SCHEDULE AND ABSTRACTS

Stowers Research Conferences: Developmental Cell Biology presents a “pop up” webinar:

**EARLY CAREER SYMPOSIUM**
1-4:30PM, MONDAY MAY 4, 2020

In support of the next generation of leaders in the field.

MORE INFO:

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SCHEDULE
Pop Up Early Career Symposium
Stowers Research Conferences: Developmental Cell Biology
May 4, 2020
1:00 PM – 4:30 PM CST

Schedule:
All talks are 15 minutes followed by 5 minutes for questions

1:00 PM
Welcome and Overview

1:05 PM – 2:05 PM
Session 1: Dynamics of Lineage and Cell Identity
Martyna Lukoseviciute | University of Oxford
Making heads or tails of an embryo: differential foxd3 regulation in the cranial neural crest and neuromesodermal progenitors
Dennis de Bakker | Hubrecht Institute
Epicardial Prrx1 guides zebrafish heart regeneration
Theodora Yung | Hospital for Sick Children, University of Toronto
Establishment of gut mesenchymal niches by Hedgehog signaling for pancreatic beta cell development

2:15 PM – 3:15 PM
Session 2: Mechanism of Morphogenesis
Ashley Rich | University of Chicago
Optogenetic dissection of gastrulation in Drosophila
Abdul Malmi-Kakkada | University of Texas at Austin
From super-resolution imaging to theory: cadherin clustering drives asymmetric glassy dynamics during convergent extension
Konner Winkley | Kansas State University
Iterative and complex asymmetric divisions control cell volume differences in Ciona notochord tapering.

3:30 PM – 4:30 PM
Session 3: Modeling, Quantitation, and Tissue Engineering
Katherine Rogers | Friedrich Miescher Laboratory of the Max Planck Society
Investigating BMP-mediated patterning with in vivo optogenetics
Stephanie Lau | New York University School of Medicine
A negative feedback loop maintains optimal chemokine concentrations for directional cell migration
Alexandra Eicher | Cincinnati Children’s Hospital Medical Center
Human enteric neurons encourage the growth, patterning, and maturation of human gastric epithelium and mesenchyme

4:30 PM
Closing Comments
Making heads or tails of an embryo: differential foxd3 regulation in the cranial neural crest and neuromesodermal progenitors

Martyna Lukoseviciute1 and Tatjana Sauka-Spengler1
Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford, UK

Foxd3 transcription factor acts either as a chromatin activator or repressor to mediate both neural crest (NC) specification and NC lineage decisions. Here, we investigated auto-regulation of the foxd3 locus during early embryonic patterning and uncovered two crucial foxd3 cis-regulatory elements, one active in the cranial and the other in the caudal embryonic region. Foxd3 directly regulates these elements bimodally, activating the cranial enhancer and compacting the caudal one within the anterior territory. Thus, the caudal enhancer, although accessible during initial activation of foxd3 in the early embryo, is later only maintained posteriorly. By exploiting new transgenic lines reporting both enhancer activities using imaging and single-cell -omics, we have uncovered that unlike cranial foxd3 enhancer active in the NC, the caudal foxd3 enhancer labelled bipotent neuromesodermal progenitors (NMp) and their derivatives. In the NMp pool, foxd3 is downregulated to minimal levels by a negative autoregulatory loop and maintained by a different set of NMp-specific enhancers. By integrating single-cell transcriptional and epigenomic profiles we reconstructed the global gene regulatory network underlying NMp specification in anamniotes, where NMp contribution to posterior axis extension was previously debatable. We show, both in vivo and ex vivo, that zebrafish embryos maintain a bipotent tailbud NMp pool after gastrulation, capable of depositing mesodermal and neural progenitors in the developing tail. Strikingly, we also identify NMp-derived neural cells expressing bona fide NC genes, suggesting shared features of trunk NC and NMp programmes and NMp contribution to NC derivatives in vivo. Bimodal autoregulation of foxd3 via distinct enhancers, one controlling high foxd3 expression required for cranial NC specification but repressed posteriorly in the specifying NMps, and the other active in the early embryo but later maintained only in the NMps, suggests common embryonic origin of at least a portion of NC and NM progenitors.

Session Theme: Dynamics of Lineage & Cellular Identity

Presenting Author: Martyna Lukoseviciute

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Consider for Talk? Yes
Epicardial Prrx1 guides zebrafish heart regeneration

Dennis E M de Bakker1, Mara Bouwman1, Esther Dronkers2, Anke Smits2, Jeroen Bakkers1 1Hubrecht institute, Utrecht, the Netherlands 2Leiden University Medical Center, Leiden, the Netherlands

The mammalian heart is unable to replace lost cardiomyocytes after ischemic injury, instead it forms an extensive and permanent scar. In contrast, zebrafish can regenerate lost myocardium by re-entry into the cell cycle of adult cardiomyocytes which replace the scar tissue. Understanding why the zebrafish regenerative response differs from the reparative response in mammals might lead to novel targets for the stimulation of mammalian heart regeneration. Expression of the transcription factor Prrx1b is strongly induced in the zebrafish heart upon cryoinjury. Knock-out mutants for prrx1b displayed reduced cardiomyocyte proliferation and impaired scar resolution, indicating prrx1b is necessary for heart regeneration. Co-staining of Prrx1 with lineage traced epicardial cells (TCF21+) shows that Prrx1 is expressed in epicardial cells covering the wound as well as in epicardial-derived cells that invade the wound area. In prrx1b mutant zebrafish epicardial cells did not show a deviation from their wildtype siblings in terms of proliferation, migration and invasion into the injury area. However, using a single cell sequencing approach of sorted TCF21+ cells we found that epicardial cells in prrx1 mutants follow an alternative differentiation path that leads to the production of activated fibroblasts that secrete TGF ligands and collagen. In addition, we identified a specific epicardial derived fibroblast population that was absent from prrx1b mutants. The increased expression of collagen genes correlated well with an exaggerated collagen deposition in prrx1b mutants which now follows the mammalian timing of extensive scar formation. From these results we conclude that Prrx1b drives epicardial cells into pro-regenerative fibroblasts. This research could lead to insights into the limited regenerative capacity of the mammalian heart.
Establishment of gut mesenchymal niches by Hedgehog signaling for pancreatic beta cell development

Theodora Yung1,2,*, Frankie Poon2,3,*, Minggao Liang2,4, Sabrina Coquenlorge-Gallon1,2, Emily C. McGaugh2,3, Chi-chung Hui1,2, Michael D. Wilson1,2,4, M. Cristina Nostro2,3,‡, Tae-Hee Kim1,2,‡

Dynamic tissue interactions and diverse signaling pathways govern the lineage dynamics of insulin-secreting pancreatic beta cells. The dysfunction of beta cells is central to diabetes, a disorder in which the body cannot produce or properly use insulin to maintain blood glucose levels. Human Embryonic Stem Cell (hESC)-derived beta cells offer a promising therapy for diabetes. However, the efficient generation of beta cells in vitro has proven difficult, possibly due to the lack of in vivo niche signals. To define organ-specific niche signals, we isolated pancreatic and gastrointestinal mesenchymal cells for transcriptomic analyses using RNA-seq. RNA-seq analyses revealed a dramatic divergence of the stomach, pancreas, and intestinal mesenchymes during mid-development, and identified downregulated Hedgehog (Hh) signaling as distinguishing the pancreatic mesenchyme from its neighbours. Employing genetic mouse models, we demonstrated the critical nature of this downregulation, where mesenchyme-specific activation of Hh signaling via targeted loss of Hh regulators, Sufu and Spop, led to impaired beta cell development and the transformation of mesenchymal identity. However, signaling inactivation via targeted loss of Smo led to a phenotype resembling congenital malformation, annular pancreas, altogether demonstrating the necessity of precise mesenchymal Hh regulation. To investigate downstream molecular mechanisms, we performed genetic rescue experiments and ChIP-seq analyses using our Hh activation models and revealed that Hh effectors can directly bind and activate gastrointestinal niche factors such as Wnt ligands that may go on to improperly activate Wnt signaling in the pancreatic epithelium. Applying these findings in pancreatic organoid and human stem cell cultures, we indeed found that epithelial Wnt signaling activation significantly impairs beta cell differentiation. Importantly, the reciprocal inhibition of Wnt signaling led to a significant boost in beta cell generation. Ultimately, this work reveals the requirement for organ-specific regulation of stromal niche signals to guide epithelial lineage dynamics.

Session Theme: Dynamics of Lineage & Cellular Identity

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Consider for Talk? Yes
Optogenetic dissection of gastrulation in Drosophila

Ashley Rich1,2, Richard Fehon2, Michael Glotzer1

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Many morphogenetic events, including convergent extension and tube formation, require modulation of the actomyosin cytoskeleton. Ventral furrow formation in Drosophila embryos is one such morphogenetic event; it results when extracellular signals activate two transcription factors, Snail and Twist, in a subset of ventral epithelial cells. These regulators then drive the expression of multiple proteins which ultimately induce Rho1 activation, apical constriction, and invagination of cells into the embryo. These apical constrictions are anisotropic and appear coordinated, but the basis for this anisotropy and coordination is unknown. To address this and related questions, we utilized optogenetics to control Rho1 activity in the embryo. Acute Rho1 activation at the onset of gastrulation induces ectopic invaginations in both the dorsal and ventral embryonic epithelium. Rho1 activation induces apical constriction in both dorsal and ventral cells, but ventral cell constriction is stronger and more anisotropic. Strikingly, ectopic Rho1 activation induces non-cell autonomous deformations outside the activation zone only in the ventral epithelium. Thus, we demonstrate that ventral cells are specifically predisposed to respond to Rho1 activation with anisotropic and coordinated deformations. To identify the factors required for ventral cell specific behavior, we analyzed acute Rho1 activation in embryos deficient in factors required for ventral furrowing. Ventral cells depleted of RhoGEF2 exhibit anisotropic apical constriction, suggesting a molecular specialization of ventral cells beyond their ability to activate high levels of Rho1. Ventral cells lacking Twist exhibit weaker and less anisotropic apical constrictions, indicating a previously unknown role of Twist. Unlike wildtype or twist embryos, Rho1-induced deformations persist in RhoGEF2-depleted embryos. Collectively, our results demonstrate that while Rho1 is sufficient to initiate invagination throughout the embryonic epidermis, the individual cell shape changes accompanying these invaginations differ between dorsal and ventral cells. Additionally, ventral cells specifically can propagate the response to Rho1 activation outside of the zone of optogenetic activation.

Session Theme: Polarity, Morphogenesis & Tissue Architecture

Presenting Author: Ashley Rich

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Consider for Talk? Yes
FrAbstracts

**som super-resolution imaging to theory: cadherin clustering drives asymmetric glassy dynamics during convergent extension**

*Abdul Malmi-Kakkada*, Robert Huebner2, Shinuo Weng2, Sena Serakaya2, D. Thirumalai1, John B. Wallingford2 1Dept. of Chemistry, University of Texas at Austin, United States 2Dept. of Molecular Biosciences, University of Texas at Austin, United States

Convergent extension is a mode of collective cell movement driven by cell intercalation, underlying tissue elongation in nematodes, arthropods and vertebrates. Defective convergent extension is implicated in catastrophic neural tube birth defects. By combining theoretical modeling and analysis of super-resolution imaging of Xenopus laevis embryos, we show that intracellular C-cadherin (Cdh3) cis-clustering regulates the local vertex viscoelasticity at subcellular length scales. We uncover that the spatially heterogenous cadherin clustering drives asymmetric vertex dynamics in convergent extension, exhibiting features of glassy dynamics observed in non-equilibrium materials such as colloidal glasses and gels. Even as defective clustering of Cdh3 can facilitate tissue coherence in vivo, we discover that clustering of Cdh3 is crucial in the more mechanically challenging context of convergent extension.

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**Session Theme:** Modeling & Quantitative Biology

**Presenting Author:** Abdul Malmi-Kakkada

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**Consider for Talk?** Yes
Iterative and complex asymmetric divisions control cell volume differences in Ciona notochord tapering.

Konner Winkley, Spencer Ward, Wendy Reeves, Michael Veeman | Division of Biology, Kansas State University, Manhattan, KS

Many chordates have tapered notochords with progressively smaller anterior and posterior tips. The cellular mechanisms giving rise to taper, however, are largely unknown. The notochord of the invertebrate chordate Ciona is an attractive system to study these mechanisms as it consists of only 40 cells in a small embryo well suited for quantitative in toto microscopy. The Ciona notochord primordium in the early gastrula consists of an arc of 10 cells from two separate developmental lineages. These cells undergo two rounds of division oriented along the AP axis to form a flat plate, followed by mediolateral intercalation to form a single-file column of cells at tailbud stages. Previous work suggested that asymmetric division might be involved in establishing cell volume differences important for Ciona notochord tapering. Here we have used in toto imaging and 3D image analysis to quantify sibling cell volume asymmetry throughout the developing notochord. We find there are distinctive, stereotyped patterns of unequal cleavage in all bilateral pairs of notochord founder cells and their descendants. A quantitative model confirms that the observed patterns of unequal cleavage are sufficient to explain all the anterior-posterior variation in notochord cell volume. Many examples are known of cells that divide asymmetrically to give daughter cells of different fate and drastically different size. Here, by contrast, a series of subtle but iterative and finely patterned asymmetric divisions, without obvious segregation of cell fate, controls the shape of an entire organ. We have used 3D imaging of spindle position in the context of cell and tissue architecture together with a novel modeling approach to infer how these asymmetries are driven by distinct cellular mechanisms including mitotic spindle displacement, mother cell shape, and effects occurring post-anaphase that potentially involve unequal cortical contractility. We find that different combinations of these mechanisms are used in each blastomere. Additionally, inhibition of Nodal signaling reverses the direction of asymmetric division in a subset of blastomeres, largely via changes in spindle displacement. These results demonstrate a new role for asymmetric division in directly shaping a developing organ and point towards complex underlying mechanisms.

Session Theme: Polarity, Morphogenesis & Tissue Architecture

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Consider for Talk? Yes
Investigating BMP-mediated patterning with in vivo optogenetics

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Signaling molecules activate spatiotemporally diverse patterns of gene expression to coordinate embryogenesis, but how this diversity is generated is an open question. In zebrafish, a spatial BMP signaling gradient patterns the dorsal/ventral axis. We used RNA-seq to systematically identify BMP target genes. Using lightsheet microscopy and NanoString molecular barcoding, we found that BMP target genes have diverse spatiotemporal expression profiles. We then used optogenetic manipulation of BMP signaling to test models explaining this diversity. Transcriptional responses to optogenetically delivered high- and low-amplitude BMP signaling pulses indicate that spatial expression profiles are not defined by differential BMP signaling activation thresholds. Additionally, we observed negligible correlations between spatiotemporal expression and transcription kinetics in response to BMP signaling pulses. In contrast, spatial differences between BMP target genes largely collapsed when FGF and Nodal signaling were inhibited. Our results challenge the basic morphogen model and indicate that much of the diversity in BMP target gene expression is due to combinatorial input from multiple signaling pathways.

Session Theme: Modeling & Quantitative Biology

Presenting Author: Katherine W Rogers

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Consider for Talk? Yes
A negative feedback loop maintains optimal chemokine concentrations for directional cell migration

Stephanie Lau1, Anna Feitzinger1, Gayatri Venkiteswaran1, John Wang1, Stephen W. Lewellis1, Chad A. Koplinski2, Francis C. Peterson2, Brian F. Volkman2, Martin Meier-Schellersheim3, Holger Knaut1

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Throughout development, homeostasis, and disease, cells use guidance cues to navigate to their destinations. One prominent guidance cue is the chemokine CXCL12 (also known as SDF1) which binds and signals through the receptor CXCR4 to guide the migration of many cell types. Despite tissue movement and stochastic noise that can affect the degree of CXCL12 signaling, CXCL12 guides cells with great accuracy and robustness. To achieve the greatest signaling over noise, biophysical theory predicts that chemokine concentrations should be around the dissociation constant (Kd) for its receptor. We thus investigated whether CXCL12 concentrations in vivo are around the Kd for CXCR4 so that cells are sensitive to small changes in CXCL12 concentrations. Using a genetically encoded CXCL12 signaling reporter in zebrafish, we measured the endogenous Cxcl12a signaling concentrations that guide the migration of the zebrafish posterior lateral line primordium. We found that the endogenous Cxcl12a signaling concentrations are around the Kd for its signaling receptor Cxcr4b, corroborating with theory. When we changed the Kd between Cxcl12a and Cxcr4b by genetically expressing the human CXCR4 receptor in zebrafish and mutating the N-terminal Cxcl12a binding site of Cxcr4b, primordium migration is slower and less directional, suggesting that maintaining chemokine concentrations around levels where cells are sensitive to differences in concentrations is important. To investigate how CXCL12 concentrations are maintained at levels for sensitive signaling, we found that the atypical chemokine receptor ACKR3 (also known as CXCR7), which scavenges and degrades CXCL12, forms a negative feedback loop with CXCL12. Upon upregulation of Cxcl12a, Ackr3b is also upregulated. We found that this is not mediated through Cxcr4 signaling or increased transcription of ackr3b but through increased stability by phosphorylation. By mutating serine and threonine residues in the cytoplasmic tail of Ackr3b, we break the Cxcl12a negative feedback loop and this also results in less directional cell migration. Altogether, we propose how an atypical chemokine receptor can form a negative feedback loop to regulate chemokine concentrations to levels that are optimal for sensitive signaling and thus allow for accurate cell migration. We hypothesize that this could be a general mechanism to ensure robust cell migration.

Session Theme: Modeling & Quantitative Biology

Presenting Author: Stephanie Lau

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Human enteric neurons encourage the growth, patterning, and maturation of human gastric epithelium and mesenchyme

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The development of human-specific model systems provides novel avenues for patient-specific clinical care, disease modeling, and drug screening. However, many remain simple compared to their in vivo counterparts and are isolated from key support networks such as nervous, immune, and vascular systems. This research aims to engineer additional cellular complexity, in the form of neuroglial cells, into human antral gastric organoids (hAGOs) and investigate key interactions between the enteric nervous system (ENS) and the developing gastric epithelium and mesenchyme. The ENS is a network of peripheral neurons and glial cells that regulates gut motility, secretion, and blood flow. It arises from a highly migratory population of cells called neural crest cells (NCCs) that migrate along the gut tube between 4 and 7 weeks of human gestation. Despite the prevalence of known gastric enteric neuropathies, little is known about the development of the gastric ENS. Analogous to the way mesenchymal-epithelial interactions drive gastric specification, we hypothesize that the human ENS encourages the growth, patterning, and maturation of human gastric epithelium and mesenchyme. To test this, we developed a human-specific, in vitro approach using human pluripotent stem cells (hPSCs) to examine the specific roles the ENS plays during gastric development. We physically incorporated hPSC-derived NCCs into developing hAGOs to recapitulate normal gastric ENS development and examined ENS impact on gastric growth, patterning, and maturation. hPSC-derived NCCs recombined with hAGOs arrange in a mesh-like network close to the developing hAGO epithelium, differentiate into neuronal and glial subtypes, and play a role in regulating not only gastric mesenchymal patterning but overall hAGO growth. Neuronal and glial lineages made up about 25% of the cells within innervated hAGOs and differentiated into specific subtypes, such as inhibitory (nNOS), dopaminergic (TH), and sensory (CALB1) neurons. Also, innervated hAGOs have expanded mesenchyme and upregulate specific mesenchymal transcription factors. Finally, the addition of NCCs to hAGOs aided in their in vivo growth under the murine kidney capsule. In the future, we will use this human organoid system to interrogate molecular pathways that are involved in ENS-mesenchyme-epithelial cross-talk and model congenital ENS defects.

Session Theme: Self-Organization, Tissue Engineering & Organoids

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