Stowers Institute

Stowers.org

STOWERS RESEARCH CONFERENCES:

Developmental Cell Biology

#SRCKC24

PROGRAM

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Speaker Instructions

Speakers **must upload their presentations at the podium** at the designated times listed below, prior to their scheduled sessions.

Speaker file upload times:

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SESSION 1 12:00 PM - 1:30 PM

Thursday, October 24

SESSION 2 8:30 AM - 9:30 AM

SESSION 3 12:30 PM - 1:00 PM

Friday, October 25

SESSION 4 8:30 AM - 9:30 AM

SESSION 5 12:30 PM - 1:00 PM

Speakers **must get equipped with a microphone at the podium** 15 minutes prior to their scheduled sessions.

Talk Lengths:

Keynotes	40 min presentation + 10 min Q&A
Invited Speakers	20 min presentation + 10 min Q&A
Abstract Talks	15 min presentation + 5 min Q&A

Conference Program

Invited Speakers and Trainees. 20 min Presentation + 10 min Q&A

Speakers Chosen from Abstracts. 15 min Presentation + 5 min Q&A

Keynote. 40 min Presentation + 10 min Q&A

All talks will be presented in the Stowers Auditorium

WEDNESDAY, OCTOBER 23

12:30 PM TO 2:00 PM	CONFERENCE CHECK-IN & POSTER SET-UP - *Light snacks and refreshments are available outside of the Auditorium*

SESSION 1:

2:00 PM	OPENING REMARKS
2:10 PM TO 3:00 PM	KEYNOTE: **CAROLE LABONNE & JOSH YORK, NORTHWESTERN UNIVERSITY** *Control of Stem Cell Attributes in Early Vertebrate Development*
3:00 TO 3:15 PM	BREAK – *refreshments available outside of the Auditorium*
3:20 PM TO 3:50 PM	**CHRISTIAN MOSIMANN & HARRISON WELLS, UNIVERSITY OF COLORADO** *Lateral thinking in cardiovascular development and disease*
3:55 PM TO 4:15 PM	**BHAVAL PARMAR, THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA** *Cyclooxygenase-2: Essential Insights into Craniofacial Development*
4:20 PM TO 4:40 PM	**YUHANG FAN, UNIVERSITY OF ILLINOIS CHICAGO** *Ultrafast distant wound response is essential for whole-body regeneration*
5:15 PM TO 7:00 PM	WELCOME PARTY at LINDA HALL LIBRARY ***A shuttle is available to take you to the Linda Hall Library. Walking Directions Tours of their Rare Books Collection will take place every 20 minutes. Small bites, desserts, and refreshments available***

THURSDAY, OCTOBER 24

SESSION 2:

9:00 AM TO 9:45 AM	*Coffee and light breakfast options are available outside of the Auditorium*
9:45 AM	OPENING ANNOUNCEMENTS
9:50 AM TO 10:20 AM	**LILLIAN FRITZ-LAYLIN & EDGAR MEDINA, UNIVERSITY OF MASSACHUSETTS** *Chytrid fungi and the evolution of morphogenesis*
10:25 AM TO 10:45 AM	**TOSHIMICHI YAMADA, UNIVERSITY OF CALIFORNIA SAN FRANCISCO** *Guiding Morphogenesis using Synthetic Organizer Cells*
10:50 AM TO 11:10 AM	**YUAN LU, TEXAS STATE UNIVERSITY** *Segregation between an ornamental and a disease driver gene provides insights into pigment cell regulation*
11:10 AM TO 11:25 AM	BREAK – *refreshments available outside of the Auditorium*
11:30 AM TO 12:00 PM	**MARCUS KRONFORST & WEI LU, UNIVERSITY OF CHICAGO** *Visual system cell type differences underlying divergent butterfly mate preference*
12:05 PM TO 12:25 PM	**ROBERTA FIORINO, STOWERS INSTITUTE FOR MEDICAL RESEARCH** *Genetic modifiers and environmental factors in craniofacial development and disease: unraveling the complexity of phenotypic variability*
12:30 PM TO 1:30 PM	LUNCH BREAK – ***join us in the Stowers Library for lunch***

SESSION 3:

1:40 PM TO 2:10 PM	**DUYGU ÖZPOLAT, WASHINGTON UNIVERSITY IN ST. LOUIS** *Tissue reprogramming in a simple worm with a complex gut*
2:15 PM TO 2:35 PM	**RANNYELE P. RIBEIRO, WASHINGTON UNIVERSITY IN ST. LOUIS** *Sex without chromosomes: sex-biased gene expression during gametogenesis in Platynereis dumerilii*

2:40 PM TO 3:00 PM	**SARAH CHRISTIAN, UNIVERSITY OF MISSOURI-KANSAS CITY** *Mutations in foxg1a Cause Sox10 Olfactory Abnormalities*
3:00 PM TO 3:15 PM	BREAK – *refreshments available outside of the Auditorium*
3:20 PM TO 3:40 PM	**SHERMIN MAK, MAX-DELBRÜCK-CENTER FOR MOLECULAR MEDICINE** *Endocytic receptor LRP2: a new role in murine neural crest dynamics*
3:45 PM TO 4:05 PM	**NEFTALÍ VÁZQUEZ, UNIVERSITY OF TEXAS AT AUSTIN** *The human ciliopathy protein RSG1/CPLANE2 links the CPLANE complex to transition zone assembly*
4:10 PM TO 5:00 PM	KEYNOTE: **MARIANNE BRONNER & JAN ŠTUNDL, CALTECH** *Differential neural crest gene regulatory subcircuits along the body axis*
5:10 PM TO 6:40 PM	POSTER SESSION 1 – ***proceed down the staircase to the Stowers Gallery*** ***Light snacks and refreshments are available at the base of the stairs.***
6:45 PM	DINNER – ***join us in the Stowers Library for dinner***

FRIDAY, OCTOBER 25

SESSION 4:

9:00 AM TO 9:45 AM	***Coffee and light breakfast options are available outside of the Auditorium***
9:45 AM	OPENING ANNOUNCEMENTS
9:50 AM TO 10:20 AM	**BERNA SOZEN & MONIQUE PEDROZA, YALE UNIVERSITY** *A balancing act: Decoupling the coordinators of early human development using a stem cell-based platform*
10:25 AM TO 10:45 AM	**MELODY YAZDI, UNIVERSITY OF SOUTHERN CALIFORNIA, LOS ANGELES** *Spatial Transcriptomics Reveals Proximo-distally Patterned Signaling Centers In Regenerating Lizard Tail Blastemas*
10:50 AM TO 11:10 AM	**PURBASA DASGUPTA, UNIVERSITY OF KANSAS MEDICAL CENTER** *PRMT1 equipoises epigenetic regulation in trophoblast development to prevent early pregnancy losses*
11:10 AM TO 11:25 AM	BREAK – *refreshments available outside of the Auditorium*
11:30 AM TO 12:00 PM	**PAWEL BURKHARDT & ANNA FERRAIOLI, UNIVERSITY OF BERGEN** *Tracking the deep evolutionary origins of neurons*
12:05 PM TO 12:25 PM	**JONATHAN PHILLIPS, UT SOUTHWESTERN MEDICAL CENTER** *The Hippo pathway regulates the cytoskeleton and the shape of multicellular structures in a close unicellular relative of animals*
12:30 PM TO 1:30 PM	LUNCH BREAK – ***join us in the Stowers Library for lunch***
1:30 PM TO 3:00 PM	POSTER SESSION 2 – ***proceed down the staircase to the Stowers Gallery*** ***Light snacks and refreshments are available at the base of the stairs.***

SESSION 5:

3:05 PM TO 3:35 PM	**MEGAN MARTIK & LARA BUSBY, UNIVERSITY OF CALIFORNIA, BERKELEY** *Neural crest and the evolution of the vertebrate "new head"*
3:40 PM TO 4:00 PM	**JOSEPH CORBO, WASHINGTON UNIVERSITY IN ST. LOUIS** *Conservation of retinal cis-regulatory codes over half a billion years of evolution*
4:05 PM TO 4:25 PM	**CATHRYN HAAS, KANSAS STATE UNIVERSITY** *Signaling Mechanisms Orchestrating Asymmetric Divisions in the Ciona robusta b6.5 Neuromesodermal Lineage*
4:25 PM TO 4:40 PM	BREAK – *refreshments available outside of the Auditorium*

4:45 PM TO 5:05 PM	**ALICE SHERRARD, YALE UNIVERSITY** *Quantification of chromatin ultrastructure in mouse embryos and human cells*
5:10 PM TO 5:40 PM	**VANESSA BARONE & JANA SIPKOVA, STANFORD UNIVERSITY** *Echinoderm embryos from cell biology to evo-devo*
5:45 PM	**CLOSING REMARKS**
6:00 PM TO 7:30 PM	**CLOSING RECEPTION** - ***join us in the Stowers Library for a reception. Small bites, desserts, and refreshments available.***

Poster Sessions

Poster Session 1	Poster Session 2
Thursday, October 24 Even numbered posters: 5:10 PM – 5:55 PM Odd numbered posters: 5:55 PM – 6:40 PM	Friday, October 25 All Posters: 1:30 PM – 3:00 PM

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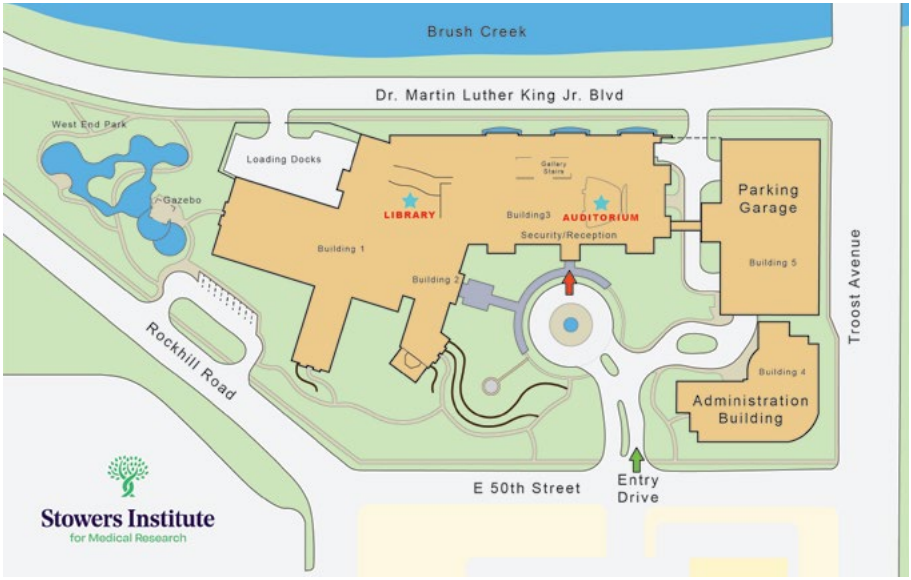
Anna Ferraioli University of Bergen	Do ctenophore have a brain? Connectome and cell type diversity of the aboral organ of the ctenophore *Mnemiopsis leidyi*	13

Campus Map


Brush Creek
Dr. Martin Luther King Jr. Blvd
West End Park
Loading Docks
Gazebo
LIBRARY
Building3
AUDITORIUM
Security/Reception
Building 1
Building 2
Parking Garage
Building 5
Troost Avenue
Rockhill Road
Building 4
Administration Building
E 50th Street
Entry Drive
Stowers Institute
for Medical Research

Shuttle Schedule

A shuttle will run between the InterContinental Hotel (401 Ward Pkwy, Kansas City, MO 64112) and the Stowers Institute for Medical Research (1000 E. 50th St, Kansas City, MO 64110) at the times listed below:

Wednesday, October 23:

12:30 PM & 1:00 PM	Pick up at Intercontinental Hotel, drop off at Stowers
4:50 PM	Pick up at Stowers, drop off at Linda Hall Library for welcome party
6:45 PM & 7:15 PM	Pick up at Linda Hall Library, drop off at Intercontinental Hotel

Thursday, October 24:

8:45 AM & 9:00 AM	Pick up at Intercontinental Hotel, drop off at Stowers
7:45 PM & 8:00 PM	Pick up at Stowers, drop off at Intercontinental Hotel

Friday, October 25:

8:45 AM & 9:00 AM	Pickup at Intercontinental Hotel, drop off at Stowers
7:30 pm & 8:00 PM	Pick up at Stowers, drop off at Intercontinental Hotel

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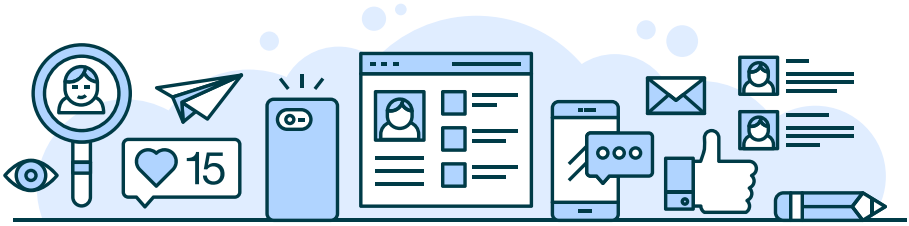

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Use hashtag **#SRCKC24**

Invited Speakers and Trainees


Carole LaBonne | Josh York
Northwestern University
Lab website


Duygu Özpolat | Rannyele P. Ribeiro
Washington University in St. Louis
Lab website


Marianne Bronner | Jan Štundl
Caltech
Lab website


Berna Sozen | Monique Pedroza
Yale University
Lab website


Christian Mosimann | Harrison Wells
University Of Colorado
Lab website


Pawel Burkhardt | Anna Ferraioli
University Of Bergen
Lab website


Lillian Fritz-Laylin | Edgar Medina
University Of Massachusetts
Lab website


Megan Martik | Lara Busby
University Of California, Berkeley
Lab website


Marcus Kronforst | Wei Lu
University Of Chicago
Lab website


Vanessa Barone | Jana Sipkova
Stanford University
Lab website

Abstracts

Mutations in foxg1a Cause Sox10 Olfactory Abnormalities

Sarah Christian[1], Roe Hendrick[1] , Hillary McGraw[1]
[1]University of Missouri-Kansas City

Sensory systems allow animals to interact and adapt to their environment. When errors in development occur, sensory function can be disrupted and can have deleterious impacts on the quality of life for the organism. Mutations in the transcription factor *foxg1* result in developmental disorders in humans and in model organisms such as the zebrafish. Research on *foxg1* mutations in human patients with Foxg1 Syndrome and in zebrafish shows defects in the olfactory system development. Our work using the *foxg1a*^{q266} mutant zebrafish line shows that loss of Foxg1a function results in the specific absence of Sox-positive olfactory neurons. Sox10-positive neurons are one set of cells within the olfactory system that lend to the complexity of the tissue and the ability to respond to a broad range of olfactory stimuli. This defect likely occurs during differentiation of olfactory neurons since neither proliferation nor cell death are affected. The goal of this research is to better understand developmental disorders like Foxg1 Syndrome for their treatment leading to enhanced lives of patients.

Protein profiling unmasks regulatory layers during zebrafish early embryogenesis

Gabriel da Silva Pescador[1], Danielson Baia Amaral[1], Joseph M. Varberg[1], Ying Zhang[1], Yan Hao[1], Laurence Florens[1], Ariel A. Bazzini[1,2]

[1]Stowers Institute for Medical Research, [2]Department of Molecular and Integrative Physiology, University of Kansas School of Medicine

The maternal-to-zygotic transition is crucial in embryonic development, marked by the degradation of maternally provided mRNAs and initiation of zygotic gene expression. While this process is widely studied at the transcript level, a systematic and global analysis on protein regulation has yet to be conducted in vertebrate models. Here, we conducted protein profiling throughout zebrafish embryogenesis using quantitative mass spectrometry, integrating transcriptomics and translatomics datasets. Our data shows that unlike RNA changes, protein changes are less dynamic over early developmental timepoints. Further, increases in protein levels correlate with mRNA translation, whereas declines in protein levels do not, suggesting active protein degradation processes. Surprisingly, genes with mRNA detected only after genome activation are present as protein since fertilization, challenging the classification of "pure-zygotic" genes based on RNA expression alone. Most of the "pure-zygotic" gene's products detected before genome activation can be correlated to mRNA expression from oocytes, and unexpectedly, from spermatozoa. As a proof of concept, we utilized CRISPR- Cas13d to target znf281b mRNA, a gene whose protein significantly accumulates within the first two hours post fertilization, demonstrating its crucial role in development. In conclusion, these results highlight the importance of looking beyond transcriptional regulation in cell fate transitions and embryonic development, providing a more comprehensive understanding of the process, and when coupled with CRISPR-Cas13d offers a new approach to unravel maternal factors function during embryonic development.
Research was funded by Stowers Institute for Medical Research and NIH grants GM136849 and R21OD03161.

PRMT1 equipoises epigenetic regulation in trophoblast development to prevent early pregnancy losses

Purbasa Dasgupta[1], Rajnish Kumar[1], Namrata Roy[1], Soma Ray[1], Asef Jawad Niloy[1], Soumen Paul[1]

[1]University of Kansas Medical Center

Introduction.
Recurrent pregnancy loss (RPL) is the failure of two or more clinically relevant pregnancies, being experienced by
~2.5% of women. The molecular pathogenesis of the early trophoblast progenitor cells and differentiation in RPL is poorly understood. The Protein Arginine Methylation (PRMT1) is the one of the major epigenetic modifiers in mammalian placentation. Defective PRMT1 is associated with pathological complications and dictates the equilibrium of trophoblast stem cells and other lineages of trophoblast, extravillous trophoblast stem cells (EVT) and Syncytiotrophoblast (ST) cells.

Objectives.
Our main aim is to understand the role of PRMT1 in maintaining a balance between cell fate choice of TSCs and other trophoblast cells in early and pathological pregnancies. We investigate how PRMT1 contributes to the defective development of trophoblast progenitors and aim to dissect the conserved transcriptional programming in mammalian placentation.

Results.
A subset of idiopathic RPLs placenta is associated with strong reduction of PRMT1 expression in trophoblast progenitor populations. Using a genetic mouse model and human trophoblast stem cells (hTSC), we provide evidence that a conserved PRMT1 is essential to maintain trophoblast progenitors and pregnancy progression, including mammals. Our mechanistic analysis provides evidence that PRMT1 regulate transcription of key genes by directly modifying histone arginine 4 methylation and their chromatin loci.

Conclusions.
Depletion of PRMT1 in human TSCs impairs their self-renewability and activates ST transcriptional program. Impaired expression of PRMT1 in RPL and hTSC is associated with the loss of TEAD4, a hippo pathway component. PRMT1 abrogation leads to the loss of trophoblast progenitors at E7.5 in post-implantation mouse embryo. Together, PRMT1 acts as the gatekeeper of trophoblast stem cells and propensity to differentiate in other lineages.

Ultrafast distant wound response is essential for whole-body regeneration

Yuhang Fan[1,2], Chew Chai[2], James E Ferrell[2], Bo Wang[2]

[1]University of Illinois Chicago, [2]Stanford University

Injury induces systemic responses, but their functions remain elusive. Mechanisms that can rapidly synchronize wound responses through long distances are also mostly unknown. Using planarian flatworms capable of whole-body regeneration, we report that injury induces extracellular signal-regulated kinase (Erk) activity waves to travel at a speed 10–100 times faster than those in other multicellular tissues. This ultrafast propagation requires longitudinal body-wall muscles, elongated cells forming dense parallel tracks running the length of the organism. The morphological properties of muscles allow them to act as superhighways for propagating and disseminating wound signals. Inhibiting Erk propagation prevents tissues distant to the wound from responding and blocks regeneration, which can be rescued by a second injury to distal tissues shortly after the first injury.
Our findings provide a mechanism for long-range signal propagation in large, complex tissues to coordinate responses across cell types and highlight the function of feedback between spatially separated tissues during whole-body regeneration.

Genesis of the Brain: Mapping the Brain's Pre-Gastrulation Origins

Kelli Fenelon[1], Priyanshi Borad[1], Anna Makridou[2], Biraaj Rout[1], Parisa Boodaghimalidarreh[1], Vanessa Avila[1], Rie Conley[1], Jacob Luber[1], Theodora Koromila[1,2]

[1]University of Texas at Arlington, [2]Aristotle University of Thessaloniki

Brain development is a complex process resulting from a lengthy progression of cell lineage transformations beginning in the early embryo. This process putatively begins well into gastrulation with the earliest conventional brain lineage markers appearing quite protracted from germ layer formation. However, we recently published evidence that the brain lineage may be specified concurrent with gastrulation onset in *Drosophila*, governed by genes with conserved function and pre-gastrulation expression patterns in mouse and human. Early zygotic factors, odd paired (Opa) and oscelliless (Oc) work together in a subpopulation of the future head to influence expression dynamics of genes involved in and important for brain development.

Further, we have demonstrated in a pending publication that the transition from maternal to zygotic direction of cell differentiation produces a complex, dynamic spatiotemporal landscape of zygotic transcriptome diversity via the Notch complex factor, suppressor of hairless, during cellularization. These pre-gastrulation regulatory dynamics support the notion that the very early embryonic anterior is comprised of greater diversity in cell identity than previously presumed. Using multiomics and live and fixed *in vivo* transcript and morphogen imaging, we begin to resolve a novel portrait of the primordial brain lineage. We propose a developmental model elucidating the dynamic regulation of pre-gastrulation cell specification engendering this diverse cell population during gastrulation. Understanding the genesis of the brain lineage is vital to answering basic questions of neurological significance as well as to finding translational solutions to neurological disorders.

Genetic modifiers and environmental factors in craniofacial development and disease: unraveling the complexity of phenotypic variability

Roberta Fiorino[1], Sharien Fitriasari[1], Thoa K.H. Truong[1], Hua Li[1], Jill Dixon[2], Michael Dixon[2], Paul A. Trainor[1,3]

[1]Stowers Institute for Medical Research, Kansas City, MO, USA, [2]University of Manchester, Manchester, UK, [3]Department of Anatomy & Cell Biology, University of Kansas Medical Center, Kansas City, KS, USA

Genetic and non-genetic factors contribute to embryonic development and this is particularly evident in craniofacial morphogenesis and the pathogenesis of congenital craniofacial anomalies. For example, Treacher Collins syndrome (TCS) is a disorder of craniofacial development, caused primarily by inherited or spontaneous autosomal dominant mutations in the *TCOF1* gene, that encodes for the nucleolar protein Treacle. TCS lacks a clear genotype-phenotype correlation and exhibits wide phenotypic variation, ranging from mild and indistinguishable, to severe hypoplasia of craniofacial bone and cartilage that can be life threatening. We have demonstrated that the phenotypic variability characteristic of TCS in humans can be recapitulated in *Tcof1*$^{+/-}$ mice, and that genetic background is a major contributor. Furthermore, non-genetic factors such as oxidative stress-induced DNA damage, which results in the apoptosis of the neural crest cells (NCCs) that generate most of the craniofacial skeleton, also drives phenotypic variation. We therefore hypothesize that the craniofacial phenotype variability associated with TCS is caused by genetic modifiers of *TCOF1* or oxidative stress, or DNA damage repair. To test this idea, we backcrossed DBA/1J;C57BL/6J F1 mice that manifest a severe TCS phenotype, with pure *Tcof1*$^{+/-}$ DBA/1J mice that exhibit a mild phenotype, and performed quantitative trait analysis (QTL) on F2 progeny. Detailed phenotyping using microcomputed tomography (micro-CT) and 3D morphometrics captured mild to severe craniofacial variation in F2 mutant progeny. We therefore combined the F2 phenotypic analyses with genetic SNP-mapping to identify loci that are detrimental for TCS via QTL. Interestingly, QTL identified a significant peak in a region of chromosome 1 containing several candidate genes involved in NCC and craniofacial development, potentially contributing to TCS phenotypic severity. *In vivo* validation using compound *Tcof1*$^{+/-}$/candidate gene mutant mice will provide new mechanistic insights into the gene-environment interactions underlying the etiology and pathogenesis of complex craniofacial disorders, and phenotypic trait variability in humans.

Signaling Mechanisms Orchestrating Asymmetric Divisions in the Ciona robusta b6.5 Neuromesodermal Lineage

Cathryn Haas[1], Michael Veeman[1]

[1]Kansas State University

In vertebrates, a large pool of mixed-lineage progenitor cells, known as NeuroMesodermal Progenitors (NMPs), give rise to the posterior spinal cord and paraxial mesoderm during tailbud morphogenesis. Tailbud development in other chordates, such as ascidians, is morphologically and mechanistically different, but there is some evidence suggesting that a particular ascidian lineage may have homology with vertebrate NMPs. In *Ciona*, the b6.5 lineage generates the posterior dorsal neural tube, tail-tip secondary muscle, and dorsal midline tail epidermis (DMTE). However, unlike most *Ciona* lineages, the mechanisms behind cell fate decisions in the b6.5 descendants remain poorly understood. Our lab's scRNAseq atlas has identified distinct gene expression patterns at the mid-gastrula stage between b6.5 descendants: b8.18/20 (DMTE), b8.19 (neural), and b8.17 (neuromesodermal). We utilized an MsxB lineage-specific reporter plasmid to confirm that b8.17 contributes both to the tail-tip muscle and cells morphologically continuous with the posterior neural tube. We further observed that b8.19 divides symmetrically to produce two neural daughters of similar size, while b8.17 divides asymmetrically, yielding a fate-restricted muscle precursor (b9.34) that is significantly larger than its sibling.
Interestingly, the previous cell cycle—where epidermal versus neural/ neuromesodermal fates are established— exhibits asymmetry through differential mitochondrial segregation without significant differences in sibling cell volume. Our findings suggest that both FGF and Wnt signaling are involved in specifying the neuromesodermal cell fates in *Ciona*'s b-line lineage, highlighting key similarities and differences in posterior neuromesodermal cell types between tunicates and vertebrates.

Spatial and temporal expression dynamics of a stinging cell-specific gene family using Hybridization chain reaction (HCR) RNA-FISH

Anna M. L. Klompen[1], Jenny K. Duong[1], Mary Cathleen McKinney[1], Jason A. Morrison[1], Jose E. Javier[1], Kevin Ferro[1], Shiyuan Chen[1], Kate Hall[1], Allison Scott[1], Kaitlyn Petentler[1], Lacey Ellington[1], Anoja G. Perera[1], Zulin Yu1, Mattew C. Gibson[1]

[1]Stowers Institute for Medical Research, Kansas City, MO, USA

The secretory organelles of cnidarian stinging cells, called cnidae, are arguably one of the most complex cellular structures in the animal kingdom. Two distinct categories of cnidae include nematocysts, with ~25 different morphologies across all cnidarians, and ensnaring spirocysts that are exclusive to sea anemones (Anthozoa: Actiniaria). A critical discovery in the 'omics age was a cnidarian-specific set of proteins called minicollagens, which appear to be a core structural products in cnidae capsules and tubules. An ongoing question in cnidarian biology is identifying genes specific to distinct cnidae type (e.g. nematocyst vs. spirocyst), which is further coupled by an assumption that higher numbers of expressed minicollagens correlates to greater cnidae diversity, implying cell-type specificity. The starlet sea anemone *Nematostella vectensis* displays four morphological types of cnidae: three types of nematocysts (mastigophores, small and large isorhizae) as well as spirocysts. Previous molecular characterizations of *Nematostella* minicollagens suggests some degree of cell-specific expression, however these studies only focused on a subset of minicollagens. Here, we used hybridization chain reaction fluorescent *in-situ* hybridization (HCR RNA-FISH) to visually explore the expression patterns of all six endogenous minicollagen genes in *Nematostella*, including three previously studied minicollagens and three uncharacterized minicollagens. We found four *minicollagen* genes were expressed throughout the ectoderm of budding polyps, as expected though with variable overlap patterns, but two were restricted to the newly-formed tentacles. In combination with public and newly generated scRNAseq datasets, we suggest these two genes are the first spirocyst specific minicollagens. Based on these results, we plan to integrate bulk RNA-sequencing from these isolated cell populations marked with each individual minicollagen using a Probe-seq approach. This work provides a foundation for future exploration into how these structurally important proteins impact the morphology and assembly of cnidae, enhancing our understanding of how cell identities emerge and diverge.

Integration of CRISPR-Cas Systems Uncovers a Critical Spatio-Temporal RNA Decay Factor Essential for Embryonic Development

Gopal Kushawah[1], Ariel Bazzini[1]

[1]Stowers Institute for Medical Research

Maternal transcripts are essential in early embryonic development, providing a foundation for zygotic genome activation. They undergo time-dependent degradation and shift the control from maternal to zygotic transcripts (MZT). Our study focuses on the unexplored aspect of spatial regulation of maternal RNA degradation, hypothesizing that both spatial and temporal control are essential for the development of complex, multicellular embryo. Investigating the regulation of dynamic spatio-temporal changes in maternal mRNA degradation, stabilization, and localization in a cell-specific manner, and understanding their impact on early embryonic development, remains a topic of intense research. To unravel this, we initially identified the spatio-temporal maternal RNAs using RNA-seq, Slam-seq, and Seurat and validated their degradation patterns by RNA *in situ*. To gain deeper insights into their functional importance, we attempted to deplete maternal RNAs by creating maternal-zygotic Cas9 mutants. However, this approach led to embryonic lethality for one of the selected candidates Cth1. This poses a challenge to validate our hypothesis, therefore we turned to CRISPR-Cas13d to specifically deplete these spatio-temporal RNAs, which resulted in specific spatial phenotypes. RNA-seq of Cas13d knockdowns of Cth1 reveals its indispensable function in gamete development evidenced by F0 Cth1 mutants' inability to fertilize as they failed to produce fertile mature gametes. Additionally, our RNA reporter assays indicate that cis-regulatory elements in the Cth1 3'UTR are responsible for spatio-temporal RNA dynamics during embryogenesis. Single-cell RNA-Seq reveals that Cth1 potential targets exhibit mutually exclusive spatio-temporal patterns during development. Cas13d-mediated Cth1 knockdown causes an early onset of accumulation of these transcripts during zygotic genome activation, suggesting Cth1 negatively regulates their targets transcripts levels during early embryonic development. Finally, our reporter assay suggests that potential targets' 3'UTRs become more stabilized upon Cth1 knockdown, suggesting Cth1 regulates potential targets level by destabilizing their 3'UTRs.

Segregation between an ornamental and a disease driver gene provides insights into pigment cell regulation

Yuan Lu[1]

[1]XGSC-Texas State University

Genetic interactions are adaptive within a species. Hybridization can disrupt such species-specific genetic interactions and creates novel interactions that alter the hybrid progeny overall fitness. Hybrid incompatibility, which refers to degenerative genetic interactions that decrease the overall hybrid survival and sterility, is one of the results from combining two diverged genomes in hybrids. The discovery of spontaneous lethal tumorigenesis and underlying genetic interactions in select hybrids between diverged *Xiphophorus* species showed that lethal pathological process can result from degenerative genetic interactions. Such genetic interactions leading to lethal phenotype are thought to shield gene flow between diverged species. However, hybrids between certain *Xiphophorus* species do not develop such tumors. Here we report the identification of a locus residing in the genome of one *Xiphophorus* species that represses an oncogene from a different species.
Our finding provides insights into normal and pathological pigment cell development, regulation and a molecular mechanism in hybrid incompatibility.

Phenotypic characterization of genes with pathogenic splice disrupting variants associated with rare pediatric disorders of brain and craniofacial development

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Background.
Most craniofacial and neurodevelopmental disorders are rare and of unknown etiology, partly because many genetic variants occur in noncoding regions with unclear functional consequences or in genes not previously associated with diseases. Consequently, it takes an average of 6-8 years for an affected individual to receive a definitive genetic diagnosis. Using the "Genomic Answers for Kids" pediatric data repository from The Children's Mercy Research Institute, we have begun profiling the molecular consequences of disease-associated intronic variants, which can lead to abnormal RNA splicing and disease, and then validating their phenotypic effects using zebrafish and patient-derived organoid models. We identified a high prevalence of seizures among undiagnosed rare disease patients and created a catalog of 4,589 intronic variants in 347 genes that affect RNA splicing.

Results.
To confirm the disease association of some of these genes, we disrupted *Arid1b, Arid2, Ankrd11, Dcc, Gli3, Pbx1, Ppp3ca, Reln, Setbp1, Sf3b4, Tnrc6a* and *Wdfy* function in zebrafish embryos using CRISPR-Cas9 mediated gene editing and assessed their neural and craniofacial development. All crispant embryos exhibited developmental delay by 24 hours post fertilization (hpf), and abnormal movement, including slower acceleration, lower maximum speed, and less directional swimming at 48 hpf. Most crispants manifest with fewer oligodendrocyte precursor cells at 5 days post-fertilization (dpf). *Ankrd11* and *Gli3* crispants had smaller brain size and increased neuronal activation as marked by pERK. *Ankrd11* and *Sf3b4* crispants also exhibited craniofacial anomalies, as a consequence of widespread cell death and fewer neural crest cells at 24 hpf, synchronously with abnormal bone and cartilage development at 5 dpf.

Conclusion.
These novel mutant zebrafish in parallel with patient-derived organoids model the effects of rare intronic variants on the function of genes associated with disease phenotypes and further our understanding of pediatric disease etiology and pathogenesis.

Ribosome biogenesis is essential for neural crest cell differentiation

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Neural crest cells (NCCs) are a migratory cell population specified during early embryogenesis that generate cartilage, bone, and connective tissue as well as neurons, glia and melanocytes in the head and face. Previous work in the lab has shown that RNA Polymerase I (Pol I)-mediated ribosomal RNA (rRNA) transcription, a rate limiting step in ribosome biogenesis, is elevated in NCCs compared to other tissues. Disruption of Pol I function in mice leads to increased pre-migratory and migratory NCC death, resulting in hypoplastic frontonasal prominences and pharyngeal arches, and consequently cranioskeletal hypoplasia. Emerging evidence also supports the tight regulation of ribosome biogenesis in differentiating cells, suggesting post-migratory roles during NCC differentiation.

To examine the importance of ribosome biogenesis in later stages of craniofacial development, we deleted *Polr1a*, which forms part of the catalytic core of Pol I, with tamoxifen-inducible Cre^{ERT2} at E9.5-E13.5, timepoints during which NCCs begin to differentiate. *Polr1a* mutant embryos exhibited craniofacial anomalies at E18.5 when gene excision took place at E10.5. To determine the mechanisms driving these changes, single-cell RNA sequencing and bulk proteomics were performed on dissected craniofacial tissues at E12.5, during the early onset of the phenotype. Transcriptomic results revealed significantly altered cell type proportions in the mutants, with reductions in mesenchymal cartilage and bone progenitors, and increases in neuronal progenitor populations. Spatial transcriptomic data in wild type mice at E12.5 determined that the most affected mesenchymal progenitor cell types, marked by *Satb2*, were specifically located in the anatomical structures phenotypically affected in *Polr1a* mutants. Differential protein analysis also revealed impaired cartilage as well as muscle development, potentially in association with metabolic changes in mutant tissues. Together, these results support a specific requirement for ribosome biogenesis in NCCs during their differentiation phase of craniofacial development.

Endocytic receptor LRP2: a new role in murine neural crest dynamics

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Vertebrate head formation encompasses successful neural tube closure and neural crest development, which ultimately give rise to the functional brain and craniofacial structures, respectively. These two processes take place during early embryonic development and involve distinct molecular events. Neural crest cells (NCCs) are multipotent, transient population of cells that originate from the neural plate border (NPB). Among different NCC subtypes, cranial NCCs (CNCCs) contribute to majority of the craniofacial features within a living organism. Low-density lipoprotein receptor-related protein 2 (LRP2) is an endocytic receptor that localises on the apical surface of neuroepithelium and plays a pivotal role during early embryonic development by mediating the internalisation of various ligands, including folate. Additionally, LRP2 regulates the subapical scaffold complex formation within the neuroepithelial cells, where it helps to ensure a proper cellular integrity during embryonic morphogenesis.

Herein, we detected LRP2 expression in the early NCC populations which prompted us to explore the role of LRP2 during NCC development. Severe craniofacial anomalies in *Lrp2*$^{-/-}$ mice are indicative of dysregulated NCC behaviours. Further analyses revealed a folate-deficient state induced by the functional loss of LRP2 that impaired cell fate decision making during NPB specification, leading to an abnormal expansion of the NPB and the subsequent pre-migratory as well as migratory NCC territories. Through time-lapse imaging, we demonstrated that murine NCCs exhibited contact inhibition of locomotion (CIL) behaviour during directed collective migration *ex vivo*. In particular, CIL behaviour during directed migration of CNCCs *ex vivo* required the function of LRP2. Finally, this study has uncovered several potential candidates that inform the possible molecular mechanisms in which LRP2 governs neural crest development using spatial proteomics approach.
Altogether, we propose LRP2 as a central regulator of vertebrate cranial morphogenesis, essential for not only embryonic brain development but also craniofacial framework formation through the regulation of NCC dynamics.

Context-dependent Interactions Between Immune Cells and Sensory Hair Cell Regeneration Programs

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Tissue regeneration following injury requires the concerted response of multiple cell types, including immune cells and stem cells. The cellular and molecular components of regeneration programs can be affected not only by the severity of the injury, tissue identity, but also the type of cell death. Determining the individual contribution of these parameters to regeneration programs has remained challenging, largely due to the variable nature of mechanical injury paradigms. Here, we established a comparative approach of regeneration programs to induce either necrosis or apoptosis in zebrafish lateral line hair cells (HCs), respectively. Keeping the identity and quantity of the ablated cells consistent, this approach allows us to specifically interrogate the influence of the cell death modality on regeneration. High resolution live imaging allowed us to visualize the rapid recruitment of tissue resident macrophages to the site of cell death, uncovering intricate differences in their phagocytic behavior depending on the cell death modality. Single-cell RNA sequencing revealed that these cellular differences were accompanied by distinct transcriptional signatures in both phagocytosing macrophages and lateral line support cells. While HC necrosis triggered a robust injury response in support cells, it was greatly diminished following apoptosis. Despite these differences in the early response to injury, both paradigms eventually converge on similar genes involved in hair cell regeneration. Lastly, blocking recruitment of immune cells using a dominant negative approach not only increased injury response gene expression in neuromast support cells but also injury-induced proliferation of support cells in response to hair cell apoptosis. In sum, our data provide evidence for distinct molecular and cellular responses to different cell death modalities in a regenerating sensory organ. Taken together, this study provides insights into the context-dependent nature of regeneration programs.

Cyclooxygenase-2: Essential Insights into Craniofacial Development

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Background.
Cyclooxygenase-2 (COX-2) is traditionally recognized for its role in inflammatory processes, yet its involvement in embryonic development, particularly in craniofacial morphogenesis, is unexplored. Craniofacial development is a complex process driven by intricate signaling pathways and cellular interactions, especially involving cranial neural crest cells (CNCCs) that contribute to the formation of facial structures such as the jaw, palate, and teeth. This study aims to elucidate the role of COX-2 in craniofacial development by exploring its expression patterns, functional significance, and impact on key developmental genes and pathways such as WNT, BMP and FGF during embryogenesis.

Results.
Our investigation revealed that COX-2 is expressed in the craniofacial region during early embryogenesis, particularly in neural plate borders that give rise to neural crest cells. At later stages, COX-2 is expressed in the mandibular arch and other NCC-derived structures. Immunolocalization studies confirmed COX-2 expression in critical craniofacial regions, especially within neural crest mesenchyme and surrounding tissues. To assess the functional role of COX-2, we inhibited its activity using Etoricoxib in chick embryos, leading to severe craniofacial defects, including micrognathia, optic cup malformation, and disrupted mandibular and maxillary development. COX-2 inhibition downregulated crucial developmental genes like PAX6, FGF8, BMP7, and MSX1, essential for normal craniofacial morphogenesis, and disrupted downstream signaling molecules, resulting in aberrant craniofacial development.

Conclusion.
The findings show that COX-2 is crucial for craniofacial development, regulating key signaling pathways and gene expression in neural crest cells and their derivatives. Reduced COX-2 activity leads to significant craniofacial malformations, emphasizing its role in facial morphogenesis. These insights enhance our understanding of the molecular mechanisms in craniofacial development and suggest COX-2 as a potential target for treating congenital craniofacial disorders.

The Hippo pathway regulates the cytoskeleton and the shape of multicellular structures in a close unicellular relative of animals

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Much remains to be understood about how developmental signaling pathways emerge and change over evolutionary time. The Hippo pathway is a conserved signal transduction pathway that regulates tissue size in animals by controlling cell proliferation. Remarkably, the Hippo pathway is conserved in some very close unicellular relatives of animals. Characterizing the functions of this pathway in these unicellular organisms could elucidate the ancestral function(s) of Hippo signaling and reveal novel properties of this biomedically important developmental pathway. To this end, we have developed genetic tools in *Capsaspora owczarzaki*, a close unicellular relative of animals that displays a simple form of aggregative multicellularity. We found that in *Capsaspora*, mutation of Hippo pathway components does not significantly affect proliferation, but instead affects cytoskeletal dynamics and the morphogenesis of multicellular aggregates. Transcriptomic analysis by RNA-seq revealed that conserved structural components of the cytoskeleton are regulated by the Hippo pathway in *Capsaspora*. Genetic perturbation of these cytoskeletal components phenocopies mutation of the key Hippo pathway transcriptional regulator Yorkie, indicating that Hippo signaling in *Capsaspora* affects multicellular shape through transcriptional regulation of structural components of the cytoskeleton. Together, our results suggest that the Hippo pathway emerged in unicellular organisms and functioned to regulate cytoskeletal structural dynamics, and was subsequently co-opted to regulate proliferation and tissue size in animals while still retaining the function of cytoskeletal regulation.

Elucidating the cellular and molecular mechanisms of segmentation in Nematostella body plan: the role of ECM

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Segmented, metameric body plans are observed throughout animals, allowing for the diversification of different axial regions of the bodyplan. In vertebrates, metameric subdivisions of the anterior-posterior axis are created by the process of somitogenesis, where the extracellular matrix (ECM) plays an important role in the maintenance of somite boundaries. Nevertheless, the precise mechanisms by which segments are formed and maintained in diverse animal phyla remains poorly understood. Here we use the sea anemone *Nematostella vectensis* to elucidate the role of ECM during the segmentation of cnidarian endo-mesoderm, a process which generates 8 bilaterally-arranged segments. We focussed our analysis on two ECM factors that are expressed in developing segments: Laminin and the Thrombospondin protein Tspear. Indicating a clear role for ECM in the establishment or maintenance of segments, knockdown of either of these ECM genes disrupted segment mophogenesis. Consistent with its role as an essential ECM component in cell-matrix adhesion, *Laminin* knockdown completely abolished formation of all segments. In contrast, *Tspear* was expressed at the segment boundaries at planula stage and its knockdown led to the fusion of specific segments. Overall, our data indicate that Laminin and Tspear participate in the formation and the maintenance of boundaries during endomesodermal segmentation and provide new insights into the role of ECM in cnidarian evolution and morphogenesis. Further, these results provide intriguing evidence for a possible evolutionary homology between vertebrate somites and cnidarian segments.

Sex without chromosomes: sex-biased gene expression during gametogenesis in *Platynereis dumerilii*

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The segmented worm Platynereis dumerilii is a gonochoric species, meaning individuals are distinctly male (sperm producers) or female (oocyte producers). However, the mechanisms driving sex differentiation remain unknown in *P. dumerilii*. This species lacks sex chromosomes (such as X/Y), making karyotyping ineffective for sex identification. Additionally, the environmental factors influencing sex differentiation are unclear, with lab cultures consistently showing a 1:1 male-to-female ratio. Since sex is defined by the production of different gamete types, studying gametogenesis can reveal early molecular changes in gamete progenitors, i.e. germ cells. To investigate sex differentiation in *P. dumerilii*, we focused on germ cells and examined sex-biased gene expression using bulk RNA sequencing. We surgically isolated germ cells from juveniles at various developmental stages and processed these samples for RNA sequencing. Our analysis identified genes with sex-biased expression, particularly during meiotic stages. Among these were genes involved in sex-related pathways conserved across metazoans, including the doublesex and mab-3 related transcription factor 1 (*dmrt1*), which was upregulated in males. Notably, we discovered a methyltransferase gene upregulated in females. This methyltransferase gene was annotated as a novel gene unique to protostomes, which we named *psmt* (protostome-specific methyltransferase). We also found several novel genes representing long non- coding RNAs. These findings offer new insights into the genetic mechanisms governing sex differentiation in segmented worms and provides a foundational resource for germ cell research in *P. dumerilii*.

Fat cadherin cleavage releases a transcriptionally active nuclear fragment to regulate target gene expression

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The conserved atypical cadherin fat (*ft*) controls cellular processes such as growth, planar cell polarity, and mitochondrial function, in organisms ranging from fruit flies to mammals. The intracellular domain of the Ft protein, FtICD, binds to and regulates a variety of partners to execute these functions. We have found that FtICD is present in the nucleus in tissue culture cells as well as in embryonic and larval tissues, and have identified nuclear localization and nuclear export signals in FtICD required for this localization. A membrane-bound version of FtICD, fused to a Gal4VP16 transactivation domain, is able to activate a reporter construct in imaginal discs, demonstrating that FtICD can be cleaved and enter the nucleus *in vivo*. To determine if Ft regulates transcription we used CRISPR to endogenously label Ft, and conducted ChIP-Seq. Ft target genes identified through ChIP experiments on *Drosophila* embryonic and larval tissues include genes involved in signaling pathways including the Hippo pathway, chromatin organization, pattern formation, neural development and others. A subset of these genes are differentially regulated in *ft* mutants and/or animals overexpressing FtICD, as determined by RNAseq experiments. Ft ChIP peaks are frequently found to overlap with previously published Yorkie (yki) and Trithorax-like) (Trl, a.k.a. GAF) peaks, as well as sometimes with Scalloped (sd) peaks, suggesting that Ft may act in conjunction with these factors to regulate gene expression. Supporting this hypothesis, we found that Ft can physically interact with both Yki and Sd in co-immunoprecipitation experiments in S2 cells. We propose that the modulation of Hippo pathway activity constitutes one of the nuclear functions of Ft, complementing its established function as an upstream regulator of Hippo signaling.

Quantification of chromatin ultrastructure in mouse embryos and human cells

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Cell identity is defined at the level of chromatin, which is fundamentally regulated by its structure. To recapitulate and manipulate diverse cell states, it is thus critical to define the mechanisms that shape chromatin structure. Our current understanding of this regulation is however limited by the lack of methodologies capable of probing chromatin structure at the base unit in which it is remodeled, the nucleosome. To overcome this, we have developed ChromGEM (Chromatin Gold Electron Microscopy), a method which combines a new strategy for endogenous protein labeling with chromatin tomography to enable nucleosome scale chromatin tracing in the context of cell and epigenetic state. We apply our technique to quantify changes in chromatin structure as cells differentiate after fertilisation in the mouse embryo. We show that states of developmental potential and epigenetic environments are defined by distinct ultrastructural properties that differ between in vitro and in vivo systems, as well as mouse and human stem cells. We further leverage this approach to investigate the ultrastructural basis for silencing of pluripotency genes in the trophectoderm, and mechanistically link changes in chromatin structure to the upregulation of Lamin A/C. We thus define the ultrastructural properties underlying mouse and human stem cells during differentiation and development and develop an approach that allows chromatin structure to be holistically quantified *in situ* and related to cell and epigenetic state.

Left-right patterning in veiled chameleon (*Chamaeleo calyptratus*), and insights from the genome into its evolution in amniotes

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Although squamates (lizards and snakes) define the largest order of reptiles, little is known about their early development, since at the time of egg laying, most squamate embryos are well into organogenesis, precluding the study of early processes like gastrulation and left-right (L-R) patterning. Veiled chameleons (*Chamaeleo calyptratus*) breed well in captivity, with pre-gastrulation embryos at oviposition. I have shown that chameleons lack motile cilia in their left-right organizer, which is a synapomorphy of all reptiles. This motile cilia- independent mode of establishing L-R asymmetry differs from most deuterostomes, which use motile cilia to establish L-R asymmetry. Moreover, unlike chickens, geckos and turtles, chameleons retained both paralogs of *Nodal*, a critical regulator of L-R patterning. Both *Nodal* genes are expressed in the left lateral plate mesoderm, indicating their likely involvement in L-R patterning. Through live imaging I observed a leftward tilt in the posterior neural plate hinge point, and this morphological L-R asymmetry precedes, and likely triggers, asymmetric expression of the Nodal cascade. In parallel, we recently sequenced, assembled and annotated the veiled chameleon genome. My analysis of different genomic regions has revealed both ancestral paralogs of the *Nodal* gene, but loss of *Dand5* and several other genes, likely in conjunction with loss of motile cilia during left-right patterning in reptiles. Our studies in chameleons therefore provide novel insights into the evolution of squamate genomes and the molecular mechanisms driving early development.

Streamlined RfxCas13d mRNA targeting in zebrafish embryos supports non-essential roles for individual, maternally encoded microproteins

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Zebrafish is an outstanding model for interrogating developmental vertebrate gene function(s) due to its genetic tractability and synchronous, external embryogenesis. After fertilization, the next critical step is the switch in developmental control from maternally provided to zygotically produced factors—known as the maternal-to- zygotic transition (MZT). Loss-of-function approaches to elucidate gene function during zebrafish MZT have been hindered by three key barriers: 1) maternally provided messenger RNAs (mRNAs) can mask the effects of mutations in the offspring alone (from either heterozygous parents or in F0 "CRISPRants"), 2) maternal-zygotic mutant generation is tedious, and 3) established, morpholino-based mRNA targeting can elicit toxicity and off-target effects. To overcome these barriers, our lab has established the CRISPR-RfxCas13d system that targets and degrades mRNA, including maternal mRNA. In this work, we are enhancing the throughput of RfxCas13d-based loss-of-function analysis during zebrafish MZT through 1) cellpose-based segmentation and staging of 2–6 hours post-fertilization embryos, 2) pre-assessment of RfxCas13d gRNAs to remove those with non-target, 28S rRNA cleavage activity, and 3) implementation of miniaturized bulk RNA-seq to gain transcriptomic insights at the cost of RT-qPCR. We are leveraging this streamlined RfxCas13d pipeline to assess the necessity of 31 maternally provided mRNAs that encode short proteins (< 100 amino acids) called *microproteins*. RfxCas13d targeting reduces mRNA levels by >50% (by RT-qPCR) for the majority of the 27 currently targeted microproteins, with negligible organismal effects observed through 24 hpf. Future work will assess the necessity of the remaining microprotein mRNAs and test for transcriptomic phenotypes in microprotein knockdowns. Overall, this work is improving the accessibility of the RfxCas13d system and bolstering our capacity for both organismal and molecular analysis during zebrafish MZT.

Guiding Morphogenesis using Synthetic Organizer Cells

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Over the past decade, remarkable progress has been made in driving the differentiation of pluripotent cells in vitro. However, this process relies primarily on treating progenitor cells with media-borne morphogens, lacking the native-like spatial information found *in vivo*. A key missing feature in many *in vitro* developmental systems is the presence of spatially asymmetric microenvironmental signals, such as those normally provided by natural developmental niches or signaling centers, such as the classic Spemann-Mangold organizer.

Here, we demonstrate the ability to program a fibroblast cell line to form "synthetic organizer" cells. By using a toolkit of native and synthetic cell adhesion molecules, we can now program cells to self-assemble around an embryoid – a 3D aggregate of mESCs – into diverse, customizable architectures. We then harness these architectures to express instructive morphogen signals in specific, spatially asymmetric patterns. Expressing the morphogen WNT3A and its antagonist DKK1 in different organizer architectures generates a set of systematically varied morphogen activity gradients, allowing us to explore how subtle changes in these gradient guides the embryoid toward distinct outcomes. These gradients were strongly correlated with morphogenetic outcomes as the range of minimum-maximum WNT activity determined the resulting range of Anterior-Posterior (A-P) axis cell lineages. Strikingly, shallow WNT activity gradients, despite showing truncated A-P lineages, yielded higher- resolution tissue morphologies, such as a beating, chambered cardiac-like structure associated with an endothelial network. Thus, synthetic organizer cells, which integrate spatial, temporal and biochemical information, provide a powerful and flexible tool to systematically direct the development of ESCs or other progenitor cells along distinct trajectories within the morphogenetic landscape.

Spatial Transcriptomics Reveals Proximo-distally Patterned Signaling Centers In Regenerating Lizard Tail Blastemas

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A branch of regenerative medicine looks to non-mammalian animals for clues to improve regeneration in human patients. Lizards are the only living amniote that form blastemas with multi-lineage differentiation capabilities as adults and present an ideal case study to determine drivers of appendage regeneration.

Immune cells, including phagocytes, play a significant role in the outcome of the regeneration process. Treatment with clodronate liposomes depletes phagocyte populations and inhibits blastema formation, resulting in loss of tail regeneration. Previous studies from our lab have also described fibroblasts as important regulators of tail regrowth. In this study, we test the roles of blastema phagocyte and fibroblast subpopulations to act as signaling centers during lizard tail regeneration.

To investigate this hypothesis, I analyzed tail samples collected from lizards (*Anolis carolinensis*) that had been treated with clodronate liposomes or control PBS liposomes. Tail samples were collected 14 days post- amputation (14 DPA) and 19 DPA, which correspond with early and late blastema stages, respectively. Having performed spatial transcriptomics sequencing on the samples using the 10x Genomics ST platform, I determined 12 distinct cell types across the samples. I observed one cluster unique to the day 14 blastema stage, indicating its identity as a regenerative fibroblast cluster, and I determined the genes expressed by this putative signaling center.

I validated candidate pro-regenerative genes via in-situ hybridization and showed that genes MSX1 and WNT16 localized to blastema tips, the location of putative signaling centers. By running CellChat in RStudio, we determined that the WNT signaling pathway, which is known to be involved with cell patterning and proliferation, played a significant role in maintaining MSX1-positive cell populations associated with signaling centers at blastema tips. Our future research aims involve investigating the mechanism by which MSX1-positive cells propagate their regenerative signal.

The human ciliopathy protein RSG1/CPLANE2 links the CPLANE complex to transition zone assembly

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Cilia are microtubule-based projections essential for development and homeostasis of nearly every organ system in the vertebrate body. The dysfunction of cilia in mammals leads to various diseases, known as ciliopathies. Ciliogenesis, the process of cilia formation, is a strict spatiotemporal process that requires dozens of proteins for its regulation, including the Ciliogenesis and Planar polarity Effector (CPLANE) protein complex. All CPLANE subunits except Rsg1 have been linked to human ciliopathies and here we describe the first human allelic variants of Rsg1/CPLANE2 associated with disease. The human patients present with similar rare orofacial anomalies and polydactyly previously reported for other subunits of CPLANE. Through *in vivo* cell biology we identified the etiology of these variants and using proteomics, we also identified novel Rsg1/CPLANE2 interactors.
Finally, super-resolution imaging reveals a role for CPLANE and its interactors in tuning the normal structure of the ciliary transition zone. This work will expand our understanding of the mechanisms involved in rare genetic diseases detrimental to human health. NIH:F31DE033290

The *Hydractinia* cell atlas reveals cellular and molecular principles of cnidarian coloniality

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Coloniality is a widespread growth form in cnidarians, tunicates, and bryozoans, among others. Despite being modular, composed of multiple zooids and supporting tissues, colonies function as a single physiological unit. A major question in the biology of colonies is the cellular mechanism of generating structurally and functionally distinct colony parts. The cnidarian *Hydractinia* establishes colonies with different types of zooids (polyps), interconnected by a gastrovascular system that is attached to the substrate and known as stolons. We obtained single cell transcriptomic profiles of ~200K *Hydractinia* cells, including isolated stolons and two polyp types. We characterised the major *Hydractinia* cell types and quantified their abundance across colony parts. Overall, we find that distinct colony parts are characterised primarily by distinct combinations of shared cell types and to a lesser extent by part- specific cell types. Therefore, we propose that both cell type combinations, as well as rarer cell type innovations, have been the main mechanism in the evolution of coloniality in cnidarians. We identified cell type-specific transcription factors (TFs) and gene networks expressed within these cell types. Notably, we discovered a previously unidentified, stolon-specific cell type, which expresses enzymes related to biomineralization and chitin synthesis, reminiscent of molluscan shell matrix proteins that may represent a crucial adaptation to the animal's habitat. An additional epithelial cell type capable of toxin production and allorecognition was also identified. In summary, the *Hydractinia* cell atlas elucidates the fundamental cellular and molecular mechanisms underlying coloniality.

Insights into Inner Ear Melanocytes: A Comparison of CreER Lines

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Background.
Stria vascularis, a multilayered highly vascularized epithelium located on the lateral wall of the cochlea, is comprised of three types of cells: marginal cells, intermediate cells, and basal cells, each derived from distinct embryonic origin. Marginal cells origin from otic epithelium (Sagara et al., 1995). Intermediate cells are cochlear melanocytes derived from the neural crest (Cable et al.,1992), Basal cells originate from otic mesenchyme and are adjacent to fibrocytes and spiral ligament (Trowe et al., 2011, Wangeman P., 2002). These cells function collectively in a synergistic manner to pump potassium ions into the scala media, generating a positive endocochlear potential. A defect in any of these cell types leads to the dysregulation of the ionic composition of the endolymph, ultimately resulting in deafness.

Surprisingly, to date, there is an absence of Cre driver mouse lines that effectively facilitate the study of individual cell functions within the stria vascularis. Furthermore, the use of CreER transgenic mice runs the risk of poor recombination or non-specific expression. Therefore, the present study aims to determine the most appropriate line that targets the intermediate cells based on the development of melanoblast.

Results.
We observed that Pax3-CreER, DCT-CreER and Tyr-CreER successfully target the intermediate cells at three different stages: E11.5 (early development of otocyst), P0 (maturation of cochlea) and P28 (adult stage). However, the recombination was non-specific at P0 of Tyr-CreER mice line as the modiolus region showed some recombination. A consistent recombination was observed in DCT and Pax3 Cre ER x Ai9 mice line in all three developmental stages.

Conclusion.
Our study reveals that both Pax3-CreER and Dct-CreER mouse lines exhibit efficient recombination in intermediate cells. Among these, the Pax3-CreER line demonstrated particularly high specificity and reliability for tamoxifen-induced recombination, making it the preferred model for spatiotemporally targeting the intermediate cell populations.

Conservation of retinal cis-regulatory codes over half a billion years of evolution

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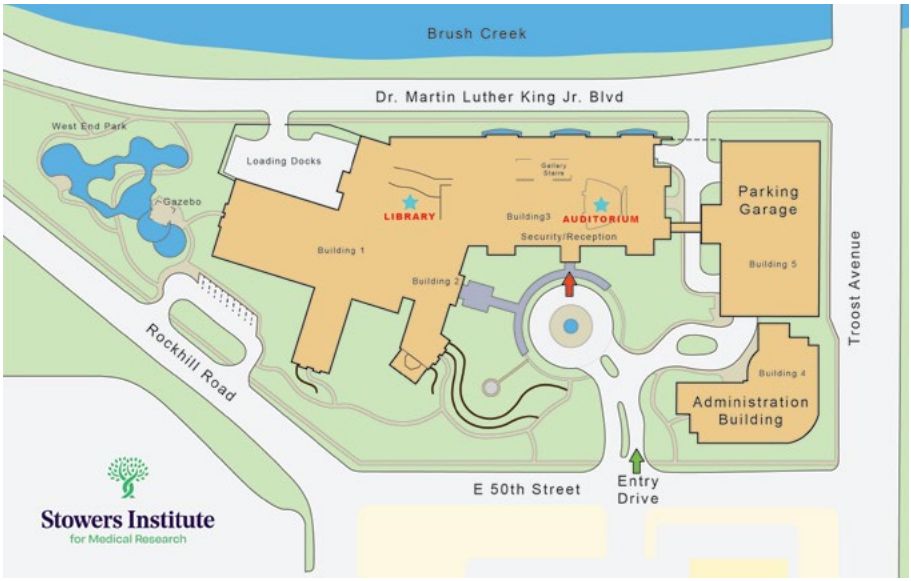
The identification of homologous cell types across species represents a crucial step in understanding cell type evolution. The retina is particularly amenable to comparative analysis because the basic morphology, connectivity, and function of its six major cell classes have remained largely invariant since the earliest stages of vertebrate evolution. Here, we show that the retina's highly conserved cellular architecture is mirrored by deep conservation of the underlying *cis*-regulatory codes that control gene expression. We use comparative single-cell chromatic accessibility analysis of lamprey, fish, bird, and mammalian retinas, which began to diverge over half a billion years ago, to demonstrate cross-species conservation of *cis*-regulatory codes in all six retinal cell classes. This conservation persists despite extensive turnover of *cis*-regulatory regions between distant species. Conservation manifests as the clustering of multiple distinct high-affinity TF (TF) binding sites toward the center of open chromatin regions with little cross-species preservation of higher-order syntax. Hierarchical clustering of machine-learning models of retinal *cis*-regulatory codes from diverse species recovers six clusters corresponding to the six retinal cell classes. Thus, the retina's cellular Bauplan is controlled by *cis*-regulatory codes which predate the divergence of extant vertebrates and persist despite nearly complete enhancer turnover.

Attendee Resources

Location: Stowers Institute for Medical Research – 1000 E. 50th Street, Kansas City, MO 64110
General Phone Number: 816-926-4000
Driving directions from Kansas City International Airport:
(approximately 30 minutes under normal driving conditions)

1. Follow the signs on International Circle to the airport exit.
2. Take Cookingham Drive to I-29 South (right ramp), heading toward Kansas City.
3. Continue to follow I-29 South as it merges into I-29 South 71 (merge in left lane), then I-35South (merge in left lane again).
4. Cross the Paseo Bridge and get in the lane for I-70 South 71 (Exit 3)
5. Merge right and follow sign to South 71 Highway (Exit 2M).
6. Take the Emmanuel Cleaver II Boulevard exit and turn right onto Emmanuel Cleaver II Boulevard.
7. Follow Cleaver II to Troost Avenue and turn left on Troost.
8. Turn right on 50th Street and take another immediate right at the Stowers Institute's entrance (1000 E. 50th Street, Kansas City, MO 64110).

Campus Map



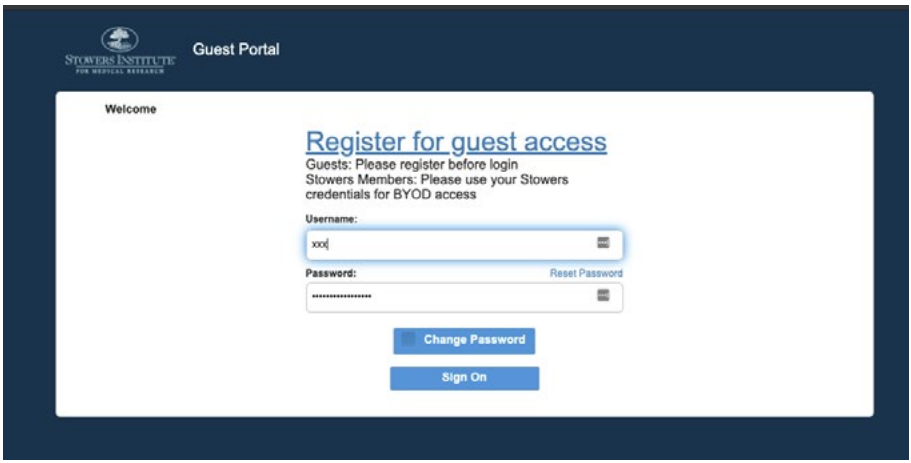
Parking: Visitor parking is available in the parking structure located between the Administration Building (to your right as you enter the campus) and the Research Building (to your left). Please park on the 5th floor of the parking garage and take the elevator down to the 1st floor to enter the Research Building.

Restrooms: Located by the two large planters on the 1st floor of the Research Building. All-gender restrooms are available at the base of the stairs on the B1 level.

Mother's Room: At the base of the stairs on the B1 level, located next to the all-gender restrooms. An entry key will be provided by security to guests that request to use it.

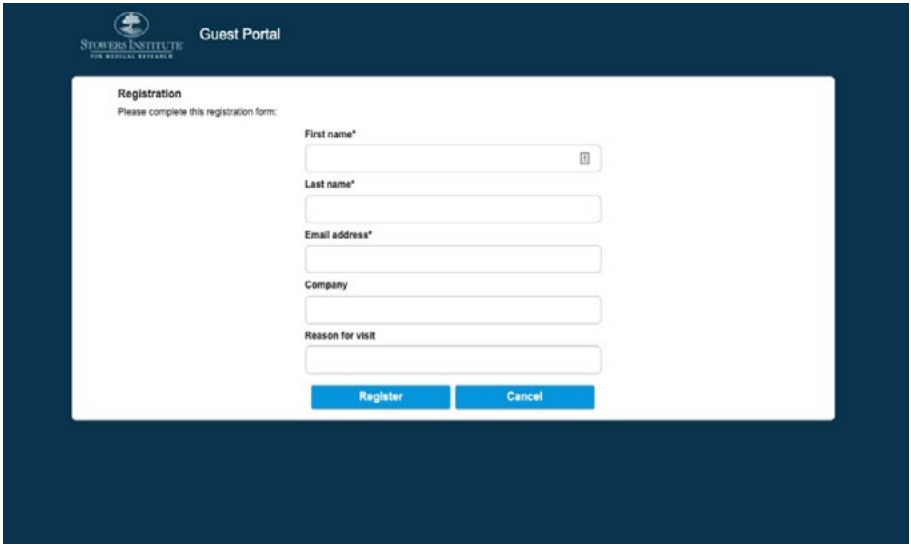
Tobacco Free Campus: Tobacco of any kind is prohibited in all the Institute facilities and on the Institute grounds. The Institute does not offer any designated smoking areas.

Wireless Access: Stowers Guests may connect to the **stowers_guest SSID**
When connected, you will see a captive portal. Click on the link at the top of the page to register for guest access.



- You will be prompted to enter their information. Name and email are the only required fields.

- After entering the required information click Register.



- You will see a page like this with sign on information. You can use the userid and password to connect other devices without going through the registration, by entering the userid and password in the first page of the captive portal.

- Click the "Sign On" button.

After clicking sign on, you will be prompted to accept the "Acceptable use Policy". After clicking accept you should now have internet access.

If you have any questions, please contact the Stowers Help Desk at 816-926-4150.

Kansas City Fun: The Kansas City area offers a wealth of cultural, educational, and entertaining opportunities to explore, many of which are inexpensive or free of charge. The following is a brief listing of local attractions, event calendars, and resources.

Useful Resources
Information about Kansas City and the surrounding area can be found at kcmo.gov or at visitkc.com. Two publications to help you discover things to do in the area are: "Insider's Guide to Kansas City" by Katie van Luchene and "Day Trips from Kansas City" by Shifra Stein. Both can be found in local bookstores.

Linda Hall Library
5109 Cherry Street, 816.363.4600
The Linda Hall Library in Kansas City is one of the world's foremost independent science research libraries. Founded in 1946 through a philanthropic bequest, it houses vast collections spanning science, engineering, and technology, serving researchers, students, and the public. Known for its international research programs and collaborations, the Library promotes lifelong learning with innovative programming, digital access, and educational resources. The Library also maintains its grounds as a public urban arboretum, offering both scholarly and community engagement opportunities.
www.lindahall.org

Nelson-Atkins Museum of Art
4525 Oak Street, 816-751-1278
Opened in 1933, the Nelson-Atkins Museum has more than 50 galleries and several period rooms. The museum's outstanding feature is its collection of Asian art. The collection of Chinese landscape paintings is one of the finest in the West, and the museum's holdings of Chinese ceramics and decorative arts are also noteworthy. Besides European paintings from the Renaissance on, the museum also has notable collections of ancient Egyptian sculpture, Japanese porcelains and lacquer, and English pottery. The E.F. Pierson Sculpture Garden was dedicated

in 1972, and the Henry Moore Sculpture Garden opened in 1989. Admission is free.
nelson-atkins.org

Kemper Museum of Contemporary Art
4420 Warwick, 816-753-5784
Founded in 1994, the Kemper Museum of Contemporary Art presents modern and contemporary art of the highest quality and significance. It collects, preserves, documents, interprets, and exhibits a growing permanent collection; develops and presents special exhibitions; and offers a variety of educational programs. Admission is always free, and the Museum serves a diverse and inclusive public population.
kemperart.org

Union Station
30 West Pershing Road, 816-460-2020
This fully restored 1914 landmark is Kansas City's most prominent destination for entertainment and cultural activities. The Station is home to a permanent rail exhibit with vintage rail cars, an interactive science center, a vibrant Theater District featuring giant screen movies and live theater, fine restaurants, unique shops, spaces for meetings and events and much more. Of course, you can still catch the train at Union Station, once again among Amtrak's busiest stops.
unionstation.org

Loose Park
Intersection: Wornall Road and 51st Street, 816-784-5300
Loose Park is one of Kansas City's most beautiful parks. The park is home to a lake, a walking path, a shelter house, Civil War markers, tennis courts, a wading pool, picnic areas and the famous Rose Garden. The Rose Garden is popular for all types of outdoor special events including theatrical performances and wedding ceremonies.

Westport
Westport is one of Kansas City's premier destinations for dining, shopping, site seeing and is the heart of the city's nightlife. Located in the midtown, Westport is just north of the Country Club Plaza and a few miles south of downtown Kansas City. Historically, Westport was built along the Santa Fe Trail as an outfitting center for wagon trains heading west. Today the area is filled with renovated and new buildings housing trendy shops, restaurants, and nightspots.

Stowers Institute

Stowers.org

Creative, innovative, fearless science

At Stowers, we aim to attract scientists who are visionary risk-takers, who seek to solve the most challenging and intractable problems in biology.

Meet the Scientists at the Stowers Institute for Medical Research
stowers.org/scientists