Caltech
Student-Faculty Programs

Session C Abstracts

Characterizing estrogen-related receptor function during nephron tubule development using zebrafish
Kara Z. Lo
Mentors: Marianne Bronner and Tianli Qin

The nephron, the kidney's fundamental structural and functional unit, performs essential physiological functions, including metabolic waste excretion, hormone production, and maintenance of fluid and electrolyte homeostasis. Each nephron consists of a blood-filtering unit followed by an epithelial tubule patterned into functionally specialized segments. Proper nephron development requires coordinated segment patterning and tubule morphogenesis to establish a functional renal tubule, and defects in these processes contribute to kidney disease. We use the zebrafish embryonic kidney, which exhibits a high degree of genetic and cellular conservation with the mammalian nephron, as a high-throughput model to investigate the roles of the estrogen-related receptors *Esrra* and *Esrrb* in nephrogenesis. Analysis of CRISPR-Cas9-generated single and double knockout embryos using hybridization chain reaction (HCR) reveals expansion of the proximal convoluted tubule (PCT) and concomitant shortening of the distal tubule, suggesting impaired tubule convolution. Because proper tubule convolution depends on cilia-driven fluid flow that directs collective migration of nephron epithelial cells toward the proximal end, as well as stretch-induced proliferation in the distal tubule that sustains this process, ongoing studies are testing whether these phenotypes arise from defects in cilia function or reduced cell proliferation.

Evaluating the effectiveness of different transposon systems for genomic integration in chicken embryos
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Mentors: Marianne Bronner and Junpeng Gao

Developmental biologists seek to understand the molecular factors regulating embryonic development. Although CRISPR/Cas9-mediated loss-of-function experiments can determine the roles of molecular factors, they do not provide stable, long-term labeling of perturbed cells. To address these limitations, this project evaluates three transposon systems (AcDs, PiggyBac, Tol2) for stable genomic integration to enable long-term lineage tracing following loss-of-function experiments. These systems mediate genomic integration through a transposase enzyme that inserts DNA flanked by transposon sequences into the genome. Chicken embryos were electroporated with each transposon system, and fluorescence was monitored over one week. The AcDs system sustained weak fluorescence for 3 days. The PiggyBac system was not evaluated due to unsuccessful cloning of its transposon. The Tol2 system maintained strong fluorescence over 6.5 days. In conclusion, Tol2 produced the most sustained fluorescence, supporting its use for future lineage-tracing studies. Future research will focus on using Tol2 in loss-of-function experiments to study molecular factors involved in the development of the peripheral nervous system.

Tbx1 gene regulatory network in mandibular development
Jaidyn M. Woods
Mentors: Marianne Bronner and Sierra S. Marable

Tbx1 is a transcription factor implicated in mandibular development within the first pharyngeal arch. Loss of Tbx1 function is associated with craniofacial and cardiovascular abnormalities, including defects observed in 22q11.2 deletion syndrome, yet its exact role in mandibular development remains unclear. Conflicting studies have reported Tbx1 expression in either the mesodermal core of the pharyngeal arches or subsets of neural crest-derived cells. To characterize spatiotemporal expression of Tbx1 in the developing chick mandible, hybridization chain reaction fluorescence in situ hybridization and Sox10E2-GFP in ovo electroporation followed by whole-mount immunostaining were performed in HH14-HH22 embryos. Tbx1 did not overlap with Sox10-positive cranial neural crest cells

in the pharyngeal arches but was detected in GFP-positive neural crest derivatives, suggesting expression after Sox10 downregulation. Ongoing experiments using mesodermal markers will determine whether Tbx1 is also expressed in the mesodermal core. CRISPR inhibition will be used to knock down Tbx1 and evaluate its effects on Sox10 expression, mandibular morphology, and candidate downstream genes to identify the cell populations and gene networks disrupted during mandibular development. Together, this work explores the molecular and cellular functions of Tbx1 during mandibular development and provides insight into how disruption of Tbx1 may contribute to craniofacial defects.

Analysis of the exploratory dynamics of microtubule search for kinetochores during cell division

Nishanth Chavourkar
Mentors: Rob B. Phillips and Minakshi Ashok

Many biological processes are designed to reach specific goals to ensure proper functioning of an organism. During cell division, microtubules emerging from the poles of a spindle must each reach individual chromosomes. Any error in this process can lead to one of the daughter cells having one too many or one too few chromosomes. Thus, biological systems must develop mechanisms for reaching their goal with high fidelity. To achieve high fidelity, many biological systems operate on a paradigm of variation and selection, where a biological process explores multiple trajectories, terminating unsuccessful outcomes until a successful outcome is reached. We aim to provide a more realistic description of the variation and selection paradigm within the context of the exploratory dynamics of the mitotic spindle. To achieve this we accounted for the dynamics of the termination of a trajectory (microtubule catastrophe), and analyzed how the structure of the mean search time for a chromosome varied as we introduced this correction. This work is part of a broader effort to describe the dynamics of biological systems using the language of trajectories. An understanding of the trajectories taken by biological systems allows us to further understand *how* a biological system reaches a specific goal.

Energetics of gradients in biology

Zitao Yang
Mentors: Rob B. Phillips, Minakshi Ashok, and Sara Mahdavi

Energy investment is necessary for biological systems to exhibit beautiful and complex patterns. Many of such patterns play crucial roles in the functions of living organisms. For instance, the Bicoid protein gradient in *Drosophila* embryo is crucial in setting up the anterior-posterior axis. Much previous work has been done to understand the molecular dynamics that give rise to the gradients. However, little is known about the power needed to build and sustain them. In this work, we first lay down the theoretical framework to compute the rate of change for various thermodynamic quantities. Specifically, we compare the minimum power needed to maintain the gradient and actual chemical work done on the system. We apply this framework to analyze several biological systems including: Bicoid protein gradient in developing *Drosophila* embryo, kinesin-2 gradient in *Chlamydomonas* flagella, polarity protein Cdc42 gradient in yeast, and Min protein gradient in *E. coli*. A careful account of energy budget reveals an apparent inefficiency of biological approaches to maintaining gradients.