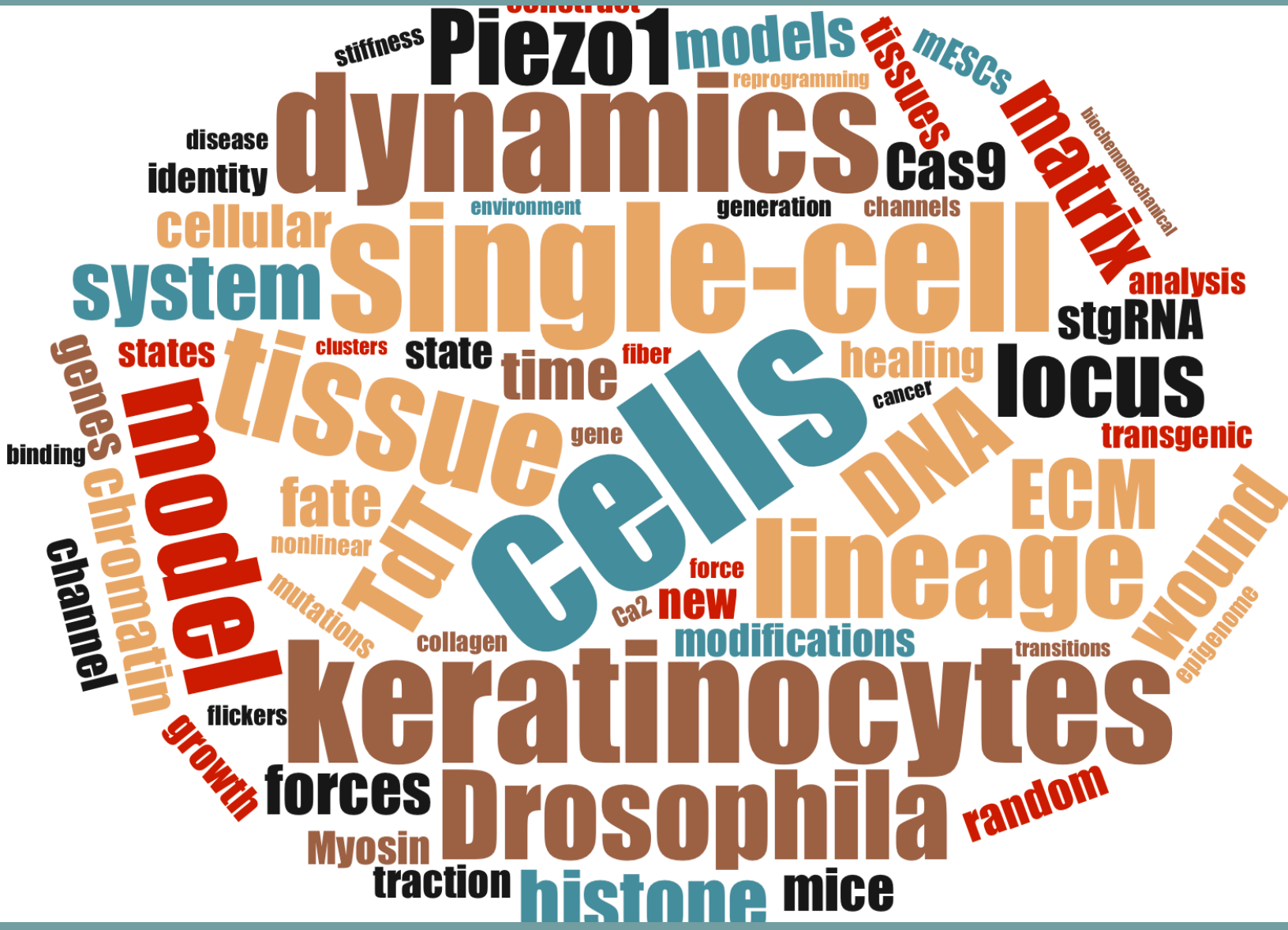
EARLY-CAREER RESEARCHER SYMPOSIUM 2019



SATURDAY, MARCH 9TH

Natural Science I

1114

PRESENTED BY :

NSF-SIMONS CENTER FOR MULTISCALE CELL FATE RESEARCH

EARLY-CAREER RESEARCHER SYMPOSIUM 2019

SCHEDULE OF EVENTS

8:30 - 8:50 — **REGISTRATION, BREAKFAST & POSTER SETUP**

8:50 - 9:00 — **INTRODUCTION**

9:00 - 11:15 — **AM SESSION TALKS**

DANIEL HAENSEL, UC IRVINE
Towards multiscale modeling: single-cell genomics and EMT regulation in cutaneous wound healing.

CHRISTINA WILCOX, UC IRVINE
Team Members: Christina Wilcox, Kate Williams, Linh Vuong

Characterizing the Transition Between Naïve and Primed States of Pluripotent ESCs in Mouse and Human.

JESSE HOLT, UC IRVINE
Team members: Jessie Holt, Wei-Zheng Zeng (Scripps)

Mechanosensing by Piezo1 in keratinocytes migration and wound healing.

MELANIE I. WORLEY, UC BERKELEY
Team members: Theresa B. Loveless, Courtney K. Carlson (UC Irvine)

Cell fate plasticity and cell lineage during regeneration in Drosophila.

CAMERON GALLIVAN, UC IRVINE
Noisy Dynamics in the Epigenome: Insights from Stochastic Modeling and Inference.

11:15 - 11:30 — **BREAK**

11:30 - 12:15 — **PANEL: APPLYING FOR FELLOWSHIPS**
Moderator: Dr. Christian Guerrero-Juarez
Dr. Mahul Chakraborty, UCI Ecology & Evolution
Professor Medha Pathak, UCI School of Medicine
Professor Albert Siryaporn, UCI Physics & Astronomy
Professor Zeba Wunderlich, UCI Developmental & Cell Biology

12:15 - 1:00	**BREAKOUT SESSIONS**
1:00 - 2:00	**LUNCH**
2:00 - 2:30	**BREAKOUT PRESENTATIONS**
2:30 - 3:30	**POSTER SESSION & COFFEE BREAK**
3:30 - 5:00	**PM SESSION TALKS**

KERRIGAN BLAKE, UC IRVINE
Single-cell analysis of the brain microenvironment in breast cancer brain metastasis.

DAVID TATAKARIS, UC IRVINE
Single Cell Profiling and Genetic Analyses of Fate Determination in the Cranial Neural Crest.

PRAVEER SHARMA, UC IRVINE
Measurement and modeling of patterning gene expression reveals importance of spatiotemporal kinetics in jaw development.

JINSU KIM, UC IRVINE, KATHERINE SHEU, UCLA
Stochastic modeling of chromatin accessibility in response to distinct inflammatory transcription factor dynamics.

DANIEL RAMIREZ-GUERRERO, UC IRVINE
Team members: Xingyu Chen (U Penn), Qingda Hu, Alicia Jagiello, Daniel Ramirez-Guerrero

Unraveling how interactions of chemical signaling and mechanical forces influence cell fate decisions and behaviors of growing tissues.

SHUXIONG WANG, UC IRVINE
Team members: Yutong Sha, Shuxiong Wang, Zi Ye

Revealing the Underlying Mechanisms of Fibroblast-Keratinocyte Transdifferentiation using a Data-Driven Approach.

5:45 - 7:30	**DINNER AT TACO ROSA**

2632 San Miguel Dr, Newport Beach, CA 92660

BREAKOUT SESSIONS

BREAKOUT SESSION A: WHAT FIELD IS THIS?

During the proposal preparation of the Center in mid-2017, the PIs originated a phrase, "multiscale cell fate", and a brief paragraph describing an area of inquiry we felt was relevant, underexplored, and that UCI was well-positioned to confront. Now, two years later, following the unrelenting advances of science at UCI and elsewhere, it is time to revisit this, and ask how the Center's potential impact is evolving. This is epitomized by the Center Fellows and the Opportunity Award projects, who together define the impact of the Center -- and not the other way around. What unifies us? What scientific questions, approaches, philosophies and goals do we share? How can we describe these succinctly?

Outcome: A paragraph describing this field

Here's the old one: *"Broadly, a quantitative understanding of subcellular or cellular dynamics that can, or has the potential to, explain supercellular function, or a quantitative understanding of cell-cell communications and supercellular-scale patterning that can, or has the potential to, explain cell fate decisions."*

BREAKOUT SESSION B: THE NEW INSTITUTIONS FOR SHARING SCIENCE

There is wide discussion, exemplified by the San Francisco Declaration on Research Assessment, that the classical venues for scientific communication and metrics of success are poorly equipped to handle the future. Are journal impact factors, h-indices, citation counts, and the traditional pre-publication peer-review process on their way out? What will be the roles of: continuous-updated model familiar in software development (GitHub); preprint servers with commenting (bioRxiv, arXiv.org), "alt-metrics" and scientific Twitter? Code and data repositories? Open publishing (Project S)? Meanwhile, the negative risks of mob mentality that come with viral mechanisms of discourse are all too apparent outside science (and in some cases, within science -- mobs can turn against a researcher in half a day). What does the future of scientific communication and scholar assessment look like? How do we prepare for it?

Outcome: What public presence should be fostered, at center-level and at single-PI-level? What social media training activities do we need?

BREAKOUT SESSION C: ENABLING EARLY-CAREER RESEARCHERS

What events should the Center organize in the next 4 years to help graduate students and postdocs succeed? Think big and small, high-cost and low-cost; 3-day workshops, 1.5hr events for next year's ECR Symposium, mailing lists or Slack groups, ...

Consider activities in two (often overlapping) categories:
Activities that enable the research happening at UCI. While there is a wide variety of research represented, the scientific enterprise is unified by things like visualization tools (Blender, Adobe Illustrator) to paper writing and talk and poster preparation. For example: software or method tutorials, peer writing workshops?
Activities that are aimed at preparing the trainees for the next steps in their careers and lives, in what is sometimes called "professional development". Here we also have opportunities to synergize with UCI's GPS-Biomed programs.

Outcome: A list of proposed future activities for UCI and area graduate students and postdocs (not necessarily restricted to Fellows and IOA teams)

ABSTRACTS

AM SESSION TALKS

DANIEL HAENSEL, UC IRVINE

Towards multiscale modeling: single-cell genomics and EMT regulation in cutaneous wound healing.

Wound healing of the skin represents a multifaceted repair process involving myriad cell types and cell biological processes, such as proliferation, migration, and differentiation, which are intertwined at various spatial and temporal scales. Here we utilize single cell RNA sequencing (scRNA-seq), in vivo murine functional assays, and stochastic multiscale modeling to investigate the cellular heterogeneity and to identify the key regulators of this regenerative process. scRNA-Seq was used to profile all cells present in adult murine skin during normal homeostasis and wound healing. Comparative analysis identified major differences in the overall cellular makeups between normal and wounded skin, uncovered previously undocumented heterogeneity within the epidermal basal populations, and revealed the enrichment of molecular signatures associated with epithelial-to-mesenchymal transition (EMT) in wound epidermal basal cells. Multiplexed in situ RNA detection validated the existence of multiple basal cell subsets, and pseudotemporal ordering and RNA velocity analyses suggested their novel lineage relationships and differentiation dynamics during homeostasis and wound repair. K14-Cre-mediated deletion of Ovol2, encoding a transcription factor that inhibits EMT, leads to delayed wound healing characterized by reduced proliferation, enhanced movement, but loss of directional migration of epidermal keratinocytes. Together, these findings describe repair-specific cellular and molecular programs, as well as identify a key factor that regulates the wound re-epithelization process. We are currently developing preliminary multiscale spatial models to incorporate the identified cellular (sub)populations and potential regulations for mechanism exploration. Our systems approach combining single-cell genomics and mouse molecular genetics with multiscale modeling promises to unravel conditions that favor optimal regeneration during wound healing.

CHRISTINA WILCOX, UC IRVINE
Team Members: Christina Wilcox, Kate Williams, Linh Vuong

Characterizing the Transition Between Naïve and Primed States of Pluripotent ESCs in Mouse and Human.

Pluripotent stem cells (PSCs) have the potential to give rise to cells of the three embryonic germ layers and can be derived from any point during the early stages of embryonic development. Both mouse and human ESCs are derived from the inner cell mass (ICM) of pre-implantation blastocyst stage but have different pluripotent states. Mouse ESCs resemble naïve cells that are found in the ICM, whereas human ESCs are closer to cells derived from the post-implantation epiblast stage of development. Primed PSCs require both epigenetic repressors and activators to keep chromatin in a poised state so that genes can be quickly switched on/off in response to differential stimuli. Naïve PSCs, on the other hand, have active marks on chromatin to keep genes on therefore having greater developmental capacity. While in-depth studies of the epigenetic and transcriptomic regulations of these PSCs have been done, a comprehensive study characterizing the dynamic intermediates that transition between the two PSCs has not been done. We are characterizing the transcriptome and chromatin structure changes that occur during the transition between naïve and primed pluripotency for mouse and human using RNA-seq and ATAC-seq. We are building interaction networks from the datasets and comparing these between the two species. We expect that the knowledge gained from determining the intermediate mechanisms of the transition between naïve and primed pluripotent states for human and mouse will provide a comprehensive model to study cellular differentiation in early embryogenesis and to understand the evolutionary developments of human and mouse.

JESSE HOLT, UC IRVINE
Team members: Jessie Holt, Wei-Zheng Zeng (Scripps)

Mechanosensing by Piezo1 in keratinocytes migration and wound healing.

Tissue repair and regeneration is not only influenced by genetic and chemical cues, but also by mechanical cues that are transduced by mechanically-activated channels. Despite the observed importance of mechanical forces in guiding processes integral to repair, such as keratinocyte reepithelization during wound healing, the molecular mechanisms by which mechanical cues influence migration remain largely unknown. We have previously found that the mechanically-activated ion channel, Piezo1, powerfully regulates the differentiation of neural stem cells by transducing cellular traction forces. The goal of this work is to understand the functional role Piezo1 plays in guiding keratinocyte migration and facilitating wound healing in mammalian skin.

MELANIE I. WORLEY, UC BERKELEY
Team members: Theresa B. Loveless, Courtney K. Carlson (UC Irvine)

Cell fate plasticity and cell lineage during regeneration in Drosophila.

Regeneration after tissue damage requires the reprogramming of adjacent cells to both proliferate and repattern to replace what was lost. What are the molecular mechanisms that regulate cellular reprogramming within an individual and groups of cells during regeneration? To address these questions we study tissue regeneration in Drosophila imaginal discs, which are the larval precursors to adult structures. We use a synthetic ablation system to produce reproducible damage in the imaginal discs and then investigate how the surviving epithelium cells are able to regenerate. We are using single-cell RNA-sequencing (scRNAseq) to profile how different cells change their transcriptional state during the reprogramming process, however, it will be crucial for the better understanding of cellular reprogramming to know the origin of these individual cells. Toward this goal, we have set out to adopt the CHYRON lineage tracing technology (Loveless et al.,) to Drosophila. The CHYRON technology relies on the cutting of a self-targeting guide RNA with Cas9, followed by insertion of random nucleotides by the vertebrate-specific template-independent polymerase TdT. Currently, the technology requires the recruitment of TdT to the double-strand break created by Cas9 via an interaction between TdT and the host cell DNA repair factor Ku. Since there is no TdT homolog in Drosophila, we will test whether human TdT can promote insertion mutations at Cas9 breaks in Drosophila tissue culture cells. If it can promote these mutations, we will create a Drosophila transgenic line with an inducible CHYRON system and test whether the RNA that carries the lineage information from each cell can be detected during scRNAseq. The combination of a 1) CRISPR based cell-labeling system that supports consecutive rounds of labeling and 2) the ability to describe a cell's current state by scRNAseq expression analysis will allow us to address a number of fundamental questions in regenerative biology.

CAMERON GALLIVAN, UC IRVINE

Noisy Dynamics in the Epigenome: Insights from Stochastic Modeling and Inference.

Increasingly, cell biological data is available at single-cell, single-nucleotide, and single-molecule resolution, revealing significant heterogeneity at these scales. Stochastic models are required to quantify this heterogeneity and understand its
origins and consequences.
I will present two areas of current research. First, stochastic modeling of gene regulatory networks can inform our understanding of cell-to-cell heterogeneity, but integrating such models with data is challenging. We propose a framework for integrating single cell RNA sequencing datasets with stochastic network models, shedding light on cell-developmental trajectories.
Second, new bisulfite-sequencing experiments reveal significant temporal heterogeneity in DNA methylation patterns post-replication. Combining statistical inference and stochastic modeling, our results inform cooperative models of DNMT1-mediated epigenetic inheritance, and point to novel potential mechanisms of dynamic epigenetic regulation.

ABSTRACTS

PM SESSION TALKS

KERRIGAN BLAKE, UC IRVINE

Single-cell analysis of the brain microenvironment in breast cancer brain metastasis.

Regeneration after tissue damage requires the reprogramming of adjacent cells to both proliferate and repattern to replace what was lost. What are the molecular mechanisms that regulate cellular reprogramming within an individual and groups of cells during regeneration? To address these questions we study tissue regeneration in Drosophila imaginal discs, which are the larval precursors to adult structures. We use a synthetic ablation system to produce reproducible damage in the imaginal discs and then investigate how the surviving epithelium cells are able to regenerate. We are using single-cell RNA-sequencing (scRNAseq) to profile how different cells change their transcriptional state during the reprogramming process, however, it will be crucial for the better understanding of cellular reprogramming to know the origin of these individual cells. Toward this goal, we have set out to adopt the CHYRON lineage tracing technology (Loveless et al.,) to Drosophila. The CHYRON technology relies on the cutting of a self-targeting guide RNA with Cas9, followed by insertion of random nucleotides by the vertebrate-specific template-independent polymerase TdT. Currently, the technology requires the recruitment of TdT to the double-strand break created by Cas9 via an interaction between TdT and the host cell DNA repair factor Ku. Since there is no TdT homolog in Drosophila, we will test whether human TdT can promote insertion mutations at Cas9 breaks in Drosophila tissue culture cells. If it can promote these mutations, we will create a Drosophila transgenic line with an inducible CHYRON system and test whether the RNA that carries the lineage information from each cell can be detected during scRNAseq. The combination of a 1) CRISPR based cell-labeling system that supports consecutive rounds of labeling and 2) the ability to describe a cell's current state by scRNAseq expression analysis will allow us to address a number of fundamental questions in regenerative biology.

DAVID TATAKARIS, UC IRVINE

Single Cell Profiling and Genetic Analyses of Fate Determination in the Cranial Neural Crest.

Neural crest (NC) cells delaminate from the developing neural tube and migrate extensively along tightly defined streams to differentiate into cartilage, bone, neurons, glia, and many other cell types. How these migratory pathways and lineage decisions are coordinated to achieve robust development in highly dynamic environments remains unclear. We are addressing this question in NC cells of the zebrafish embryo using a novel paradigm for single-cell RNA-seq (scRNA-seq) that maintains spatial and temporal information combined with genetic perturbations that disrupt migration of specific NC cell lineages. Using a transgenic line carrying photoconvertible nuclear EOS driven in the NC by a sox10 promoter we label NC cells in targeted locations and isolate them via FACS for scRNA-seq.. Premigratory NC cells show considerable heterogeneity, including a small subpopulation with a putative neurogenic signature including expression of several hairy-related (her) genes. In addition, a critical window becomes apparent during early stages of migration when signatures of skeletal and pigment lineages appear. Hybridization Chain Reaction (HCR) in situs confirm expression of her4 in a subset of NC cells, and we are constructing developmental trajectories for these and other signatures of putative lineage decisions. In addition, scRNA-seq profiles of NC cells from zebrafish mutants that disrupt migration of specific subsets of NC lineages suggest a fate switch from the her4+ neurogenic subpopulation to another signature characterized by expression of an epithelial cell adhesion molecule (epcam). Computational models built using our scRNA-seq data suggest that these defects result from changes in the transition states of migrating NC cells, and that both Notch and Wnt signaling influence these states. Taken together, our results promise to lead to insights into the mechanisms coordinating a NC cell's migratory path with its cell fate decisions.

SHUXIONG WANG, UC IRVINE

Team members: Yutong Sha, Shuxiong Wang, Zi Ye

Revealing the Underlying Mechanisms of Fibroblast-Keratinocyte Transdifferentiation using a Data-Driven Approach.

The transdifferentiation from fibroblasts to keratinocytes provides an efficient source for keratinocytes, which are the key progenitor cells that maintain the self-renewing epidermis. We proposed a data-driven approach to gain mechanistic insight into the poorly understood process of transdifferentiation by studying the engineered conversion of fibroblasts to keratinocytes. The method quantifies the cell stability index to detect transitional cells between the relatively stable states, intermediate cell states and the trajectories capturing cell lineage in which cells are undergoing transitions. By applying the method to the single-cell data, we identify distinct cell states of keratinocytes and fibroblasts and differentially expressed genes that mark the two types. In addition, two intermediate states and transition cells are inferred between keratinocytes and fibroblasts on the trajectory along with the highly varying genes that capture the transdifferentiation process.

PRAVEER SHARMA, UC IRVINE

Measurement and modeling of patterning gene expression reveals importance of spatiotemporal kinetics in jaw development.

Mobile jaws are the defining feature of the gnathostome group of vertebrates, which includes bony fish and all land animals. Jaws develop from neural crest-derived embryonic structures called pharyngeal arches, which are patterned on the dorsal-ventral axis into dorsal, intermediate, and ventral domains, and further subdomains, as defined by the expression of various transcription factors. This patterning, which is regulated by signaling pathways including Bmp, Endothelin (Edn), and Notch, is critical for proper jaw development and disruptions in patterning result in birth defects such as auriculocondylar syndrome, where the mandible acquires a maxilla-like identity. Although many of the factors involved in patterning have been identified, understanding of the details of their interactions remains incomplete. We have recently addressed arch dorsal-ventral patterning using two complementary approaches. In our first approach we used transcriptional measurements of core patterning gene expression and live imaging of transgenic zebrafish expressing neural crest cell and patterning markers to quantify in detail the spatiotemporal characteristics of arch patterning. We used these measurements, along with known genetic interactions, to develop a computational model of arch patterning which revealed that patterning is most sensitive to changes in Bmp signaling, and that the precise temporal order of gene expression modulates the response of the patterning network to noise. In our second approach, we extended our transcriptional measurements to the broader arch development network, encompassing 55 zebrafish genes and 65 mouse genes. We found that genes in the same pathway with similar spatial expression often had very different temporal expression profiles, suggesting differences in baseline expression kinetics, and that genes in different pathways sometimes had very similar temporal profiles, suggesting previously unknown shared regulation.

We are building on these approaches to better understand craniofacial development. Our modeling indicates that the precise levels of Bmp signaling and gene expression timing are critical for proper patterning. We are seeking to modulate Bmp signaling using light-activated Bmp receptors (Chen lab) and patterning gene expression using light-activate Gal4-UAS (GAVPO, Chen/Mruk labs) to observe how patterning noise changes (as measured by precision, accuracy, and sharpness of domain boundaries). We are also analyzing the enhancers that regulate the wider patterning network by expressing candidate craniofacial enhancer sequences from mouse (Vista) in zebrafish using the Ac/Ds transposase system (Sauka-Spengler lab). Revealing the enhancer network will help us understand how the expression of similar genes can differ and that of different genes can be similar.

JINSU KIM, UC IRVINE, KATHERINE SHEU, UCLA

Stochastic modeling of chromatin accessibility in response to distinct inflammatory transcription factor dynamics.

Inflammation plays central roles in disease processes such as autoimmunity, diabetes, and cancer. Immune responses involve dramatic activation of stimulus-dependent transcription factors (SDTFs). The transcription factor NF□B is a SDTF that acts as a central mediator in inflammatory gene programs. In macrophages, NFkB activation can demonstrate oscillatory or non-oscillatory dynamics, which correlate with the propensity to form open enhancers and promoters. It remains unclear how information contained in signaling dynamics is decoded at the level of chromatin. Both the temporal dynamics and molecular stochasticity of NFkB signaling may serve to modulate chromatin accessibility. In this project, we construct a deterministic model to propose mechanisms that explain how oscillatory or persistent signaling dynamics lead to changes in chromatin accessibility in a bulk population. Based on the study of the deterministic model, we next study relationships between chromatin accessibility and NFkB dynamics for a single nucleosome with two coupled stochastic processes. One of the main goals of this model is to mathematically analyze the impacts of NF□B signaling on the first full eviction time of histone from DNA. We also incorporate histone modifications into the single nucleosome model and extend our stochastic model to a spatially heterogeneous population model.

DANIEL RAMIREZ-GUERRERO, UC IRVINE

Team members: Xingyu Chen (U Penn), Qingda Hu, Alicia Jagiello, Daniel Ramirez-Guerrero

Unraveling how interactions of chemical signaling and mechanical forces influence cell fate decisions and behaviors of growing tissues.

Cells interact reciprocally with their local environment using biophysical and biochemical signals. These signals influence the fate of individual cells as well as their migration, proliferation, differentiation and death, which collectively influence tissue behaviors. Here, we will investigate the hypothesis that complex patterns of tissue growth, such as invasive fingering, emerge as a consequence of the nonlinear interactions arising from feedback loops involving cell fate, proliferation, mobility and contractility, and properties of the extracellular matrix, namely fiber realignment and strain stiffening. We will use mathematical modeling and in vitro experiments to test this hypothesis. In particular, we will develop a novel multiscale biochemomechanical model of tissue growth that incorporates mechanical forces, biophysical and biochemical signaling and integrates single cell level processes. We will test model predictions using a 3D model of melanoma spheroids in collagen using an in vitro system developed in Prof. Botvinick's lab.

ABSTRACTS

POSTER SESSION

ROBERT TAYLOR, UC IRVINE

Stochastic cell surface reorganization for cell-cell interactions explored via Weighted Ensemble methods.

The formation of both long-term or transient cell-cell contacts is often accompanied by the rearrangement of surface molecules. In some cases, like the T cell during immunological synapse formation, the evacuation of large molecules is necessary for receptor triggering. Preliminary calculations demonstrate that passive mechanisms like diffusion are far too slow to make sufficient space in sufficient time, raising the question, what additional biophysical phenomena allow these cells to “make space” so quickly? We are developing computational methods to simulate the stochastic rearrangement of surface molecules. Since the diffusion-only case is rare (in the sense of a separation of timescales between individual molecules and evacuation of an ensemble molecules), direct simulation is unfeasible. We therefore use the Weighted Ensemble framework, a stochastic rare-event sampling method. We are variously including interactions between surface molecules, like dimerization and oligomerization, and heterogeneity of the cell surface, like lipid rafts, microvilli and corrals. In our preliminary results, we find a surprisingly large reduction in evacuation time from relatively weak dimerization reactions. The exploration of phenomena that drastically alter surface molecule dynamics will have implications for immune cell function. Since some of these phenomena are extracellular, they may also present opportunities for engineering immune cell therapeutics.

MICHELLE NGO, UC IRVINE

A biclustering method for single cell RNA sequencing of multi-functional cells.

We extend a clustering scheme that allows for the simultaneous clustering of both genes and cells (''biclustering'') to model instances where a gene or cell are involved in more than one biological function. Rather than clustering over the gene by cell product space directly, we apply a non-parametric approach referred to as the Mondrian process (Roy and Teh 2009). This is a multidimensional generalization of the Poisson process and constructs random partitions of the product space with nested, axis-aligned cuts. We are able to generate biclusters of genes and cells without pre-specifying the number of partitions and apply our model to single-cell RNA sequencing data.

LIANNA FUNG, UC IRVINE

Measuring Gene Expression Noise and Mechanisms of Robustness in Zebrafish Hindbrain Development.

Hindbrain development displays remarkable robustness with sharp segment boundaries consistently forming from embryo to embryo despite variations in genetic background, environmental factors (temperature, diet), and noise inherent in biological processes. While noise is often detrimental, computational models suggest that specific levels of gene expression and morphogen noise facilitate sharpening of hindbrain segment boundaries between krox20 and hoxb1a expression domains by driving cell switching at the boundaries. To further investigate the role of gene expression noise in this context, we are developing the MS2-RNA labeling system in zebrafish to measure gene expression noise. This system functions by binary expression of a fluorescent MS2 bacteriophage coat protein (MCP) and an RNA of interest tagged with multiple RNA hairpin MS2-binding sites (MBS) enabling analysis of gene expression dynamics in living cells. We have generated transgenic lines expressing variants of EGFP-tagged MCP which reduce/eliminate background aggregates enabling more reliable gene expression quantification and will use these lines to analyze noise in krox20 expression during boundary sharpening. Previous results in our lab have also suggested that specific levels of noise in the morphogen, retinoic acid (RA), are important for boundary sharpening. RA transport is particularly important since RA is hydrophobic and relies on cellular RA binding proteins (Crabps) for intracellular transport to either degradation enzymes or receptors in the nucleus. Morpholino studies in the lab have shown that Crabps modulate noise in the RA gradient. To examine further the role of Crabps, we have generated CRISPR mutant lines for all four crabp genes in zebrafish and are examining changes in gene expression and morphogen noise during hindbrain development.

ZIXUAN CANG, UC IRVINE

Single cell data in space.

The spatial information lost in single cell data can be partially retained using in situ data. We present a data driven physical model that combines single cell data, in situ data, and prior knowledge to reconstruct the spatial and possibly temporal dimensions of single cell data. This model is applied to early embryo of mouse revealing the spatial arrangement of single cells in the embryo geometry and identifying spatial expression patterns of gene.

CHRISTIAN GUERRERO-JUAREZ, UC IRVINE

Single-cell analysis identifies heterogeneity of fibroblasts and myeloid-derived adipocytes in regenerating mouse skin wounds.

During healing of large excisional skin wounds in adult mice, hair follicles and then dermal adipocytes regenerate de novo in the wound center. New hair follicles regenerate from wound epidermis and wound fibroblasts by reactivating embryonic hair morphogenesis program. New adipocytes regenerate around new hair follicles from myofibroblasts, a specialized contractile wound fibroblast, via the process of reprogramming. We studied diversity of fibroblasts in large skin wounds using single-cell RNA-sequencing. We show that wound fibroblasts group into twelve clusters. Pseudotime and RNA velocity analyses shows that some clusters likely represent sequential states during fibroblast differentiation toward a contractile phenotype, while other clusters appear to represent distinct fibroblast lineages. One subset of wound fibroblasts expresses hematopoietic markers, suggesting their myeloid origin. We validated this finding using single-cell western blot as well as single-cell RNA-sequencing on genetically labeled wound myofibroblasts. Furthermore, using bone marrow transplantation and Cre recombinase-based lineage tracing experiments, we rule out cell fusion events and confirm that hematopoietic lineage cells give rise to a subset of wound myofibroblasts and rare regenerated adipocytes. In conclusion, we show that wounding in skin induces a high degree of heterogeneity among fibroblasts and recruits highly plastic myeloid cells that contribute to adipocyte regeneration..

XIAOJIE WANG, UC IRVINE

The Dark Side of Stem Cell Niche.

Adult stem cells play a key role in tissue homeostasis and repair after injury. They are surrounded by stem cell niche components which regulate self-renewal and stemness. The interaction between stem cells and their niches regulate cell fate. In this study, I used murine skin as a model to study cell-cell interaction.
Hairs are mini-organs that reside in the skin. Hair follicle stem cells undergo cyclic regeneration, progressing through anagen for growth; catagen for regression; and telogen for resting. Prolonged growing phase in Tyr-NrasQ61K mouse model is a prototype of human hairy nevi that rely on the constitutive expression of an Nras mutant under a tyrosinase promoter and trigger melanocytes to enter a cellular senescent state. In this model, persistent anagen hair growth phenotype was apparent in the hyper-pigmented skin, suggesting melanocytes as a stem cell niche component that functions to hyper-activate hair follicle stem cell (HFSC). Two key characteristics of senescent cells have been well established in previous studies. However, how two features of cell cycle arrest and senescent associated secretary phenotype (SASP) alter stem cell function is unclear. Single-cell whole transcriptome analysis showed that melanocytes which could activate HFSC are heterogeneous. I hypothesize that distinct clusters of melanocytes may function differently. The result of this study will shed light on cell how cell-cell interactions determine cell fate.

ALAN JIANG, UC IRVINE

Label-free Mouse Neural Stem Cell Sorting with Hydrodynamic Oblique Angle Parallel Electrode Sorter.

Neural stem and progenitor cells (NSPCs) are a heterogeneous population of cells that self-renew, migrate, and differentiate into neurons and glia. Since these cells can secrete beneficial factors and differentiate into mature central nervous system (CNS) cells, they are ideal candidates for treating many different diseases and injuries affecting the CNS. However, the damaged areas and affected cell types differ across CNS ailments; therefore, the ability to tailor the cell population for a particular disease or injury could drastically improve the efficacy and consistency of cell therapies. To assess the tailored cell transplantation approach, a robust cell sorting system is needed to partition NSPCs into fractions that are largely different in cellular compositions. Furthermore, the system should be scalable to achieve throughput that can meet the needs of large scale studies. We found populations of NSPCs with more neurogenic progenitors (NPs) can be distinguished from those with more astrogenic progenitors (APs) by their inherent biophysical properties. Dielectrophoresis (DEP) can be used as a viable label-free method to enrich either population by varying the frequency of the non-uniform electric field. Based on these concepts, we developed a hydrodynamic oblique angle parallel electrode sorter (HOAPES) that incorporates hydrodynamic pressure gradients and DEP to focus and separate cells in a single microfluidic chip with a single-step operation. Our device yields two populations in a single sort, providing enriched and depleted populations that differ more from each other than from unsorted controls. Our results show an a 3.0-fold difference in APs between the enriched and depleted sorted population, and achieved a throughput of 240,000 cells/h. The new sorting method could break the theoretical AP enrichment maximum (3.2 fold) previously reported.

JULIEN MORIVAL, UC IRVINE

Testing the potential of deactivated DNA methyltransferase 1 for epigenetic editing.

DNA methylation is an epigenetic regulator of gene expression and cell fate. Previously, DNA methylation has been modulated using inhibitors of DNMT1, the enzyme responsible for maintaining the majority of methylation patterns during DNA replication. However, these drugs have poorly characterized mechanisms of action, act non-specifically in the genome, and are cytotoxic. The functional protein domains of DNMT1 have been well characterized. Prior studies showed a specific domain deletion in DNMT1 could reduce its catalytic activity, enabling it to act as a dominant negative DNA competitor. Another inactive version of DNMT1 was shown to repress certain genes through histone-related chromatin interactions, suggesting DNMT1 interacts with the epigenetic landscape in diverse ways. Building off this prior work, we aim to create DNMT1-inspired biomolecules that alter the epigenome to better engineer cell fate. We hypothesize that catalytically deactivated versions of DNMT1 (dDNMT1) will compete with wild-type DNMT1 as it carries out its epigenetic functions, similar to DNA methylation inhibitors, but without their deleterious and imprecise effects.

TESSA MORRIS, UC IRVINE

Assessing Continuous Z-line Length as a Metric for Cardiac Function.

The heart is organized into laminar sheets of cardiac fibers - myofibrils. Furthermore, the structural remodeling of the myofibrils contributes to declined cardiac function in a diseased heart. Within a myofibril, each sarcomere produces a contractile force parallel to the actin fibrils and ideally perpendicular to its z-lines, which are the boundaries between sarcomeres. Because many sarcomeres work together to produce the force necessary for cardiac contractions, their spatial organization affects the heart's ability to pump blood. Therefore, measuring/quantifying precise sarcomere organization is an essential part of understanding the mechanisms that influence cardiac function. Based on qualitative experimental observations, it is hypothesized that cardiac tissues with sarcomeric z-lines that are registered between myofibrils tend to produce a greater contractile force. This hypothesis is supported by looking at single myofibrils in isolated cells. Additionally, visibly shorter z-lines are considered a hallmark of immature stem-cell derived cardiac tissue. Based on these qualitative experimental observations, the degree of continuity and/or registration of sarcomeric z-lines is expected to correlate with cardiac force production that deviates from existing predictions. In this work, we measure the distance over which z-lines from multiple myofibrils are continuous, termed the continuous z-line length. The continuous z-line length can be automatically and accurately measured from images of alpha-actinin stained cardiac tissue using the pixel and orientation information. The continuous z-line length has the potential to be used as a tool to better compare tissues and differentiation methods across labs and contribute a robust understanding of how tissue level structure affects the hearts mechanical function.

LIHUA ZHANG, UC IRVINE

scAIMF: an efficient approach to dissect single cells by integrative analysis of transcriptomic and epigenomic profiles.

Recent technological development of single cell enables measuring multiple molecule types from the same individual cell, which provides a great chance for deepening understanding of single cells' heterogeneity from multiple layers (e.g., gene expression, chromatin accessibility and DNA methylation). However, computational approach for integrating these datasets is still lacking. And high sparsity, various data type-specific characteristics especially near binary chromatin data pose enormous challenges for integrating multi-layer information in individual cells. Here we developed scAIMF, a novel integrative method used to reveal latent cell subpopulation-specific or cell lineage-specific transcriptional and epigenomic regulation features. To demonstrate the effectiveness of our approach scAIMF, we first applied it to a set of simulated datasets with various scenarios. Then we applied scAIMF to three real single cell datasets profiled by simultaneous sequencing of transcriptome and chromatin accessibility or transcriptome and DNA methylation. Our approach facilitates general integrative analysis of continuous scRNA-seq and sparse, near binary epigenomic data, potentially deepening our understanding of how distinct cell states coordinate transcriptomic and epigenomic layers to regulate cell fate decisions.

LARA CLEMENS, UC IRVINE

Membrane-associated intrinsically disordered signaling proteins can exhibit emergent cooperativity, even under symmetric reversible kinetics.

Recent evidence has demonstrated that the intrinsically disordered regions of signaling molecules can be more than just flexible linkers of functional domains, but rather confer non-linear functionality themselves. As a specific example, the T Cell Receptor zeta chain has been suggested to dynamically dissociate from the membrane upon receptor triggering, allowing it to become further phosphorylated. This implies a delicate balance: For membrane dissociation to have a substantial impact, the membrane must significantly occlude tyrosines from kinases, but not so much that initial triggering is inhibited. We present a general model of a multi-site disordered signaling molecule in simplified, theta-solvent, freely-jointed chain dynamics. We use this model to study the zeta chain association with the membrane. We find that for a wide range of molecular properties, the zeta-chain can be membrane-associated yet still accessible to kinases, and accessibility is dramatically increased upon phosphorylation. This leads to cooperativity of phosphorylation that allows membrane-association to serve as an effective binary switch in the signal transduction cascade. Surprisingly, we find that cooperativity persists even if phosphatases, and thus dephosphorylation, are assumed to operate with identical molecular features as the kinases. Our work adds to a growing body of research suggesting that disordered regions themselves may act as modules in signal transduction cascades.

DANIEL RAMIREZ, UC IRVINE

Mechanical Feedback and stress relaxation in growing tissues.

The dynamics of tumor growth is simulated using a continuum model of tissue elasticity in an Eulerian frame. The model incorporates nonlinear elastic stresses, elastic relaxation and growth-promoting factors that regulate cell behavior. The model reduces to linear elastic models and fluid models in appropriate parameter regimes. Focusing on stress-induced feedback inhibition of proliferation, parameters are estimated from experiments on tumor spheroids. Model results are in good agreement with experiments of tumor spheroids embedded in agrose gel. An extension to simulate tumor growth in a host tissue is proposed.