in *C. elegans* sperm development and post-fertilization signalling

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Sexually reproducing species require fertilization and proper zygotic development for survival and propagation. The transition of a fertilized zygote to an embryo involves complex signalling pathways and strict cell-cycle regulation. Oocytes can arrest at multiple stages of meiosis to coordinate their maturation with fertilization. In vertebrates, oocytes arrest at metaphase of meiosis II (MII) and only resume after fertilization delivers an unknown signal that triggers MII completion. Using C. elegans, the Srayko lab previously identified memi-1/2/3 (meiosis-to-mitosis), oocyte-specific genes required to sense sperm entry upon fertilization and initiate proper female MII. Loss of memi function results in a “skipped MII” phenotype and embryonic death. In contrast to the loss-of-memi phenotype, a gain-of-function mutation, memi-1(sb41) allows entry into MII but failure to exit MII. Genetic screens for memi-1(sb41) suppressors identified other factors required for female MII regulation in fertilized embryos, including two nearly identical sperm-specific PP1 phosphatases, GSP-3/4, previously implicated in spermatogenesis. The suppressor screen also recovered a regulatory subunit of GSP-3/4PP1. A FLAG-tagged version of the regulator localized to the plasma membrane within the cell body of active sperm, enriched at the base of the sperm pseudopod and colocalizing with GSP-3/4PP1. Furthermore, co-immunoprecipitation revealed its physical interaction with GSP-3/4PP1, suggesting a mechanism for the spatial regulation of PP1 within crawling sperm. In early fertilized embryos, the regulator localized to a compact ring-like zone surrounding the condensed sperm DNA. Database searches revealed two potential paralogs of this regulator. Double and triple mutant combinations of the paralogs led to severe defects in spermiogenesis, low sperm motility, and increased embryonic lethality. Mutant embryos also exhibited a “skipped MII” phenotype, suggesting that sperm PP1 regulation is important for the completion of female meiosis. Molecular and genetic characterization of the PP1 regulatory subunits and a model for their role in post-fertilization signalling will be presented.

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Effects of Energy Drink Stimulants Caffeine and Taurine on Fruit Fly (*Drosophila melanogaster*) Circadian Rhythm & Locomotor Behaviour

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Energy drinks are utilized as a stimulant to increase wakefulness and energy, often containing high concentrations of caffeine and taurine, which negatively effect circadian rhythms. We investigated the effects of different caffeine and taurine concentrations on circadian rhythm and locomotor behaviour in fruit flies (*Drosophila melanogaster*) in 12:12 hr light:dark cycles over 72 hours. White-eyed (w-) flies and circadian rhythm mutants: periodL1, period01, and timeless01 were used to study the effects of 0.05% caffeine and 0.50% taurine on the baseline circadian rhythm and locomotor behaviour. Quantification of locomotor behaviour and circadian rhythm activity of D. melanogaster was performed using a Drosophila Activity Monitor with continuous caffeine or taurine exposure in food media. Compared to baseline w- flies, periodL1 had reduced or absent activity peaks, and irregular sleep: wake rhythms in the caffeine treatment. period01 had lower activity levels compared to periodL1 and timeless01 caffeine treatment, but higher and irregular activity in the taurine treatment. timeless01 had increased activity levels in the caffeine and taurine treatments, but normal circadian rhythm cycles with enhanced activity peaks at the start of the dark cycle (similar to period01). Caffeine supplementation can increase locomotor behaviour and extend the sleep:wake cycles in circadian rhythm mutants. Taurine supplementation increases locomotor behaviour but typically maintains the circadian rhythm cycles of the mutants. These findings show the impacts of energy drink ingredients, caffeine and taurine, on altering baseline circadian rhythm cycles and locomotor activity in fruit flies.

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Cajal Retzius cell-derived PD-L1 modulates memory and neuroimmune interactions.

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Neuroimmune interactions shape brain development in homeostasis and disease. We recently found that Cajal-Retzius (CR) cells, a population of neurons important for embryonic and early postnatal brain development, uniquely express the programmed death-ligand 1 (PD-L1) in the forebrain under physiological conditions. PD-L1 and its receptor PD-1 form an immune inhibitory checkpoint involved in auto-tolerance and immune evasion. Although this inhibitory pathway has been widely studied in conditions such as cancer, new evidence points to a role in neuroinflammation and cognition. Here, using a conditional knockout of PD-L1 in CR neurons, we investigate the role of this protein on neuroimmune interactions in the hippocampus and learning and memory. PD-L1 was deleted from all hippocampal CR neurons. When analyzing the impact of PD-L1 loss on PD-1 expressing cells, we found that the numbers of microglia and astrocytes were unaltered. Interestingly, we found an increased number of perivascular macrophages in the hippocampal fissure of adult knockout mice that was not observed in juvenile mice. Selective PD-L1 deletion in CR neurons also enhanced memory retention in the Morris water maze test, without altering spatial learning. Our study identifies CR neurons as a previously unrecognized source of PD-L1 in the hippocampus, emphasizing the function of this immune checkpoint as a regulatory mechanism for memory formation. Additionally, we show evidence of a potential interaction between CR neurons and the brain immune system via the PD-L1/ PD-1 axis, an aspect of brain immune system development that remains largely unexplored. Future studies will approach the molecular mechanism by which PD-L1 in CR neurons modulates memory and the impact of CR neuron-specific PD-L1 deletion on PVMs function in physiological and pathological conditions.

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Investigating the Paradoxical Role of mGluR5 in Traumatic Brain Injury

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Traumatic Brain Injury (TBI), defined as any injury to the brain that impairs its function, is a major risk factor for the development of dementia. Previous research suggests that post-traumatic seizures are a mechanistic pathway through which TBI can lead to dementia. My project focuses on the role of metabotropic glutamate receptor 5 (mGluR5) in mediating synaptic degeneration and excitotoxicity post-TBI. I hypothesize that TBI induces synaptic injuries in part through dysregulation of mGluR5, leading to seizures that ultimately contribute to cognitive decline. I administered a combination of CHPG (agonist) and MPEP (antagonist) to examine mGluR5's role in post-traumatic seizures in larval zebrafish, our preclinical animal model of TBI. Previous results suggest that CHPG may alleviate some biomarkers of dementia (tauopathy) associated with TBI. Paradoxically, my preliminary results suggest that CHPG induces an increase in seizures. To explain this apparent paradox, I hypothesize that mGluR5's role is different in the acute phase (seizures) and chronic phase (development of dementia biomarkers). I am using qRT-PCR to track changes in markers of the glutamate signaling pathway to determine how it is affected at different timepoints post-TBI. We expect to observe an initial upregulation of glutamate pathway markers such as mGluR5, mGluR1, and PSD-95 at 1 and 3 hours post-TBI, indicating a hyper-excitatory response. This is likely to be followed by a significant downregulation at 24, 48, and 96 hours, suggesting a subsequent phase of glutamate system depression as the brain attempts to mitigate excitotoxic damage. If we see that the glutamate pathway is initially elevated and then depressed, we can infer that CHPG's neuroprotective effect may be due to the restoration of the loss of glutamate signalling. This research will help determine optimal timepoints for treatments after TBI to best mitigate its devastating short-term (seizures) and long-term (cognitive decline) impacts.

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Characterizing the Role of Unc-45a in Zebrafish Gut Development

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Myosin proteins are vital for the development and maintenance of the GI tract. To reach a functional state, myosin motor heads must be chaperoned as they are unable to fold independently. One such chaperone is UNC45A, homologs of which have been identified in all metazoans, demonstrating its essential nature in acting as a molecular chaperone. Recently, rare human diseases, O2HE Syndrome and Aagenaes Syndrome, have been linked to UNC45A mutations. While O2HE patients present similar symptoms, like cholestasis and congenital diarrhea, there are no overlapping mutations across all studies. In contrast, all Aagenaes patients carry a 5'UTR causative mutation, with some also carrying unique coding mutations. Despite the shared cholestasis symptom between these two rare diseases, there is a notable absence of other symptoms in Aagenaes patients. Altogether this suggests a complicated pathogenicity underlying UNC45A mutations. The unc45a mutant zebrafish line is a good model of this disease as the smooth GI phenotype is apparent. However, a thorough analysis of gut morphology has not been conducted. To address this limited understanding, I will generate novel mutations using CRISPR/Cas9 followed by in-depth histological characterization of the GI tract. Bioinformatic analysis indicates that mutations in human UNC45A are not limited to the myosin head binding site, co-chaperone binding sites and other conserved motifs, suggesting that domain disruption is not necessary for disease phenotype. Moreover, in-silico modeling of the amino acid changes in patients demonstrates a range of effects on protein stability, suggesting that the pathogenicity of these variants is complex. Together, my work can provide an animal model and information to aid in researching the pathogenicity of UNC45a mutations and effect on protein function. This, in turn, can provide insight into the mechanism of pathogenesis of UNC45A mutations in O2HE syndrome, thereby allowing for effective diagnosis, counselling, and treatment of O2HE patients.

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Foxc1 induces Hh pathway activity via transcriptional regulation of Arhgap36, a novel neuroblastoma prognostic factor.

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An intriguing feature of biology is the way in which attributes essential to morphogenesis are efficiently coopted by cancer cells to drive malignancy and metastasis. This is evident in the neural crest, where lineage specific transcription factors that regulate migration and differentiation represent frequent drivers of malignancy. The paradigm is illustrated by the forkhead transcription factor FOXC1, that when overexpressed, is a recurrent feature of cancer with levels of FOXC1 a prognostic factor for metastasis. Using RNA-, ChIP-sequencing and CRISPR interference, we show that Foxc1 binds a locus in a region of closed chromatin to induce expression of Arhgap36, a tissue-specific inhibitor of Protein Kinase A. Because PKA is a core Hedgehog (Hh) pathway inhibitor, Foxc1's induction of Arhgap36 expression increases Hh activity. This increased output of the pathway is associated with impaired function of Sufu, a PKA substrate and second core Hh pathway inhibitor: the pathway also exhibits reduced dependence on Smoothened inhibition. The Foxc1-Arhgap36 relationship identified in murine cells was next evaluated in neuroblastoma, a neural crest derived pediatric malignancy, where we demonstrate at a population level (n=1348 patients) that ARHGAP36 predicts five-year survival. Furthermore, in individual neuroblastoma cell lines that express high levels of ARHGAP36, shRNA inhibition of ARHGAP36 induced apoptosis and rapid cell death. Accordingly, this study has identified as the first transcription factor to enhance ARHGAP36 expression, one that induces Hh activity in multiple tissues during development. It also establishes a model by which increased levels of FOXC1 via ARHGAP36 and PKA inhibition, dysregulate multiple facets of Hh signaling, and provides evidence demonstrating relevance to a common neural-crest derived malignancy.

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Investigating the role of CUP-2 (Derlin) protein in Niche positioning in C. elegans

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Stem cells are undifferentiated cells that can either self-renew or develop into different specialized cell types. The delicate balance between stem cell self-renewal and differentiation is crucial for an organism's growth and development. This balance is maintained by interactions with the stem cell niche, which signals to stem cells to self-renew. Germline stem cells (GSCs) are a specialized type of adult stem cells that eventually differentiate into the gametes. In the nematode C. elegans, the distal tip cell (DTC) acts as a niche for GSCs, maintaining and interacting with them through Notch Signaling. We have identified a protein, CUP-2 (Derlin), which plays a role in regulating DTC positioning. In cup-2 mutants, the DTC becomes displaced proximally, migrating away from its normal position in a significant number of animals; however, the mechanism underlying this phenotype remains to be elucidated. Interestingly, in some animals where the DTC has migrated quite far, the stem cell pool relocates to this new position, and differentiation occurs in both directions from the DTC. This demonstrates that the DTC retains functionality despite its new location. Additionally, we found that disrupting the movement of germ cells, or germline flux, suppresses the DTC displacement phenotype. Therefore, germ cell movement contributes to the displacement of the DTC in cup-2. We propose that the DTC is attached to the moving germ cells and that they 'drag' the DTC more proximally as they move. Therefore, in cup-2 mutants there may be more adhesion between the DTC processes and the germ cells. Alternatively, there may be less adhesion between the DTC body and the basement membrane at the distal end, which would normally counteract the dragging force of the moving germ cells. More broadly, our study aims to understand how niche cell positioning is normally maintained, contributing to the patterning of tissues.

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The role of Held-out wings protein in regulating expression of tumor suppressor gene Snr1

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Mutations in the human gene SMARCB1, a core component of the SWI/SNF chromatin remodelling complex, has been linked to the development of atypical teratoid rhabdoid tumor in children. SMARCB1 has been shown to be important for cellular identity and differentiation; however, little is known about how it is regulated. Through RNA immunoprecipitation assay, our lab has identified the novel interaction between Merlin, a nerve sheath tumor linked protein, and Snr1 transcript, the SMARCB1 homolog in *Drosophila melanogaster*. Loss of Merlin led to a significant reduction in Snr1 protein level and mis-localization of Snr1-RNA, but no change in Snr1-RNA expression was observed, indicating that Merlin regulation occurs at the post-transcriptional level. Previous work in the lab showed that Merlin genetically and physically interacts with the eIF4E3, an RNA cap binding protein, suggesting that these two proteins form a regulatory complex to control SMARCB1/Snr1 expression. However, Merlin has no known RNA binding domains, suggesting a role of an unidentified RNA binding protein which functions as part of the regulatory complex to specifically interact with Snr1/SMARCB1-RNA. Multiple proteomic screens have been done to identify a list of candidate proteins that interact with Snr1-RNA, including Held-out wings (How). Interestingly, binding sites of How are identified in Snr1 transcript, including sites in the untranslated region (UTR). Knockdown of How in BG2-c2, a neuronal derived cell line, leads to significant decrease in Snr1 protein levels independent of UTRs presence in Snr1 mRNA, similar to when Merlin expression is reduced. Unexpectedly, knockdown of How in BG-c2 cell shows to also negatively affect Merlin protein levels, indicating that How may act upstream of Merlin to indirectly regulate Snr1 protein levels. Further experiments to confirm the interaction between How and Snr1-RNA will be done to elucidate the potential direct and indirect regulatory roles of How on Snr1 expression.

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Investigating the Roles of Cdk8 in JNK Signalling Using *Drosophila melanogaster*

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C-Jun N-terminal kinase (JNK) signalling pathway is one of the MAPK signalling pathways conserved in all eukaryotes. While commonly associated with death and apoptosis, the JNK signalling regulates many other key processes like embryonic development and tissue homeostasis. During Drosophila development, JNK signalling is essential for both dorsal and thorax closure. Other contributions of JNK signalling include, but are not limited to, wound healing, metabolism regulation and immune response. Dysregulation of JNK has been implicated in neurological disease and cancers. Cyclin dependent kinases (CDKs) are a family of serine threonine protein kinases that have a variety of functions. The Drosophila cdk8 is an essential gene for development, with null mutants failing to reach adulthood. This protein has been well characterized for regulating transcription by being a part of the mediator transcription complex. Tissue specific reduction of Cdk8 presents ectopic vein formation in the wing, thicker myofibrils in the muscle and motor defects. New research indicates an additional function in regulating mitochondrial dynamics by phosphorylating Drp1. Here we present preliminary data supporting that Cdk8 regulates JNK signalling. Flies either over or under expressing cdk8 result in two distinct cleft thorax phenotypes, similar to altering proteins related to JNK signalling. We used qPCR to confirm that JNK readout puc-lacZ and Mmp1 levels are altered compared to the control when transgenically modifying the levels of cdk8. Uncovering the potential interaction between Cdk8 and JNK signalling during development will provide additional insights as to how signalling pathways regulate growth, differentiation and wound healing.

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UNC-43/CaMKII regulates presynaptic assembly in *C. elegans*

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Neurons communicate via a specialized interface known as the synapse comprised of pre- and postsynaptic specializations. At the presynaptic specialization, a group of conserved proteins form an electron dense region called active zone (AZ), where AZ proteins regulate the recruitment and release of neurotransmitter-containing synaptic vesicles (SVs). While AZ proteins are well characterized, less is known about how they assemble into a functional synapse. Here we found that unc-43, a sole ortholog of calcium/calmodulin-dependent protein kinase II (CaMKII), is crucial for proper presynaptic assembly in *Caenorhabditis elegans*. Using a split-fluorophore method, we examined the localization of endogenous AZ proteins specifically in the DA9 neuron. We found that CLA-1/Piccolo-Bassoon, SYD-2/Liprin-α, and RIMB-1/RIM-BP localization are disrupted in unc-43(n498n1186) loss-of-function mutants. However, UNC-10/RIM localization appears to be unaffected while the signal intensity is reduced, suggesting that unc-43 is required for proper presynaptic assembly by regulating the localization of many AZ proteins at presynapses. Consistently, the clustering of AZ proteins is increased in unc-43(n498) gain-of-function mutants. Neuron-specific, but not muscle-specific knockdown of UNC-43/CaMKII using the auxin-inducible degron (AID) system suggests that UNC-43/CaMKII functions in the presynaptic neuron. Postembryonic degradation of UNC-43/CaMKII using the AID system resulted in disruption of CLA-1 localization, while postembryonic activation of CaMKII using a photo-activatable CaMKII rescued presynaptic localization of CLA-1, suggesting that UNC-43/CaMKII functions to maintain presynaptic structure. To test the functional conservation between human CaMKII and unc-43, we replaced the endogenous unc-43 locus with human CaMKIIA (hCaMKIIA). hCaMKIIA animals exhibit normal locomotion and presynaptic structure, suggesting the conservation between unc-43 and human CaMKIIA. We introduced a recessive, and a dominant mutation identified in the CaMKII gene in patients with intellectual disabilities. These mutations phenocopied presynaptic defects of unc-43(n498n1186) and unc-43(n498) mutants. Our work reveals a conserved role of CaMKII in regulating the proper assembly of presynaptic components.

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Understanding the development and functions of the I5 neuron in C. elegans

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During neurodevelopment, neurons rely on intrinsic and extrinsic guidance cues for the proper formation of axonal and dendritic arborizations, as well as for the precise targeting and formation of synapses. A combination of guidance cues allows for neurons to form their stereotyped structures and to form precise synaptic connections along their neurites. The I5 neuron in *Caenorhabditis elegans* exemplifies a neuron with a complex geometrical structure. I5 is located in the pharynx of the worm, with its cell body on the ventral side of the terminal pharyngeal bulb. I5 forms two symmetric axon branches from its soma, each of which bifurcates multiple times at the stereotyped positions, then fuse to form closed loop structures that wrap around both the terminal bulb and the metacorpus of the pharynx. The mechanisms by which I5 forms its stereotyped structure and its function are unknown. To uncover the cues that instruct I5 neurite and synapse patterning, we generated a transgenic *C. elegans* strain in which I5 neurites and synapses are labeled with fluorescent proteins. Using this marker strain, we found that the position of synapses are asymmetric between the left and right sides of I5 neurites. From a candidate screening, we identified CWN-2/Wnt, an axon guidance molecule highly expressed in the pharyngeal region, and CFZ-2/Frizzled (Fz) receptor are crucial for proper I5 structure. In both mutants, we observed a variety of structural defects, including the loss of loop structures and abnormal synapse patterning. To understand how CWN-2/Wnt acts through CFZ-2/Fz, we are currently examining the localization of CFZ-2/Fz in I5 in relation to the locations of synapses. To determine if CWN-2/Wnt is a permissive or instructive guidance cue, we will rescue the I5 structure in CWN-2/Wnt mutants by expressing CWN-2/Wnt in different tissues around the pharynx.

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Investigating the Conservation of a tp63-Driven Gene Regulatory Network in Teleost Epithelium Development

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Tooth development tissues and processes are conserved across vertebrates, making teeth a valuable model for studying animal development, adaptation, and diversification. While mice are used widely to study mammalian teeth, mice lack the dental diversity seen in non-mammalian vertebrates, such as teleost fishes with oral and pharyngeal dentitions. We investigate the conservation of a gene regulatory network (GRN) driven by the gene tp63. This gene is crucial to dental epithelium and its derivatives in mouse. Here we probed for the tp63 GRN in two teleost species: zebrafish (*Danio rerio*), which lacks oral teeth, and medaka (*Oryzias latipes*), which has oral and pharyngeal teeth. We hypothesized that the tp63 GRN is active in zebrafish and medaka dental epithelia and derivatives during early tooth morphogenesis. We performed RNA sequencing on dissected mandibular and posterior pharyngeal tissues from zebrafish at 4.5 days post-fertilization and medaka at stage 40 (early tooth development stages). We focused on transcript expression of tp63 and its known downstream target genes. Next, via immunohistochemistry we tested for the protein expression of select tp63 GRN genes in zebrafish and medaka oral and pharyngeal dental epithelium, among other structures. Our RNA-Seq data identified many tp63 GRN transcripts, including cbln1, cldn23, fermt1, jag1, notch1, osr2, and tp63, in zebrafish and medaka oral and pharyngeal tissues. Immunohistochemical analyses found P63, FERMT1, and TRIM29 proteins in multiple epithelial tissues, including of tooth organs. Together these findings support our hypothesis that this GRN is active in oral and pharyngeal dental epithelia of both teleost fishes. The power of teleost models featuring distinct dentitions indicates deep conservation of the tp63-GRN across vertebrate dentitions, emphasizing its fundamental role in epithelium, tooth development, and morphogenesis more broadly.

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Investigating the functions of F-actin regulators in neurodevelopment in *C. elegans*

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The formation of functional neural circuits depends on several neurodevelopmental processes, including neurite outgrowth, axon branching, and synapse formation. These processes require branched filamentous actin (F-actin) formation by the Arp2/3 complex and its regulators, such as the Arp2/3-activating WAVE complex and Arp2/3-inhibiting Coronins. Despite this, the precise roles of branched F-actin regulators in neurodevelopment remain elusive as their loss of function mutants are embryonic lethal. To directly test the functions of branched F-actin regulators in neurodevelopment, we use the auxin-inducible degron (AID) system to knock down branched F-actin regulators, specifically in the nervous system (Kurashina & Mizumoto, 2023; Zhang et al., 2015). Using the AID system, we found that ARX-2/Arp2, a subunit of the Arp2/3 complex, WVE-1/WAVE, and POD-1/CORO7 are necessary for synapse formation but not for maintenance in the DA9 motor neuron. Specifically, continuous neuronal knockdown of ARX-2/Arp2 and WVE-1/WAVE throughout larval development resulted in decreased, whereas continuous POD-1/CORO7 knockdown resulted in increased synapse numbers in DA9 at the L4 stage compared to wild type. On the other hand, synapse numbers were unaffected in day 1 adult animals when we induced knockdowns at the L4 stage. These results confirm previous work showing that branched F-actin is required only during synaptogenesis but not for the maintenance of existing synapses (Chia et al., 2014). Furthermore, we found that ARX-2/Arp2 is necessary for axon branch formation in the PLM mechanosensory neurons. Specifically, continuous neuronal knockdown of ARX-2/Arp2 throughout larval development resulted in the loss of axon branches of the PLM neurons. Currently, we are investigating the functions of WVE-1/WAVE and POD-1/CORO7 in PLM axon branch formation and maintenance and the genetic and molecular mechanisms that mediate the localization of branched F-actin regulators in neurodevelopmental processes.

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Role of RNA-Induced Silencing Complex (RISC) Component VIG-1 in the maintenance and differentiation of stem cells in *Caenorhabditis elegans*

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Stem cells, with their ability to differentiate into various cell types, are integral to the body's tissue development, replenishment, and repair. A balance between self-renewal and differentiation is essential for proper functioning, as disruptions can lead to tumor formation or stem cell depletion. Our research focuses on the *C. elegans* germline stem cell population to investigate how this balance is maintained. We have found that vig-1 functions to inhibit self-renewal of the stem cell population. VIG-1 interacts with microRNAs in both *C. elegans* and humans. We have found that vig-1 likely inhibits stem cell self-renewal by inhibiting the Notch signaling pathway. In addition to vig-1(0) enhancing gain-of-function mutations of glp-1, which encodes the GLP-1/Notch receptor, loss of vig-1 also enhances a weak gain-of-function allele of lin-12, and suppresses a weak loss-of-function allele of lin-12, which also encodes a Notch receptor. Therefore, VIG-1 is likely to be a general negative regulator of Notch signaling. We are currently determining whether this VIG-1 function involves microRNA function, or if it could involve a novel function of VIG-1.

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Functional analysis of APL-1/amyloid precursor protein (APP) signaling in the nervous system

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Amyloid Precursor Protein (APP) has been the subject of extensive research as the “Alzheimer’s Disease Gene”, yet its normal biological role remains poorly understood. APP intracellular domain (AICD), a γ-secretase cleavage product of APP, translocates into the nucleus to control gene expression, analogous to the mechanism of Notch signaling. However, the activity of APP signaling and its function in the nervous system are unclear. We investigate the role of APP signaling in the nervous system using *Caenorhabditis elegans*, which has a sole APP ortholog, APL-1. While null mutants of apl-1 are lethal, mutants lacking the transmembrane and intracellular domains are viable (PMID: 17267616). Consistent with the previous work, we found APL-1 mutants lacking the transmembrane domain and AICD, or just the AICD, are hypersensitive to the acetylcholinesterase inhibitor aldicarb, suggesting a role for AICD in synapse function (PMID: 20862215; DOI: 10.1101/305284). We confirmed that transgenically expressed AICD::mStayGold can localize in the nuclei of neuronal cells. To visualize APL-1 signaling activity in vivo, we adapted a genetically encoded biosensor called SALSA developed for detecting Notch signaling (PMID: 35413239) and observed high APL-1 signaling activity in the nervous system. We found that the APL-1 signaling activity is reduced but not abolished in the sel-12/Presenilin mutant, suggesting there may be additional γ-secretases such as HOP-1 involved in the cleavage of APL-1. Currently, we are investigating the cell types in which APL-1 functions to control synapse function by conducting tissue-specific rescue experiments with APL-1 cDNA as well as conditional knockout experiments using a floxed apl-1 allele we generated with CRISPR/Cas9 genome editing.

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Elucidating the role of niche cell positioning on differentiation and proliferation decisions in stem cells

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Stem cells (SCs) are required for the survival of all multicellular organisms by playing roles in development and tissue repair. Dividing SCs must decide to either make specialized cells (differentiation) or to create more SCs (self renewal). My project aims to further our understanding of how SCs balance between self renewal and differentiation. Similar to known stem cell populations, the *C. elegans* gonad contains a SC environment that is created through signaling from a niche cell. In the *C. elegans* gonad this niche cell is known as the distal tip cell (DTC). Signaling from the DTC triggers SCs to self renew and as new SCs are created they push the older SCs away from the niche causing them to differentiate. Although the structure and morphology of niche cells and stem cells may change depending on organism and organ, every stem cell population requires niche cell signaling to retain their ability to proliferate. We have identified that a coelomocyte uptake defective 2 (cup-2) knockdown in *C. elegans* causes the DTC to be displaced from its normal position to a position more proximally along the gonad. cup-2 is a Derlin family protein that normally functions to identify and degrade misfolded proteins through the endoplasmic reticulum associated degradation (ERAD) pathway. Tissue specific RNAi shows that cup-2 function is needed in the DTC for it to contribute to proper positioning. cup-2 mutants versus wildtype *C. elegans* from 1-3 days past adolescence have been imaged and analyzed to identify any morphological differences in the gonads between displaced and non displaced DTCs. We conclude that there is not a significant difference when it comes to overall shape, projection number, length, branching, or orientation. A forward genetic enhancer screen with a cup-2 background is know being performed to identify additional factors contributing to niche cell positioning.

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Characterizing expression patterns of lysine deacetylases across neurodevelopment

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Myelination, the process by which oligodendrocytes form an insulating membrane around axons, is crucial for efficient nerve conduction. In the developing brain, neural progenitor cells differentiate through specific stages to become mature, myelinating oligodendrocytes. This process is tightly regulated by complex transcriptional and epigenetic mechanisms, including histone post-translational modifications. The lysine deacetylases are comprised of the Zn2+-dependent histone deacetylase (HDAC1-11) and NAD+-dependent sirtuin (SIRT1-7) families. Initially identified for their role in modifying histones, these enzymes regulate various cellular processes by targeting both histone and non-histone proteins. To explore their role in postnatal brain development, we first used qPCR to analyze mRNA expression in cortical tissue from C57BL/6 mice at postnatal ages P0-P84. Four distinct expression patterns were identified: i) gradual decline followed by a plateau, ii) early decline followed by a return to baseline, iii) early increase followed by a plateau, and iv) gradual increase followed by a return to baseline. In the sirtuin family, Sirt1, Sirt4, Sirt5, Sirt6, and Sirt7 were downregulated whereas Sirt2 and Sirt3 were upregulated with Sirt2 peaking during active myelination. In the CG4 oligodendrocyte cell line, Sirt1 was downregulated whereas Sirt2, Sirt3, and Sirt7 were upregulated. Western blot analysis of SIRT2 protein expression continued to increase from birth into adulthood in the developing cortex and increased during oligodendrocyte differentiation in cell culture. Based on these expression patterns, we examined myelination in the corpus callosum of SIRT2-KO and C57BL/6 mice using electron microscopy. At P15, SIRT2-KO mice exhibited a decrease in the percentage of myelinated axons. Further analysis also revealed enlarged mitochondria in both myelinated and unmyelinated axons suggesting a role for SIRT2 in cellular energetics. These findings underscore the critical role of sirtuins in brain development, especially for SIRT2 in oligodendrocyte differentiation and myelination, with potential implications for myelin-related disorders.

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Tumor growth in Drosophila larval epithelial tissue induces distant organ wasting through fat body metabolic dysregulation

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We have previously established a Drosophila tumor model by co-expressing constitutively active Salt inducible kinases (Siks) with Homeodomain interacting protein kinases (Hipk) in larval epithelial discs. Both kinases modulate key signaling pathways involved in developmental decisions and disrupted during tumorigenesis. Larvae harboring these epithelial tumors exhibit significantly delayed metamorphosis. This developmental delay is accompanied by increased tumor burden, larval volume expansion, body transparency (indicative of fat body loss), reduced locomotion and mitochondrial depletion in the larval body wall muscles. Additionally, the presence of the muscle component myosin in the larval hemolymph suggests muscle wasting—all phenotypes reminiscent of cachexia-like syndrome Cancer-associated cachexia is a systemic paraneoplastic syndrome characterized by substantial weight loss due to skeletal muscle and adipose tissue wasting. In the recent decade, Drosophila tumor model studies have elucidated numerous cachexic factors and underlying pathways associated with systemic organ wasting. In cachexic larvae, we observed morphological changes such as rounding and dissociation of fat cells, along with reduced triacylglycerol levels, indicative of increased lipolysis. One major lipolysis pathway in Drosophila is Adipokinetic hormone (AKH)-dependent, and since AKH is exclusively secreted by the Prothoracic gland (PG), we examined its role. We found elevated transcriptional and translational levels of AKH in the PG, likely induced by Jak/Stat activation according to published literature. We also found upregulation of Unpaired (Upd) cytokines from tumor tissue, which act as ligands for the Jak/Stat pathway in distant organs. Based on these findings, we hypothesize that tumor-derived Upd cytokines stimulate Jak/Stat signaling in the PG, triggering AKH release and subsequent fat wasting. We will present our work on this novel cachexia model which aims to uncover mechanistic insight to the signaling pathways, metabolic changes and potential secreted factors driving these phenotypes, with the goal of gaining insight into mechanisms of cancer cachexia in humans.

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Genetic suppressors of memi-1(sb41) reveal putative proteolytic pathways for MEMI regulation

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Fertilization triggers the correct cell-division program in newly fertilized oocytes. In *Caenorhabditis elegans*, maternal MEMI (MEiosis-to-MItosis) proteins are required for the completion of female meiosis I (MI) and entry into meiosis II (MII) in response to fertilization. Loss of MEMI results in a skipped MII program. A gain-of-function mutation, memi-1(sb41), causes persistence of MEMI-1 protein, MII delays, and embryonic death, suggesting that MEMI must also be degraded to allow the MII-to-mitosis transition. To identify factors required for MEMI activity, we screened for genetic suppressors of memi-1(sb41). We identified mbk-2 and cul-2, which encode maternal factors that could regulate MEMI degradation. MBK-2 (Mini-Brain Kinase) promotes the MI-MII-mitosis transition by coordinating activities and degradation of oocyte-specific proteins. CUL-2 (CULlin-E3 ligase) also targets proteins (e.g., Cyclin B) for destruction in MII. We previously showed that CUL-2/ZYG-11 complex activity is required for MEMI degradation in MII; hence, we were intrigued to find a cul-2 mutation in our suppressor screen. Genetic analysis revealed that the suppressing mutation in mbk-2 is likely a weak loss-of-function (lf) allele, as homozygotes are relatively healthy (92% viability) compared to strong lf mutants (0.3% viability). mbk-2(lf) mutants exhibited reduced MEMI levels in MII compared to controls, providing a plausible explanation for the suppression of memi-1(sb41). Therefore, MEMI stability, not its destruction, seems dependent on MBK-2. In contrast, CUL-2/ZYG-11 is required for MEMI destruction; thus, the suppressing cul-2 mutation is likely not a simple lf allele but most likely causes either increased or precocious CUL-2 activity. We are also pursuing the idea that MBK-2 and CUL-2 work together to regulate MEMI levels, either as part of a single pathway or as distinct, parallel pathways. Experiments are currently underway to determine the molecular mechanism of memi-1(sb41) suppression and the relationship, if any, between mbk-2 and cul-2 in MEMI regulation.

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Genetic interactions between Foxc1/2 and Sox9 genes grow and shape the mouse embryonic skeleton

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The Forkhead box transcription factors Foxc1 and Foxc2 function in chondrocyte differentiation during embryonic skeletal development. FOXC binding sites are enriched in DNA-regulatory elements bound by the chondrocyte master regulator SOX9, suggesting FOXC and SOX9 proteins may function in common pathways during chondrocyte differentiation. To test whether genetic interactions between Foxc1, Foxc2, and Sox9 occur in the developing skeleton, we generated mice containing floxed alleles for all three genes and crossed with mice containing prx1-cre that is active in the limb skeleton and sternum. Progressive reduction in Foxc1, Foxc2 and Sox9 gene dosages led to worsening phenotypes affecting the limb skeleton and sternum. Loss of one Sox9 copy accentuated the bone malformations observed in compound Foxc1 and Foxc2 homozygous mutant embryos (Foxc1Δ/Δ;Foxc2Δ/Δ). Mutant embryos displayed severe bowing of the radius and tibia, absence of a mineralized fibula and ulna along with a shortening of the femur and humerus. Malformations to the scapula were also observed that worsened as Foxc1;Foxc2;Sox9 gene dosages were reduced. Compound heterozygous Foxc1+/Δ;Foxc2+/Δ;Sox9+/Δ embryos displayed reductions in bone length, delays in sternum chondrocyte differentiation and malformations in the scapula and growth perturbations in structure facilitating muscle attachment and joint articulation. Such phenotypes were observed in Foxc1+/Δ;Foxc2+/Δ or Sox9+/Δ embryos suggesting that Foxc1,Foxc2, and Sox9 activity was reduced below a threshold needed for correct skeletogenic events to occur. Lastly, we performed gait analysis with compound heterozygous Foxc1+/Δ;Foxc2+/Δ;Sox9+/Δ mice to assess the function impact of bone malformations. We observed altered gait and a reduced range of motion in many joints, indicating a functional effect of bone malformations in Foxc1;Foxc2;Sox9 compound heterozygous mutants. Together these findings indicate that Foxc1,Foxc2 and Sox9 genetically interact in skeletogenesis.

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Sox9 and Runx2: Once evolutionary buddies, now parted paths

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Changes in transcription factors and/or their binding sites cause evolutionary changes in gene expression. Osteoblasts of earlier-diverged vertebrates express more genes typical of chondrocytes than osteoblasts of later-diverged vertebrates, suggesting that the chondrocyte transcription factor Sox9 used to work more closely together with the osteoblast transcription factor Runx2 during early skeletal cell evolution. We hypothesized that changes in Sox9 and Runx2 caused changes in osteoblast gene expression during vertebrate evolution. To test the hypothesis, mouse pre-osteoblastic cells (MC3T3-E1.4) were transduced with recombinant FLAG-tagged Runx2 or Sox9 from either gar or mouse. Resultant gene expression and genomic binding sites were analyzed using RT-qPCR and ChIP-qPCR, respectively, on genes downstream of Sox9 and Runx2. Transduction was confirmed by anti-FLAG western blotting and immunofluorescence. Overexpression of either mouse or gar Sox9 in osteoblasts induced the chondrocyte gene Col2a1. On the other hand, mouse Runx2 induced, but gar Runx2 did not, the osteoblast gene Sp7. Interestingly, mouse Sox9 reduced, whereas gar Sox9 increased, expression of mouse Runx2. ChIP-qPCR revealed that gar Sox9 was enriched on a mouse Col2a1 binding site, and mouse Runx2 was enriched on Col2a1 and Col1a2. These data begin to support the hypothesis that changes to Sox9 and Runx2 caused evolutionary changes in osteoblast gene expression. A simple explanation for cartilage gene expression in gar osteoblasts is Sox9 expression. However, induction of mouse Runx2 by gar Sox9 suggests that in earlier-diverged vertebrates, Sox9 and Runx2 used to work more synergistically, with Sox9 regulating both cartilage and bone genes. These data also support the evolutionary theory that osteoblasts evolved from chondrocytes. Subsequent compartmentalization of Sox9 and Runx2 transcriptional networks, seen in mouse, may have occurred in response to environmental factors associated with the transition from water to land.

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Unveiling the Photoreceptor Outer Segment Cage Formed by Calyceal Processes, Müller Glia, and the Retinal Pigment Epithelium

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Photoreceptors are the primary light-sensing cells of the retina, with morphologies precisely tailored to their function. In particular, a large sensory ending known as the outer segment (OS) is adapted to efficiently capture photons. By imaging live and fixed larvae, we observed the apical portion of photoreceptor precursors undergoing impressive remodelling: initial filopodia-like tangential processes are followed by a formation of an actin dome and emergence of nascent microvilli. Those microvilli, known as calyceal processes, surround the growing and mature OS. While prioritizing the phototransduction process, photoreceptors outsource many maintenance tasks to supporting cells - Müller glia (MG) and retinal pigment epithelium (RPE). MG and RPE are considered to be physically separate, with each cell type approaching photoreceptors from an opposite direction. Nonetheless, we recently discovered through confocal microscopy an overlap between MG/RPE apical processes alongside UV cone photoreceptors in the zebrafish retina. We next used Focused Ion Beam Scanning Electron Microscopy (FIB-SEM) to obtain a more detailed view of interactions in the juvenile outer retina. The imaging revealed a double cage-like arrangement surrounding the OSs of UV cones: an inner ring of calyceal processes - and an outer ring of aligned MG and RPE apical processes. Further, MG processes feature a defined structure as they extend towards the tip of the OS: initially narrow, the distal region expands to house distinct and organized bundles of actin. RPE processes are less ordered but contain abundant endoplasmic reticulum. Using confocal microscopy, we found that MG/RPE contact is established by 7 days post fertilization and subsequent to OS development. In conclusion, our data reveal extensive and unexpected interactions in the outer retina, reshaping our understanding of how photoreceptor development and homeostasis is supported. Current experiments are investigating conservation across species and how disrupting contacts alters retinal health and function.

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CTCF is essential for limb muscle development by preventing ectopic gene expression in myogenic progenitors

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During vertebrate development, multipotent progenitor cells (MPC) within the somitic mesoderm differentiate into muscle of the trunk and limbs, dorsal dermis, brown fat, as well as endothelial and smooth muscle cells of the vasculature. The transcription factor paired box 3 (PAX3) is expressed in MPCs of the somite and can instruct myogenic cell fate specification by directing the local chromosome architecture. The 3D genome is organized by CCCTC-binding factor (CTCF), a major architectural protein that binds to topologically associating domain (TAD) boundaries and chromatin loop anchors. However, it remains unknown whether CTCF is required for the coordination of transcriptional programs that drive cell fate specification of MPCs. Here, we show that CTCF is required for migratory myogenic progenitors of the limb musculature but is largely dispensable for non-migratory myogenic progenitors of the trunk and not required for endothelial cells. In the mouse, genetic ablation of Ctcf in Pax3-expressing cells led to a pronounced reduction of migratory myogenic progenitor specification at embryonic day (E) 10.5 with consequent loss of all forelimb musculature by E13.5. Pax3-derived endothelial cells - whose presence in the limb bud is essential for myogenic progenitor migration - were maintained. Lineage analysis using mosaic analysis with double markers (MADM) strongly revealed a cell-autonomous requirement for CTCF in migratory myogenic progenitors. CTCF-depleted MPCs and specified myogenic populations displayed marked upregulation of ectopic non-lineage genes. Additionally, several Hox genes, which are implicated in positional identity, were upregulated in the absence of CTCF. These transcriptional changes indicate a repressive function of CTCF and reveal migratory myogenic progenitors as particularly sensitive to disruption.Our data suggests a contextual requirement for CTCF, where some PAX3-dependent lineages are specified, while others are not. We propose a model in which CTCF insulates migratory myogenic progenitor cells from ectopic gene expression, thereby preserving their migratory identity.

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Exploring Tendon Regeneration using a Tenocyte Genetic Ablation Model in Zebrafish

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Tendons are highly specialized fibrous tissue with tremendous tensile strength that connect muscles to bones. In the hypocellular tendon tissue, specialized fibroblast cells called tenocytes are the key extracellular matrix (ECM) producing cells that maintain tendon integrity. Various tendon conditions, including accidental tears and tendinopathy, are common due to repeated exposure to high loads and forces during physical activities. Unfortunately, tendon regeneration in mammals is incomplete and degenerative following an injury due to prolonged inflammation and the formation of fibrous scar tissue, resulting in functionally impaired tendons that impair movement. Zebrafish have a remarkable ability to regenerate a wide variety of tissues and cell types, including tenocytes. In this study, I established a tenocyte genetic ablation model to study the mechanisms underlying tendon regeneration in zebrafish. Using a nitroreductase-based system, I showed that tenocytes at the myotendinous junction can be efficiently ablated. This injury model results in the ablation of ~40% of tenocytes throughout the fish without directly affecting the surrounding ECM and tissues. Long-term time-course imaging using a tenocyte-specific reporter scxa:mCherry revealed that tenocytes have a slow regeneration dynamics post-ablation, requiring about 2 weeks to fully regenerate the tenocyte numbers to control level. Interestingly, in a mosaic transgenic line with only ~15% tenocyte ablation, regeneration occurred more rapidly, suggesting that the scale of tenocyte loss determines the speed of regeneration. I will combine time-lapse imaging and lineage tracing to determine the identity of tenocyte progenitor cells during regeneration following genetic ablation. My work will provide new insights into regenerative mechanisms in zebrafish and contribute towards therapeutic applications, leading to scar-free tendon regeneration in mammals.

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Determining the role of Moesin protein palmitoylation

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Cooperative cell differentiation to form tissues during animal development requires establishment of unique domains in the plasma membrane and underlying cytoskeleton, which together comprise the cell cortex. Organization and dynamic remodelling of the cell cortex is tightly regulated in development during cell division, motility and morphogenesis. Dysregulation of the cell cortex has been implicated in developmental disorders, and epithelial-to-mesenchymal transition in metastatic cancers. Moesin, a crucial protein linking the plasma membrane and actin cytoskeleton, plays essential roles to maintain epithelial integrity and proper cell division. While Moesin activation has been traditionally linked to phosphatidylinositol 4,5-bisphosphate (PIP2) binding and phosphorylation, recent studies using phosphorylation and PIP2 binding mutants have suggested that activation of Moesin can occur in the absence of these processes, indicating that other regulatory mechanisms may be involved. Two large-scale screens in different cell types have identified Moesin as a protein modified by addition of lipid moieties, including S-palmitoylation, though the role of this post-translational modification and its impact on Moesin's function is unknown. This study investigates the role of palmitoylation in Moesin activation. Our results support the hypothesis that palmitoylation is necessary for Moesin recruitment to the plasma membrane, facilitating its binding to PIP2 and downstream activation steps. In cell culture experiments, disruption of palmitoylation through mutation of putative sites led to increased cytosolic Moesin and reduced phosphorylated Moesin (pMoe) at the plasma membrane, as well as disruption of the actin cytoskeleton. In dividing neural stem cells in the developing brains of third instar Drosophila larvae, mutation of putative palmitoylation sites resulted in defects in asymmetric cell division and spindle orientation, supporting the hypothesis that palmitoylation has an essential role in Moesin function during mitosis. These findings provide insights into the role palmitoylation may play in Moesin activation and its subsequent effects on cell morphology and division.

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Dissecting the mechanism and function of polarized actomyosin dynamics during Wnt-dependent asymmetric cell division

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Asymmetric cell division is essential for generating cell type diversity during development. In *Caenorhabditis elegans*, the endomesodermal precursor EMS cell undergoes asymmetric cell division along the anterior-posterior body axis, a process dependent on a Wnt ligand expressed by the posteriorly located P2 cell. Disruption of either Wnt signalling, or actin filaments results in the loss of endoderm fate specification (Goldstein, 1995). However, the mechanisms by which actin cytoskeleton regulates Wnt-dependent asymmetric cell division remain unclear. Here, we identify asymmetric cortical dynamics and its potential regulators during Wnt-dependent asymmetric cell division. We found that the cell cortex is more contractile in the anterior during cytokinesis. A mathematical simulation demonstrates that asymmetric cortical contractility accounts for the asymmetric cortical dynamics observed in vivo. Specifically, the model can explain polarized cortical flow, the asymmetric distribution of actomyosin, and asymmetric orientation of F-actin. These results suggest that asymmetric cortical contractility is the upstream event mediating various observed cortical asymmetries. Next, we sought to identify the regulators of asymmetric cortical contractility. We found that actin nucleators, such as the Arp2/3 complex and formin, localize asymmetrically during EMS cell division. We also found that both Wnt signalling and receptor tyrosine kinase Src signalling regulate the asymmetric localization of these actin nucleators. Strikingly, these actin nucleators are required for proper endoderm specification. By imaging non-muscle myosin and using it to evaluate cortical contractility, we are investigating the role of actin nucleators in regulating asymmetric contractility during EMS cell division. Furthermore, we are investigating the role of asymmetric cortical dynamics in cell fate specification as well as cell size asymmetry. This study should illuminate a novel interplay between Wnt signalling and cytokinesis during animal development.

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Insights into developmental consequences of Robinow Syndrome DVL1 mutations using Drosophila

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Insights into how development is regulated by signal transduction networks can be gained from studies on the mechanisms leading to developmental disorders. We are characterizing the effects on signaling of Dishevelled 1 (DVL1) variants identified in Robinow Syndrome (RS) patients. RS is a rare developmental disorder that was linked to mutations in several genes which are components of the non-canonical/Planar Cell Polarity (PCP) pathway of Wnt signaling. DVL1 is a relay molecule for both canonical and non-canonical/PCP Wnt signaling cascades. PCP signaling regulates cytoskeletal processes and directs cell polarity within the epithelial plane. Canonical Wnt signaling plays a role in both tissue homeostasis and embryonic development. The DVL1 variants we study have frameshift mutations that replace the C-terminus with a long novel peptide sequence with no known homology. To understand their altered function, we generated fly strains expressing both wildtype and variant human DVL1 genes and assayed their effects on flies. We also expressed DVL1 protein lacking the C-terminus, to investigate whether the neomorphic phenotypes are caused by the novel peptide sequence in the variants or by the absence of the well conserved C-terminus. Our studies have demonstrated that these DVL1 variants ectopically stimulate PCP/JNK signaling, trigger apoptosis, and interfere with the stability of Armadillo, thus inhibiting canonical Wnt signaling. Additionally, the mutations cause several novel phenotypes in wing and leg tissues, including abnormalities in tissue morphogenesis and cell adhesion. We find that imaginal disc development is disrupted and accompanied by increased cell death, without changes in cell proliferation. Furthermore, we find altered dynamics of basement membrane components and modulators. Notably we find increased Mmp1 expression and tissue distortion, which is dependent on JNK signaling. Through these studies we have gained more insight into the developmental consequences of DVL1 variants implicated in autosomal dominant Robinow Syndrome.

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Transposable Elements May Hijack Zygotic Genome Activation During Early Drosophila Embryogenesis

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Transposable elements (TEs) are mobile DNA sequences capable of replicating themselves by inserting into new genomic locations. Such insertions can disrupt gene function and threaten genome integrity. Despite robust cellular defenses, TEs constitute a substantial proportion of eukaryotic genomes, indicating frequent evasion of these mechanisms. Recent studies highlighting elevated TE expression during early embryogenesis suggest that the early embryonic syncytium may provide an opportune environment for TE activity. Newly laid Drosophila embryos are predominantly occupied by maternal transcripts. Approximately one hour post-fertilization, the embryonic transcription initiates through Zygotic Genome Activation (ZGA). Zelda, a pioneer transcription factor, regulates ZGA by binding to its motif, CAGGTAG, thereby remodeling inaccessible chromatin, promoting the recruitment of additional transcription factors, and initiating zygotic gene expression. We hypothesize that TEs may exploit Zelda's role during ZGA, utilizing its chromatin-remodeling function to enhance their own expression. Consistent with our hypothesis, in RNA-seq datasets of Zelda-Knockout mutants, we identified several transposable element families that are significantly downregulated, including the LTR retroelement roo. Interestingly, we found a potential Zelda binding site (ZBS) with the motif, CAGGTAC, located near the 3' end of roo's ORF. We hypothesize that through this incorporation of ZBS, roo might take advantage of Zelda's activating function, thereby enhancing its own activity. To test this hypothesis, we engineered a GFP-tagged roo element containing either an intact or a disrupted ZBS. Constructs are introduced into the Drosophila genome using site-specific integration. We expect the ZBS-disrupted roo insertion to show reduced zygotic expression. Phenotyping of roo activity through qPCR and fluorescent microscopy is currently in progress.

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A non-linear response to Hedgehog signalling provides a mechanism for novel directions of shape change during development

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Due to the requirement of robustness during development, there are many examples of processes that have a high tolerance for a certain degree of loss. This is perhaps best demonstrated by most systems being able to tolerate a heterozygous loss of function. This threshold acts protectively, preventing major deleterious effects and preserving correct development. Cell signalling pathways that are reused many times throughout development, such as Hedgehog (Hh) signalling, function with a high degree of finetuning, likely due to a requirement for level control past simply on and off. To study this deeper, we utilise the zebrafish as a model and selectively inhibit Hh at specific timepoints with varying concentrations of the small molecule inhibitor cyclopamine. Through increasing the concentration, we can alter the amount of Hh signalling the embryo receives from low inhibition to complete inhibition. Using 3D geometric morphometrics, we uncovered a clear threshold of Hh inhibition required to generate severe phenotypes and further defined a non-linear relationship between Hh signalling output and craniofacial morphology. Interestingly, this non-linear relationship also appears to allow for new directions of shape change that differ from the major “Hh loss” axis. This is particularly noticeable at below-threshold levels of inhibition, where the phenotypic changes are subtle and non-severe, but the direction is distinctly different. This change in covariance structure along the non-linear response curve suggests an evolutionary mechanism for exploring the morphospace to find novel directions of shape change while maintaining developmental robustness.

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Evolved embryonic suppression of recently expanded retroelements through heterochromatin nucleation

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Transposable elements (TEs) occupy large swaths of eukaryotic genomes and are some of the most rapidly changing constituents. Repression of TEs requires the production of complementary piRNAs for post-transcriptional degradation and the local formation of heterochromatin for transcriptional silencing. In Drosophila, H3K9me3, the key repressive histone modifications, is established during zygotic genome activation. Previously, ChIP-seq on *D. miranda* embryos revealed that while the bulk of H3K9me3 appear at embryonic stage 4 (nuclear cycle 10-13), a small number of retroelement families show position-specific nucleation of H3K9me3 at stage 3, the onset of ZGA, which then spread to neighboring nucleosomes. Because TE families with such targeted H3K9me3 nucleation have lower expression but, counterintuitively, higher copy number, we hypothesize that they are recently active elements that are now being targeted for silencing. Phylogenetic comparisons to the sister species *D. pseudoobscura* revealed that the elevated copy number resulted from recent expansions specific to *D. miranda*. Further, in *D. pseudoobscura*, the same families, despite fewer copies, are more highly expressed and do not nucleate H3K9me3 during early embryogenesis, suggesting that early nucleation likely arisen in *D. miranda* as an adaptative silencing mechanism. While piRNAs can mediate heterochromatin formation at TE insertions, we found no association between their abundance and the nucleation sites arguing against a piRNA-dependent mechanism. Instead, we propose that maternally deposited DNA-binding proteins target specific motifs in these TEs to recruit histone methyltransferases inducing site-specific H3K9me3 nucleation. Consistently, we observed targeted nucleation only when *D. miranda* is the mother of hybrid embryos produced by reciprocal crosses of the two species. Overall, our work reveals the potential for rapid emergence of countermeasures beyond the piRNA pathway amidst insidious and selfish spread of TEs.

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Investigating the roles of zoo-1/TJP in synapse patterning in C. elegans

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Nervous system function depends on precise regulation of synapse number, arrangement, and target recognition. While many extrinsic cues that spatially control synapse formation have been elucidated, the intracellular effectors of these pathways remain poorly understood. Zonula occludens (ZO) proteins are cytoplasmic scaffolding proteins encoded by the TJP genes that link transmembrane proteins to cytoplasmic proteins and the actin cytoskeleton at intercellular junctions. Recent studies in zebrafish showed that ZO proteins promote the formation of electrical synapses by localizing Connexins. However, the role of ZO proteins in chemical synapse formation is not understood. The cholinergic DA9 motorneuron of *C. elegans* forms approximately 20 synapses onto the posterior dorsal body wall muscles within a small subaxonal region. We and others have shown that the position of DA9 synapses is regulated by a prosynaptogenic cue, Neurexin, and the antisynaptogenic cues Plexin, Frizzled, and Netrin. In the loss-of-function mutant of zoo-1, the sole ortholog of TJPs in *C. elegans*, we observed synapse loss in the posterior synaptic domain and ectopic synapse formation in the anterior asynaptic domain, resulting in an anterior shift of the DA9 presynaptic domain. Interestingly, the ectopic synapse formation in the anterior asynaptic domain of plx-1/Plexin mutants, which is more severe than in zoo-1 mutants, was partially suppressed by loss of zoo-1, suggesting that zoo-1 may function downstream of plx-1. We previously found that PLX-1 negatively regulates RAP-2 activity to restrict synapse formation. As ZOO-1 and GTP-RAP-2 are both enriched in the presynaptic domain, we are investigating whether zoo-1 may function with RapGEFs to regulate synapse formation. Currently, we are testing whether transgenic expression of ZOO-1 cDNA is sufficient for a gain-of-function condition and to determine the tissues in which zoo-1 functions. Together, our data show a novel role for zoo-1 in mediating multiple signaling pathways to specify precise synapse patterning.

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The functions of CaMKII and PKC in Wnt-dependent neurite pruning in *C. elegans*

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During development, neurons remove excessive neuronal processes, or neurites, via a mechanism called developmental neurite pruning. Previously, we showed that in *Caenorhabditis elegans*, lin-44/Wnt instructs neurite pruning of the postembryonic cholinergic motor neuron, PDB, at the L2 stage by recruiting its receptor lin-17/Frizzled (Fz) to the pruning neurites (Lu and Mizumoto, 2019). In this study we found that itr-1/IP3 receptor (IP3R) loss of function (lof) mutation exhibits the PDB pruning defects, while the irt-1/IP3R gain-of-function (gof) mutation suppresses the pruning defect of lin-44/Wnt(lof) mutant, suggesting that Wnt regulates PDB neurite pruning through IP3R-dependent calcium signaling. We then looked for calcium-dependent protein kinases as potential effectors of Wnt-dependent neurite pruning, and found that loss-of-function (lof) mutant of unc-43/calmodulin-dependent protein kinase II (CaMKII) exhibits ectopic neurites in PDB at the L4 stage, suggesting that CaMKII is required for neurite pruning. unc-43/CaMKII(lof) does not enhance the pruning defect of lin-44/Wnt(lof), while the unc-43/CaMKII gain-of-function (gof) allele suppresses the pruning defect of lin-44/Wnt(lof). Additionally, expressing CaMKII cDNA in PDB rescues the pruning defect of unc-43/CaMKII(lof) mutant. These data suggest that CaMKII functions downstream of Wnt, and acts cell-autonomously in PDB to regulate neurite pruning. We also found that, while pkc-2/Protein Kinase C (PKC) lof mutant exhibits a mild pruning defect in PDB, the unc-43/CaMKII(lof); pkc-2/PKC(lof) double mutants exhibit a severe neurite pruning defect comparable to lin-44/Wnt(lof) mutant. Moreover, unc-43/CaMKII(lof) and pkc-2/PKC(lof) do not enhance the pruning defect of lin-44/Wnt(lof), suggesting that CaMKII, and PKC function in the same genetic pathway as Wnt. Lastly, we observed the accumulation of early endosome marker, rab5, in the pruning neurite in a PKC-dependent but CaMKII-independent manner. Consistently, genetic epistatic analysis suggested that the clathrin-dependent endocytic pathway acts in the same genetic pathway as PKC but in parallel to CaMKII, suggesting PKC and CaMKII regulate distinct downstream cascades.

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Inter-class axon-axon interaction defines tiled synaptic innervation of DA-class motor neurons in *C. elegans*

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Axon-axon interaction plays a crucial role in topographic map formation during nervous system development. However, we have limited knowledge about how it regulates neuronal map formation at the level of synapse patterning. Previously, we showed that Semaphorin-Plexin signaling mediates the inter-axonal interaction between two DA-class cholinergic motor neurons, DA8 and DA9, to define their tiled synaptic innervation (Mizumoto and Shen, 2013). As the axons of DA8 and DA9 have contact with other axons within the dorsal nerve cord we tested the potential contributions of other neuron classes in the synaptic tiling of DA neurons. We found that genetic ablation of the DB class of cholinergic motor neurons using the recCaspase-3 system resulted in a synaptic tiling defect between DA8 and DA9. Consistently, DA8 and DA9 synaptic tiling is disrupted in the vab-7(e1562) mutant, in which the axons of DB neurons are often misguided anteriorly. Genetic ablation of D-type GABAergic motor neurons (DDs and VDs) did not cause a synaptic tiling defect of DA8 and DA9, suggesting that DB axons, particularly the DB7 axon, which overlaps with the DA8 and DA9 synaptic regions, are specifically required for the synaptic tiling of DA8 and DA9. We conducted a candidate screening to identify cell adhesion and signaling molecules mediating DA-DB interaction and found that a null mutant of syg-2(miz185) exhibited a synaptic tiling defect between DA8 and DA9 similar to vab-7(e1562) mutants. Additionally, syg-2(miz185) did not enhance the synaptic tiling defect resulting from DB ablation, suggesting that syg-2 may act through DB7 to control DA8-DA9 synaptic tiling. Interestingly, syg-2(miz330) mutants lacking the cytoplasmic domain also displayed synaptic tiling defects between DA8 and DA9, suggesting that SYG-2 may signal through its cytoplasmic domain to control DA8-DA9 synaptic tiling. Currently, we are continuing to investigate the role of syg-2 and its known binding partner, syg-1.

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Neural role in biomineralization for the slipper snail *Crepidula atrasolea* (Calyptraeidae, Gastropoda)

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While we understand biomineralization as a chemical process, we know little about how organisms like molluscs (e.g., snails, Nautilus, clams, etc.) control mineral deposition. Several studies suggest that biomineralization in molluscs is under neuronal control, however, there is currently no direct evidence to support this hypothesis. Specifically, the neural control hypothesis (NCH) proposes that neurosecretory networks signal to the mantle, the organ that secretes the shell, to control biomineralization. Our goal was to test the NCH by determining if nerves are present throughout all stages of shell formation in the slipper snail *Crepidula atrasolea*. To date, the ontogeny of mantle neural anatomy was observed across shell developmental stages using the neural antibodies serotonin and FMRF-amide. Preliminary data suggests that neural tissue may be present early during shell formation. However, further markers and higher-resolution studies are required to confirm this. This research is the first step in understanding how molluscs control shell secretion, as well as developing an antibody staining protocol for future studies with *C. atrasolea*. Furthermore, it will aid future molluscan research on shell form variation and alternate shell control hypotheses.

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Cytokine stem cell priming promotes gut homeostasis under conditions of hypoxia-reoxygenation in Drosophila.

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Adult epithelial organs maintain homeostasis through continuous cellular turnover regulated by stem cells that self-renew and differentiate to replace lost cells. The intestinal epithelium exemplifies this adaptability, dynamically responding to environmental perturbations to maintain proper organ size and function. Hypoxia represents a significant intestinal stressor. Although the intestine typically functions in a physiologically low oxygen environment, conditions like ischemia, lung disease, or sleep apnea can further reduce oxygen availability, often causing repeated bouts of intermittent acute hypoxia. However, the mechanisms maintaining intestinal homeostasis under fluctuating oxygen conditions remain poorly understood. Using Drosophila as a model system, we are investigating gut homeostasis during hypoxia-reoxygenation cycles. We find hypoxia (1% oxygen, 6 hours) induces significant intestinal remodeling, characterized by a dramatic ~40% reduction in gut length, followed by restoration to normal size over 48 hours upon reoxygenation. Interestingly, this hypoxia-reoxygenation cycle operates independently of the canonical hypoxia-inducible factor-1 (HIF-1) pathway. Instead, we demonstrate that this process relies on the Unpaired (Upd) cytokines, homologs of mammalian interleukins. During hypoxia, we observe rapid induction of both caspase activity and Upd expression in mutually exclusive populations of differentiated gut epithelial cells (ECs) and activation of Upd downstream JAK/STAT signaling in intestinal stem cells (ISCs). Despite these changes, acute hypoxia alone triggers neither increased EC apoptosis nor ISC proliferation. Rather, upon reoxygenation we observe a significant increase in TUNEL-positive apoptotic ECs accompanied by a wave of ISC proliferation. Genetic inhibition of caspases in mature ECs blocks reoxygenation-induced ISC proliferation, while inhibition of JAK/STAT signaling in ISCs inhibits their proliferation and prevents intestinal resizing during reoxygenation and reduces survival during repeated cycles of intermittent hypoxia. We propose a model wherein hypoxia induces Upd to prime ISCs to rapidly proliferate upon reoxygenation to replace caspase-dependent loss of mature ECs, thus facilitating intestinal adaptation to oxygen fluctuations.

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Lens Regeneration in Newts: A Developmental Perspective

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Newts are unique among salamanders as they regenerate the lens of the eye at any stage of their lives. Upon lens loss, iris pigment epithelial cells transdifferentiate and give rise to the new lens. This regenerative competence is restricted to the dorsal iris, and not the ventral. To interrogate this enigma, we have performed RNAseq/scRNAseq, spatial transcriptomics/ Hybridization Chain Reaction Fluorescent In-Situ Hybridization (HCR-FISH) and immunohistochemistry to delineate regional patterns in gene expression across the irises of two newt species, the Eastern newt (*Notophthalmus viridescens*) and Iberian Ribbed newt (*Pleurodeles waltl*). We identified a highly conserved dorsal-ventral patterning axis associated with vertebrate eye development that is maintained across the iris into adulthood that includes the Ephrin signaling pathway. In response to lentectomy, the BMP signaling pathway undergoes an injury-responsive “switch” that is associated with the dorsal regenerative ability. We found evidence that this signaling axis acts along with the Ephrin signaling axis to maintain iris polarity, and that pharmacologic disruption of this axis is sufficient to confer regenerative competence to ventral iris pigment epithelial cells. Our data suggests that this molecular axis was co-opted to dictate regenerative competence in newts.

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Roles of lat-2/Latrophilin and ten-1/Teneurin in Developmental Neurite Pruning in *C. elegans*

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Neurite pruning is a crucial developmental process required for the maturation of functional neural circuits. Defects in this process have been linked to neurodevelopmental disorders, including Autism Spectrum Disorder, ADHD, and schizophrenia (Moreno-Salinas et al., 2019). However, the molecular mechanisms regulating neurite pruning remain largely unknown. We previously showed that the PDB motor neuron in *Caenorhabditis elegans* undergoes stereotyped neurite pruning (Lu and Mizumoto, 2019). Through a candidate approach, we found that null mutants of lat-2, an orthologue of Latrophilin, result in neurite pruning defects in PDB, indicating that lat-2 plays an important role in this process. Latrophilin is an adhesion-GPCR that undergoes auto-proteolytic cleavage into the N-terminal and C-terminal fragments, which remain non-covalently associated. It is currently accepted that the dissociation of the extracellular N-terminal fragment exposes the tethered agonist domain in the C-terminal fragment, thereby activating the GPCR signaling. By using CRISPR-Cas9, we generated lat-2 mutants carrying mutations in the autoproteolytic cleavage site or the tethered agonist domain. Our preliminary data suggest that these mutations do not result in pruning defects, suggesting that lat-2 function in neurite pruning may not depend on its GPCR activity. In mammals, Latrophilins can also act as ligands of a large type-II transmembrane protein, Teneurin (del Toro et al., 2020; Wang et al., 2024). Consistently, we found that the null mutant of ten-1(ok641), the sole ortholog of Teneurin in *C. elegans*, also exhibits a pruning defect in PDB. We are currently examining the genetic and molecular interactions between lat-2 and ten-1 in neurite pruning by examining the pruning defects in the lat-2; ten-1 double mutants. Additionally, we are investigating the co-localization patterns in the L2 stage when PDB undergoes neurite pruning. Together, our findings suggest a novel role for Latrophilin and Teneurin in developmental neurite pruning.

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Dynamics of Nephric duct elongation during kidney development: Insights from the mouse model

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Collective cell migration is essential for shaping tissues during development. In the early embryo, the nephric ducts (NDs) form in a precise location and extend toward the posterior through coordinated cell movement, triggering kidney formation. While most knowledge of ND elongation comes from non-mammalian models (axolotl, frog, zebrafish, and chick) due to their accessibility, understanding this process in mammals remains limited. To address this, we analyzed ND elongation in mice by tracking cell protrusions along the anterior-posterior axis at different developmental stages. This allowed us to study how actin dynamics influence migration directionality. Additionally, we assessed cell proliferation and death rates using whole-mount immunostaining to identify regional differences along the ND. We also examined whether left and right NDs exhibit distinct migration dynamics. By defining the cellular mechanisms of ND elongation in mammals, we highlight both shared and unique features compared to other vertebrates, advancing our understanding of kidney development.

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Transient TNF-α Signaling Regulates Ank-Dependent Mineralization in Bone Cells

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Tumor necrosis factor-alpha (TNF-α) is a pro-inflammatory cytokine secreted in response to stress, injury, and infection. Studies show that TNF-α upregulates NF-κB and normal bone mineralization is disrupted that depends on exposure and concentration. Ank is a gene encoding a transporter that exports pyrophosphate (PPi) and ATP to the extracellular matrix. PPi donates phosphate for physiological mineralization and at higher concentrations inhibits pathological calcification. It is proposed that NF-κB downregulates Ank via TTP (Tritetrproline, encoded from Zfp36 gene) however, the impact of Ank suppression on mineralization remains unclear. In this study, MC3T3-E1 cells were treated with 10 ng/mL TNF-α from day 0 to day 3 and differentiated over 21 days. Samples collected on days 4, 7, 14, and 21 were analyzed for expression of Ank, NF-κB, and Zfp36, as well as osteogenic markers (Runx2, Osx) and mineralization markers (Alp, Ocn). Mineral deposition was assessed via Alizarin Red staining. NF-κB and Zfp36 were elevated at day 4, with significant downregulation of Ank, Runx2, Osx, Alp, and Ocn, indicating impaired differentiation. However, by day 7, Ank returned to baseline, and osteogenic markers began recovering. Mineralization remained low at this time. From days 7 to 14, NF-κB and Zfp36 expression remained elevated relative to the control group, but the overall trend showed a gradual downregulation over time. Ank and osteogenic markers were upregulated at this time, resulting in a strong mineralization response as evident by Alizarin Red and the pronounced increase in nodules. Mineral content remained elevated at day 21 despite declining gene expression, which is likely due to the transient peak in Ank and osteogenic markers. Future experiments using SN50 and siRNA targeting Ank will determine whether these effects are directly mediated through Ank. These findings, however, suggest short-term TNF-α exposure enhances bone formation via an Ank-dependent pathway.

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Integrins in *Drosophila melanogaster* Peripheral Axon Ensheathment: Insights from RNA-Interference Knockdowns in Wrapping Glia

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In most animals, peripheral axonal ensheathment by non-myelinating Schwann cells (NMSCs) provides physical protection from environmental insults, such as pathogens, as well as functional support, such as enhancing nerve conductance velocity. Across many species, failure of NMSC axonal ensheathment leads to negative outcomes such as impaired animal locomotion, decreased axonal conductance velocity and regeneration, and axonal death. In *Drosophila melanogaster*, the wrapping glia is morphologically and functionally alike to NMSCs. The highly conserved transmembrane protein, integrin, has been shown to play a major role in peripheral glial ensheathment *in D. melanogaster*. Integrin are heterodimers, with α and β subunits, that exist with unique subunit combinations. In *D. melanogaster* peripheral glial cells, the existence of αPS2, αPS3, and βPS subunits suggest the existence of αPS2/βPS and αPS3/βPS complexes. Loss of the βPS subunit leads to disruption of the wrapping glia, but the role of the αPS2 and αPS3 subunits is not known. This study aims to explore which α subunits play a role in wrapping glia ensheathment. Immunohistochemistry and confocal fluorescent microscopy were used to identify α integrin subunits in the wrapping glia. RNA-interference knockdowns of both αPS2 and αPS3 were employed and disruption of the wrapping glia sheath was assessed. Preliminary findings from this study show that αPS2 primarily exists outside of the wrapping glia, while αPS3 primarily exists within the wrapping glia in the center of the peripheral nerve. Moreover, RNA-interference knockdowns of αPS3 seem to have a greater disruption to wrapping glia ensheathment compared to αPS2 knockdowns. This study may reveal the role of αPS2 and αPS3 integrin subunits in wrapping glia ensheathment of D. melanogaster peripheral axons. Given the deep evolutionary conservation of both NMSCs and integrins, these results will shed light on the fundamental role of integrins in peripheral axon ensheathment, offering biological insights across species.

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Shaping Kidney Development by Nephric duct leader cells

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The nephric duct (ND) plays a crucial role in kidney development by coordinating the collective migration of its cells. This movement allows the ND to reach and fuse with the cloaca, a key step in forming the metanephric kidney. Previous studies suggest that ND cells follow a functional hierarchy, similar to migratory birds. Cells at the migration front, known as leader cells, guide ND elongation by sensing environmental cues and directing movement along a precise pathway. The failure to form leader cells is a major cause of congenital anomalies of the kidney and urinary tract (CAKUT), as seen in Gata3 mutant mice, which exhibit severe ND elongation defects leading to kidney agenesis. However, the molecular regulators of ND leader cell dynamics remain poorly understood. Here, using single-cell RNA sequencing (scRNA-seq) on normal and Gata3-deficient ND cells, combined with whole-mount staining in mouse embryos, we have identified novel gene markers of ND leader cells and Gata3 target genes. These findings provide new insights into renal development and the mechanisms underlying CAKUT.

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Shaping kidney development with cell fate decisions and collective cell migration

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During early kidney development, renal progenitor cells undergo dynamic changes and rearrange to form a pair of nephric ducts. These ducts extend through collective cell migration toward the embryo's posterior, initiating the formation of the definitive kidneys and ureters while also contributing to the reproductive tract. Although these developmental events are well documented, the precise mechanisms that coordinate nephric duct progenitor fate and collective migration remain unclear. My lab focuses on understanding how renal progenitor cells develop and organize to shape the kidneys and how disruptions in these processes contribute to diseases such as congenital kidney disorders and cancer. Using newly developed fluorescent mouse models and advanced techniques like single-cell multiome analysis at different stages of progenitor lineage progression, we have uncovered the gene regulatory dynamics that govern cell fate and allocation during early kidney development.

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The transmembrane proteins integrin and Kon-tiki are important for the insulation of Drosophila peripheral axons by the wrapping glia

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Glial cells comprise a substantial portion of cells in the nervous system. In the human peripheral nervous system, most of the nerves consist of small-diameter axons ensheathed by a class of glial cells known as the non-myelinating Schwann cells in non-myelinated (Remak) bundles. Loss of non-myelinating Schwann cells leads to degeneration of sensory neurons such as the unmyelinated nociceptive C-fibers, resulting in severe neuropathic pain. Non-myelinating Schwann cells play a key role in human peripheral neuropathies associated with conditions like diabetes and aging, which cause the degeneration of small-diameter non-myelinated axons. However, the mechanisms underlying the development of non-myelinating Schwann cells remain poorly understood, and their contribution to peripheral neuropathies is often underappreciated. In *Drosophila melanogaster*, the wrapping glia serves as the morphological equivalent of non-myelinating Schwann cells in vertebrates. We found that knockdown of the beta-subunit of integrin (βPS, myospheroid), results in the loss of axon ensheathment by the wrapping glia, but the binding partners of Integrin in the wrapping glia remain unclear. Integrins can interact with proteins containing Laminin globular (LamG) domains and we carried out a screen to knock down LamG domain-containing proteins specifically in the wrapping glia. Through our RNAi screen, we identified Kon-tiki (Kon). Kon is a transmembrane protein containing two extracellular LamG domains expressed in the Drosophila peripheral glia. RNAi-mediated knockdown of Kon mimics the Integrin knockdown phenotype, in which the wrapping glial layers fail to wrap around the axons. Immunostaining revealed that Kon colocalizes with the Integrin beta-subunit in both the wrapping and outer glial layers. We are currently investigating the genetic interactions between Kon and Integrin in promoting wrapping glia ensheathment of axons. Given the conservation of integrins and Kon-tiki, results from this work will shed light on the protein interaction pathways involving the development of non-myelinating glia in all animals.

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Sd-complex regulation of cell identity

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The evolutionarily conserved family of 'TEAD' DNA binding proteins require 'cofactor' proteins with transactivation domains to act as transcription factors. TEAD cofactors include Vestigial-like proteins (VGLLs) and Yes-associated protein or Transcriptional coactivator with PDZ domain (YAP, TAZ). In *Drosophila melanogaster*, the paralogs are Scalloped (Sd) for TEAD; Vestigial (Vg) and Tondu-domain-containing growth inhibitor (Tgi) for VGLLs; and Yorkie (Yki) for YAP/TAZ. Multiple TEAD cofactors may be present simultaneously in a cell, and it is hypothesized they compete to bind Sd and drive separate cellular processes. One major regulatory event in VGLL, YAP/TAZ, and TEAD transcription factor activity is the sequestering of one or more protein components in or out of the nucleus. Thus far, interactions of Yki and Tgi with Sd have been the primary focus of study, with only minor consideration for the role of Vg. This study demonstrated using immunofluorescent imaging in Schneider 2 cells (derived from Drosophila embryos) that overexpression of transgenic Vg affected cellular localisation of co-overexpressed Sd, Yki, and Tgi. A second regulatory event in TEAD transcriptional activity is the phosphorylation of TEAD cofactors, which alters cellular localization and protein stability. Post-translational phosphorylation of serine 215 in Vg had been previously reported to affect Vg functionality in developing tissue, though the exact mechanism behind this effect was not well understood. Overexpression of phosphomimic or non-phosphorylatable VgS215 mutant isoforms produced an observable shift in localization in or out of the nucleus, especially when Sd or other cofactors were co-overexpressed. Finally, Sd cofactors were shown to colocalize with one another even in the apparent absence of Sd, both in and out of the nucleus.

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Modeling biological resilience in the embryo using a cleft lip induction model

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Non-syndromic cleft lip with or without cleft palate (NSCLP) is the most common craniofacial congenital anomaly, affecting 1 in 700 live births. Gene variants explain only 30% of the clefting risk, leaving a significant role for environmental influences. Our goal is to develop a model of induced cleft lip so that we can identify mechanisms of embryo resilience and susceptibility to environmental stress. To induce cleft lip, chicken embryos were treated with hydrocortisone (HC), to simulate increased stress. A dose of 40 µg of HC was administered once at HH24 (E4.5) or sequentially (5µg, 24h apart). HC was rapidly absorbed within 2hrs and fully metabolized by 24hrs as determined by mass spectroscopy. In addition, glucocorticoid receptors translocated to the nucleus 2hrs after a 40µg dose of HC, suggesting rapid changes in gene expression were induced. A dose of 40 µg caused externally visible cleft lip at 96hrs in 26% of embryos, with 27% lethality. The sequential treatment gave a higher penetrance and better survival at 96h (43% clefts, 13% lethality). The earliest phenotypes were a significant reduction in the sizes of facial processes at 24h (N = 10, 20% reduction, P = 0.001). Interestingly there was no change in proliferation or apoptosis (24h), but embryos did have a persistent epithelial seam which is a transient structure present just at the start of lip fusion (10/10 at 48hrs and 8/17 at 72hrs). Thus, even though all embryos experienced equal environmental stress, many were able to restore normal development after an initial delay. Our next goal is to identify the key molecular feedback loops that are induced by HC and that could be targeted to further decrease risk of developing cleft lip. The findings will shed light on the mechanisms of biological resilience that could be harnessed in future preventive strategies.

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Gene deserts as essential regulatory regions of SHOX homeobox genes during mammalian limb development

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We have previously reported the genomic distribution and spatial activity of a group of developmental limb enhancers located within a gene desert downstream of the mouse Shox2 gene. Our results showed that development of the proximal limb (the humerus and femur) is partially dependent on the Shox2 gene desert. Here we report that a similar array of limb enhancers is found downstream of the human SHOX gene, a paralog of SHOX2. Deficiencies of human SHOX cause deformities of the middle segment of the limb that are characteristic of Léri-Weill dyschondrosteosis (LWD) and idiopathic short stature (ISS). Deletions of noncoding sequences within the SHOX gene desert are a common cause of the limb defects of LWD and ISS. Since rodents lack the SHOX gene, identification of the relevant enhancer sequences in the deleted regions has been hampered by the lack of an animal model. Therefore, we adapted the domestic cat as a model to identify SHOX limb enhancers and their human orthologs. The conserved synteny of coding and noncoding elements in the human and cat pseudoautosomal region 1 where SHOX resides, and a similar pattern of SHOX expression in cat and human embryos support the use of the cat model for studying SHOX regulation. Toward this end, we used circular chromosome conformation capture (4C-seq) to identify enhancers regulating SHOX expression in cat embryonic limbs. The corresponding human orthologs were identified through sequence conservation and tested for enhancer activity using transgenic mice. Using this strategy, we identified previously uncharacterized human enhancers with specific activity in the proximal embryonic limb. Importantly, these enhancers are within an interval downstream of the SHOX gene that is deleted in a subset of LWD and ISS patients, suggesting that these sequences are critical for proper development of the human limb.

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Exploring the function of SHOX2 in the developing mouse limb using single-cell RNA sequencing

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Patterning and outgrowth of the developing limb requires the complex coordination of several signaling molecules and intracellular proteins that must be precisely regulated spatially and temporally during embryonic development. Shox2 codes for a homeodomain transcription with an important role in the patterning and development of several structures in the developing mouse embryo including the sinoatrial valves and pace making region of the heart; the forebrain, midbrain, and hindbrain; mechanosensory neurons in the dorsal root ganglia; the secondary palate; interneurons of the spinal cord; and the proximal segments of the limb. The severe limb truncation phenotype associated with a loss of Shox2 expression indicates that it is absolutely required for normal development of the proximal stylopod bones, the humerus and femur. While this loss of function phenotype is robust and well characterized, an understanding of the mechanism of SHOX2's contribution to limb development is not complete. Recent single-cell sequencing studies on embryonic mouse limb bud cells have allowed for a better understanding of the cellular composition of the early limb bud mesenchyme, with more precise definitions of cell-types already defined in the limb and the identification of new sub-types, including a proximal progenitor population in the early limb mesenchyme marked by the expression of Shox2. Here, single-cell RNA sequencing analysis of datasets from wild-type and Shox2-null embryonic limb buds was performed to investigate the impact of a loss of Shox2 expression on early limb progenitor populations. We employed RNA velocity analysis to compare predicted cell trajectories and characterize the reprogramming of progenitor populations in the absence of Shox2 expression. A combined analysis of clustering, RNA velocity, and differential gene expression between control and Shox2-null datasets reveal broad-scale differences in mesenchymal, progenitor, and chondrocyte populations in the early limb bud, indicating the reprogramming of cellular trajectories and a loss of proximal lineages.

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Rod photoreceptor specialization by NRL is conserved across vertebrates

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The evolutionary origins of vertebrate rod and cone photoreceptors remain blurry. A crucial factor in this discussion is the transcription factor NRL (neural retina leucine zipper), which is essential for rod photoreceptor specification in the vertebrate retina. When expressed in developing Sws1 (UV sensitive) cones in zebrafish, NRL redirects their fate toward a rod identity. Interestingly, the same is not true for other cone subtypes. We used mutant and transgenic zebrafish to investigate the conservation of NRL's role as a rod fate determinant across vertebrates. Immunohistochemistry and qPCR revealed that each vertebrate homolog of NRL, including that of hagfish, one of the earliest branching vertebrates, was sufficient to induce a fate shift from UV cones to rod photoreceptors in zebrafish. These findings suggest that the ability of NRL to direct rod photoreceptor identity is deeply conserved throughout vertebrate evolution. This study challenges centuries-old dogma of photoreceptor classification by demonstrating the unique and remarkably well-conserved relationship between rods and Sws1 cones, supporting the hypothesis that rods and UV cones have a shared evolutionary and developmental history. Furthermore, our results suggest that the ambiguous photoreceptors of hagfish are most likely rods. By elucidating the molecular mechanisms underlying photoreceptor development and specification, this work has implications for strategies such as stem cell therapies in treating retinal degenerative diseases.

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In vivo and live imaging studies define RhoA signaling roles during lip fusion

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Most orofacial clefts (OFC) are not due to single gene mutations but rather variants in about 40 genes. One of the most replicated genes is ARHGAP29, which converts Rho GTPase from the active GTP-bound state to the GDP-bound inactive state. The variants in ARHGAP29 are thought to cause a loss of function and thus increase RhoA activity. Here we began by testing 4 coding region variants (3 truncating and 1 missense) of ARHGAP29 in vitro and found that only one truncating variant caused nonsense-mediated decay (Lys326*). The others were expressed and increased cytoskeletal tension using measurements of phalloidin intensity and nuclear shape. These data are consistent with an increase in RhoA activity. Next we simulated a local increase in RhoA activity in the embryonic face using beads soaked in a RhoA agonist placed in the chicken embryo. The beads induced cleft lip due to a local increase in cell tension, which caused contraction of the facial prominence accompanied by increased cell density and increased expression of the downstream mediator, non-muscle myosin. In contrast, the ROCK inhibitor Y27632 did not affect cell tension. To determine whether RhoA was regulating other aspects of cell behavior we also carried out live imaging experiments during the period of lip fusion. In control organ cultures, there were three types of movement of epithelial cells - directed movement towards the fusion zone, movement from deeper to more superficial layers in the center of the fusion zone and oscillatory movements. The oscillations decreased the closer cells approached the fusion zone. The RhoA agonist caused cells to change the direction of movement, and oscillations decreased in amplitude resulting in a failure of fusion. Taken together our data support the idea that local increases in RhoA signaling, mediated by ARHGAP29 variants, are sufficient to cause orofacial clefting.

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A Model of the Middle: How do cell behaviours shape the embryonic face?

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To build a healthy face during embryogenesis, genes coordinate tissue shape by influencing cell behaviours. As few as 6 fundamental cell behaviours, including proliferation, mitotic orientation, and extracellular matrix deposition, drive nearly all tissue shape changes; however, the roles of these behaviours in the face are poorly characterized. Understanding how patterns of cell behaviours drive shape change throughout growth is critical to understanding how syndromes arise and what is necessary for healthy development.Here, I develop the first cell behaviour-driven model of facial morphogenesis, integrating 3D microscopy and computational simulations to understand how proliferating cells shape developing murine faces. Using light sheet microscopy, I capture tissue shapes and the 3D distribution and orientation of cell proliferation in wild-type mouse embryonic faces. I relate tissue-wide patterns in the distribution and orientation of cell divisions to shape change, discovering how cell behaviours cooperate to drive development. To understand the precise roles of different cell behaviours, I build physics-based simulations of tissue development based on imaging data.Results indicate that although spatial differences in proliferation across E10.0 mouse facial prominences are small and diffuse. An average of 17% of cells are mitotic in facial prominences, carrying only by 2%. However, these small changes can drive significant changes in tissue shape in simulation. Conversely, mitotic orientation is tightly aligned with the primary growth axis in the medial mandibular prominence, but randomly distributed in the lateral side. This may provide a mechanism for early snout elongation in the mouse. Additionally, we found that in areas where mitotic orientation is more uniform, nuclear geometry of mitotic cells is also more uniform. This co-coordination between cellular behaviours and geometry suggests a two-way relationship between tissue properties and cellular activity, where both areas of tight coordination and random growth are important to properly shape the embryonic face.

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The unexplored relationship between proteoglycan sulfation, cartilage maturation, and short stature

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Due to an abundance of proteoglycans (PGs) in skeletal tissues, mutations to PG genes cause dozens of human skeletal diseases. Many of these (e.g., mucopolysaccharidosis (MPS)), result in short stature (dwarfism), which might be expected given the known structural roles of PGs in the extracellular matrix. However, research from our lab and others suggest that cartilage PG-dependent growth factor (GF) signalling underlies skeletal defects of many PG-deficient humans. Specifically, we recently used fam20b mutant zebrafish to show that cartilage PGs delay the timing of chondrocyte maturation—a major driver of endochondral ossification—by inhibiting bone morphogenetic protein (Bmp) signalling. In addition, PG sulfation is required for PGs to interact with GFs. We recently characterized biochemical and physiological functions of a novel PG sulfatase, Arsi, which is related to known MPS-causing genes, during endochondral ossification. Future work seeks to use confocal imaging to reveal at cellular resolution how PG-dependent GF signalling drives skeletal morphogenesis, especially since many PG mutants, including fam20b, have short stature.

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Progressive coverage of brain blood vessels by pericytes from different embryonic sources

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Pericytes are mural cells that provide support to the endothelium of small vessels. Defects in the association of pericytes with the developing endothelium are implicated in stroke and neurodegenerative diseases. Here, we examine the relationship between pericytes and endothelium during brain angiogenesis and identify the developmental sources of pericytes. We focus on pericytes developing on the small Central Arteries (CtAs) that form as naked endothelial tubes before pericyte recruitment. In embryonic stages, CtA pericytes rapidly expand their numbers and extend their processes in parallel with the growth of the endothelial network. Inhibiting CtA angiogenesis via inhibition of Gpr124 (Canonical Wnt) or Vegfr (VEGF) led to a decrease in the vessel network length, pericyte number, coverage, and density; nonetheless, the remaining pericytes are well-spaced across the vessels, similar to the spacing in wildtype animals. This suggests each pericyte has an endothelial territory as we did not observe them clumped together in any scenario. We next assessed the mechanisms that drive pericyte expansion on the vessels. Using time-lapse imaging and markers for proliferation, we confirmed proliferation accounts for slow and steady pericyte population growth, but additional sources or pericytes are also present. Using genetic labeling and cell ablation studies, we show that a previously undescribed col1a2-expressing mesodermal population of cells gives rise to a subset of CtA pericytes. Some col1a2 cells migrate into the brain, attach to brain vessels and express the pericyte marker pdgfrb. A second set of col1a2-expressing cells migrate into the brain and contribute to a distinct population of brain fibroblasts on the larger vessels that are negative for pericyte (pdgfrb) and vascular smooth muscle cell (acta2) markers. These experiments reveal different migration routes, behavior and origins of brain pericytes, and potentially imply that there are also functional differences, based on cell of origin.

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Co-option of epithelial-to-mesenchymal regulators for biomineralizing cell fates in the gastropod *Crepidula atrasolea*

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The molluscan shell is a striking example of an evolutionary innovation and considered key to the success of this phylum. In molluscs, biomineralization underlies the formation of shells, spicules, and other calcium carbonate structures that display remarkable elegance and diversity. A comprehensive understanding of the genes and developmental pathways driving this process is still lacking. While the biomineralization gene regulatory network (GRN) in echinoderms has been extensively characterized, studies in molluscs have primarily focused on more terminal effector genes, leaving the initial regulatory mechanisms of this process relatively unresolved. GRNs provide a framework to understand the complex; hierarchical interactions of genes and pathways driving such biological processes. Transcription factors, as initiators of GRNs, activate signaling cascades that ultimately turn on downstream effector genes. In gastropods, transcription factors associated with epithelial-to-mesenchymal transitions (EMTs) are expressed early in the embryonic shell field—the region that gives rise to the larval shell —implicating them in the specification of biomineralizing cells. EMTs are driven by deeply conserved regulatory genes and occur at stereotyped developmental stages, suggesting a fundamental link between cell fate transitions and the evolution of novel morphological structures such as the shell. We aim to explore how GRNs orchestrating embryonic EMTs may play a role in specifying a biomineralizing cell fate in the developing shell field of the gastropod *Crepidula atrasolea*. By identifying the early regulatory architecture of the molluscan biomineralization GRN, this work will enhance our understanding of shell ontogeny and contribute to broader insights into the role of cellular movements in the evolution of complex traits.

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The Role of TMED2 on Murine Cardiac Development

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The transmembrane emp24 domain (TMED) proteins are essential components of the secretory pathway, mediating protein transport via coat protein (COP)-coated vesicles. TMED proteins act as cargo receptors, regulating selective protein trafficking between the endoplasmic reticulum (ER) and the Golgi apparatus. Disruptions in this transport pathway can impair cell differentiation and fate determination, leading to congenital malformations. TMED2, a member of the TMED β subfamily, is particularly critical for normal embryonic development in mice. Previous studies have demonstrated that a single-point mutation in Tmed2 (Tmed299J/99J) results in embryonic lethality by mid-gestation, with early-stage embryos exhibiting abnormal development, including randomized heart looping. These findings suggest a key role for TMED2 in cardiac morphogenesis, though the underlying molecular mechanisms remain unclear. We hypothesize that disrupted protein transport between the ER and the Golgi in Tmed2 mutants disrupts cardiac development, leading to abnormal heart morphogenesis. To investigate this, we employ a conditional mutant mouse model with LoxP sequences flanking exons 2 and 3 of Tmed2. Using Mesp1-cre, which is expressed in mesodermal cells contributing to the developing head and heart, we mutated Tmed2. Additionally, we conditionally mutated Tmed2 in neural crest cells using Wnt1-cre, as cardiac neural crest cells contribute to outflow tract elongation and septation as of E10.5, forming the pulmonary trunk and aorta. Preliminary histological analysis at E11.5 has showed that Tmed2LoxP/LoxP; Mesp1-cre+/- embryos display normal heart looping but may exhibit defects in endocardial cushion development. Furthermore, at E11.5 Tmed2LoxP/LoxP; Wnt1-cretg/+ embryos show normal neural crest cell migration but fail to septate the outflow tract into two distinct vessels needed to separate the systemic and pulmonary circulation, suggesting a critical role for TMED2 in this process.

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