MIT DEPARTMENT OF BIOLOGY

Biology Undergraduate Research Symposium 2016

January 28th | 1–4:30 pm | 68-181

1:00 pm Opening Remarks

1:10–2:10 pm	2:25–3:25 pm	3:40–4:25 pm
Shi Yun Wang Jacks Lab	**Ching Pin Cheng** Dedon Lab	**Sarah Osmulski** Hung Lab
Erika Arias Yilmaz Lab	**Will Conway** Cissé Lab	**Xiao Chen** Sive Lab
Lei Ding Xu Lab	**Sean Corcoran** Jaenisch Lab	**Alina Li** Housman Lab
Rachel Lily Terry Amon Lab	**Anastassia Bobokalonova** Weng Lab	

4:25 pm Closing Remarks

Refreshments will be served. All are welcome!

Contents

Colorful Cancer: Lineage Tracing and Generating Tumor Heterogeneity Using Brainbow

Shi Yun Wang, Carman Man Chung Li, Tyler Jacks
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Cancer is fundamentally an evolutionary disease that relies on mutations in cancer cells to progress; thus, cells in a single tumor often have different gene expression profiles. This heterogeneity in gene expression may activate signaling pathways of surrounding tumor cells, causing changes in properties such as tumor growth, proliferation, and potential for metastasis[‡]. Although widely known to be a crucial factor of cancer, tumor heterogeneity cannot be studied efficiently without directly altering the cells' gene expression. In this project, we developed and tested a lentiviral construct, based on the popular recombination-based lineage tracing method of Brainbow, to create specific, traceable differential gene expression (either $mCherry^+$ or $EGFP^+$) in a mixed population of cells. Since both possible recombination events induce a loss of hygromycin resistance, we assayed for correct recombination by treating the cells with hygromycin and checking for cell death. We also quantified the relative number of cells that underwent each type of recombination event using flow cytometry. We found that in a human lung adenocarcinoma cell line, our construct recombined correctly and induced expression of either mCherry or EGFP, but only at a 1:100 ratio. Our proof-of-concept demonstration shows that our current method may serve as a good system to generate and investigate tumor heterogeneity within a population of tumor cells that are derived from a single cell-of-origin.

‡ Marusyk, Andriy, and Kornelia Polyak. "Tumor heterogeneity: causes and consequences." Biochimica et Biophysica Acta (BBA)-Reviews on Cancer1805.1 (2010): 105-117.

Faculty Supervisor: Tyler Jacks
Graduate Student Mentor: Carman Man Chung Li

Diet-Activated PPAR-delta Enhances Stemness and Tumorigenicity of Intestinal Progenitor Cells

Erika Arias, Miyeko Mana, Ömer Yilmaz
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Little is known about how pro-obesity diets regulate tissue stem and progenitor cell function. Here we find that high fat diet (HFD)-induced obesity augments the numbers and function of Lgr5+ intestinal stem cells (ISCs) of the mammalian intestine. Mechanistically, HFD induces a robust peroxisome proliferator-activated receptor delta (PPAR-d) signature in intestinal stem and progenitor cells, and pharmacologic activation of PPAR-d recapitulates the effects of HFD on these cells. Like HFD, *ex vivo* treatment of intestinal organoid cultures with fatty acid constituents of the HFD enhances the self-renewal potential of these organoid bodies in a PPAR-d dependent manner. Interestingly, HFD- and agonist-activated PPAR-d signaling endow organoid-initiating capacity to non-stem cells, and enforced PPAR-d signaling permits these non-stem cells to form *in vivo* tumors upon loss of the tumor suppressor *Apc*. These findings highlight how diet-modulated PPAR-d activation alters not only the function of intestinal stem and progenitor cells, but also their capacity to initiate tumors.

Faculty Supervisor: Ömer Yilmaz
Postdoc Mentor: Miyeko Mana

Quantitative Synaptic Proteomics Elucidates Whole-Synapse Proteome Changes

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Recent genetic studies have highlighted associations between genes encoding excitatory postsynaptic components with schizophrenia and autism spectrum disorders (ASDs), suggesting that disorders in excitatory synaptic function may play an important role in the pathomechanisms underlying ASDs. It is essential to understand the overall functional impact of candidate genes on the synaptic proteome and neuronal signaling cascades. This project seeks to apply quantitative analysis of the proteome and phosphoproteome to the excitatory postsynaptic compartment using the rodent neuronal culture system with virus-mediated gene manipulation. In our preliminary studies, a SILAC-based quantitative proteome approach was used to identify specific changes in the composition of the postsynaptic density when different postsynaptic scaffold proteins (PSD-95 vs PSD-93) were knocked-down. The expression of a small number of synaptic proteins was found to be strongly affected by knockdown of PSD-95 and/or PSD-93, with distinct subsets of proteins up or down regulated in response to manipulation of each scaffold. Additionally, TMT labelling was used to detect changes in the neuronal phosphoproteome resulting from manipulation of the calmodulin-binding protein Neurogranin. The set of differentially phosphorylated proteins displayed significant overlap with previously discovered disease pathways for schizophrenia and ASD. Additionally, functional annotation analysis revealed that substantially different families of proteins are targeted for phosphorylation and dephosphorylation in response to Neurogranin knockdown.

These experiments constitute an exploratory proof-of-concept for using quantitative proteomics to investigate whole-synapse changes in response to targeted protein manipulations. The ultimate goal of this project is to produce an efficient proteomic pipeline that will allow the researchers to throughput ASD gene targets and curate elaborated functional interactomes with an ASD focus.

Faculty Supervisor: Weifeng Xu

Increased cell size slows cell cycle progression through activation of the DNA damage checkpoint

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Saccharomyces cerevisiae cells increase in cell size with every cell division, such that they, and other eukaryotic cells, are larger at death than at birth. Previous studies of *S. cerevisiae* suggest that a maximal cell size limits the cell's ability to proliferate, consequently limiting the number of divisions a cell can undergo during its lifespan*. How cell size interferes with cell proliferation and whether it actually contributes to the onset of senescence and aging is not clear. To understand how changes in cell size affect proliferation, we manipulated cell size using a temperature sensitive allele of Cdk1 to temporarily arrest young cells in G1. During this cell cycle block, the cells continue to grow, resulting in large, oversized cells. We have found that increased cell size results in slowed cell cycle progression. By systematically deleting various cell cycle checkpoint genes, we have determined that large cells experience a significant delay in metaphase that depends on the activation of the DNA damage checkpoint. This result implicates that an increased persistence of DNA damage is at least partially responsible for slow cell cycle progression in large cells. Furthermore, we have determined that large cells are more sensitive to double strand breaks in comparison to other types of DNA damage. We are now working to elucidate whether these large cells have more DNA damage or DNA repair is dysfunctional. Additionally, we are determining whether DNA damage is also responsible for the cell cycle delays observed in aged cells.

**Yang, J., Dungrawala, H., Hua, H., Manukyan, A., Abraham, L., Lane, W., Mead, H., Wright, J., and Schneider, B.L. (2011). Cell size and growth rate are major determinants of replicative lifespan. Cell Cycle Georget. Tex 10, 144–155.*

Faculty Supervisor: Angelika Amon
Postdoc Mentor: Gabriel Neurohr

Hypochlorite Induces DNA Cleavage and Phosphorothioate Turnover in Bacteria

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Phosphorothioate (PT) is the only known backbone modification in DNA. Previous studies have indicated its potential implications in a novel restriction modification system, as well as oxidant resistance. Here, in contrast to literature, we found significantly higher sensitivity toward a different oxidant, sodium hypochlorite (NaOCl), in bacteria bearing PT compared with the PT knockout strain in both survival curve and growth curve assays. Moreover, with a stable, isotopically-labeled kinetic assay that allowed us to observe the dynamics of PT in bacteria culture, we detected a higher PT turnover rate in NaOCl-treated bacteria than in untreated controls, indicative of PT-specific hypochlorite attacks. Finally, according to mass spectrum data of NaOCl-treated PT dinucleotide, we hypothesized two outcomes following the attack: a single strand break or conversion from a phosphorothioate to a phosphate. Our prediction was then verified by both gel electrophoresis and the TUNEL assay, where we successfully visualized the strand breaks generated by NaOCl attacks in PT-bearing bacteria. Our results confirmed the previously uncharacterized reactions between NaOCl and phosphorothioate ***in vivo***, and shed light on our understanding of biological functions for phosphorothioate.

Faculty Supervisor: Peter Dedon
Mentors: Stefanie M. Kellner and Michael S. DeMott

Real-Time Organization and Dynamics of RNA Polymerase I in Living Cells

Will Conway[1,2], Namrata Jayanth[1], Ibrahim Cissé[1]
Dept. of Physics[1], and Dept. of Biology[2], MIT, Cambridge, MA 02139, USA

Eukaryotic transcription is spatially confined in nuclear subcompartments. Within the nucleus, RNA Polymerase I (Pol I) is responsible for transcribing ribosomal RNA (rRNA) while RNA Polymerase II (Pol II) transcribes messenger RNA (mRNA). We recently uncovered that Pol II clusters in the nucleoplasm of living cells. These Pol II clusters are very short-lived and correlate with number of messenger RNA synthesized. Though Pol I displays significant structural homology with Pol II, little is known about how RNA Pol I is recruited instead to the nucleolus and what the relevant organization and dynamics are. Here we develop a method to capture the spatio-temporal distribution of RNA Polymerase I in living cells. We observe stable Pol I clusters distinct from the transiently clustered Pol II. Our study provides new insights in the organization and regulation of rRNA transcription in contrast to mRNA transcription in live mammalian cells.

Faculty Mentor: Ibrahim Cissé
Postdoctoral Mentor: Namrata Jayanth

MitoCas9: Utilizing Genome Engineering Techniques to Create Double Strand Breaks in Mitochondrial DNA

Sean Corcoran, Julien Muffat, Yun Li, Grisilda Bakiasi, and Rudolf Jaenisch
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CRISPR/Cas9 is a genome editing technology that creates targeted double strand breaks (DSBs) in DNA which are often repaired by non-homologous end joining (NHEJ). NHEJ may insert or delete bases as it stitches two strands of DNA together, leading to mutations in the DNA sequence of interest. In order to expand on the CRISPR/Cas9 system, my research aims to use the system to create DSBs in mitochondrial DNA (mtDNA). To do this, we modified the two components of the CRISPR/Cas9 system. We modified the Cas9 nuclease sequence to include a mitochondrial localization sequence in order to change the cellular localization from the nucleus to the mitochondria. Concurrently, we added mitochondrial trafficking sequences to the chimeric guide RNA, the small nucleic acid that brings the Cas9 nuclease to a complementary locus in the genome. We also added a stem-loop that allows the import of certain RNAs into the mitochondria. Immunohistochemistry on HEK 293 cells shows localization of Cas9 to the mitochondria, and multiple assays show reduction in total amount of mtDNA in the cell, reduced respiration rate, and increase in glycolytic activity of treated cells. We plan to expand on this work in different cell types, and in cells with heteroplasmic populations of mtDNA in which we can target specific populations with a unique guide RNA. We believe that this system could help model the role of mtDNA damage in diseases such as cancer, neurodegenerative disorders, and aging.

Faculty Supervisor: Rudolf Jaenisch

Postdoc Mentor: Julien Muffat

Forward genetics screen of suppressors of *fah1-10* identifies negative regulators of the plant phenylpropanoid pathway

Anastassia Bobokalonova, Jing-Ke Weng
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The phenylpropanoid pathway in plants synthesizes a rich variety of metabolites including flavonoids, hydroxycinnamates, pigments, UV protectants, and lignin structural monomers. The enzyme ferulate-5-hydroxylase (F5H) encoded by the Arabidopsis *FAH1* gene functions at a key metabolic branch point and diverts carbon flux from guaiacyl (G) lignin precursors to the biosynthesis of syringyl (S) lignin as well as sinapoylmalate, a major UV-fluorescent compound present in Arabidopsis leaves. The *fah1-10* mutant has a T-DNA insertion in the enhancer region 3'-downstream of *FAH1*, resulting in a reduced epidermal fluorescence phenotype when observed under UV light. We conducted an EMS-based forward genetic screen on the *fah1-10* background and isolated seven suppressor of *fah1* (*sof*) mutants with recovered fluorescence. We hypothesize that these suppressors may encode previously uncharacterized negative regulators of phenylpropanoid metabolism. To identify the causal mutations and provide a mechanistic basis for the *sof* mutants, we adopted a combinatorial approach involving metabolomic profiling and mapping by next-generation sequencing. LC/MS analysis of methanolic leaf extracts revealed 3,092 differentially affected ($p<0.05$) metabolite features among the *sof* mutants and several control lines. The diversity and pattern of altered metabolite groups suggest multiple modes of action by the underpinning suppressors. This is further supported by our full genome re-sequencing data, which reveals enhanced mutation selection in several unique regions on the genome. We filtered a total number of 22,023 EMS-generated SNPs present in the *sof* genomes down to 4 to 52 candidate causal mutations for each *sof* mutant. Complementation and knock-out tests are currently underway to pinpoint the causal mutation. Once identified, we aim to elucidate the mechanism of action of the suppressor gene, which will shed light on the regulation of metabolic flux in plant phenylpropanoid metabolism.

Faculty Supervisor: Jing-Ke Weng

Lab Manager Mentor: Valentina Carballo

A Comprehensive Analysis of Essential and Conditionally Essential Genes in *Pseudomonas aeruginosa* Using Transposon Insertion Sequencing

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Pseudomonas aeruginosa is a multidrug-resistant opportunistic pathogen capable of colonizing many human tissues. There is a pressing need for novel antibiotic therapies to combat these infections, but the development of antibiotics is hindered by a lack of understanding of gene essentiality in *P. aeruginosa*. To address this deficit in knowledge, we created transposon insertion libraries and assessed growth abilities of the mutants in five diverse bacterial growth media: a standard laboratory rich medium (LB); a minimal medium (M9); a rich medium supplemented with blood (TSA+5% sheep's blood); a biologically relevant rich medium (Sputum Cystic Fibrosis Medium, SCFM); and a biologically relevant minimal medium (urine). Using high-throughput transposon insertion sequencing (TnSeq), we were able to identify novel genes in *P. aeruginosa* that are essential and conditionally essential. We found 299 genes to be essential for growth in all media tested, one gene to be essential only in LB, six genes to be essential in TSA+5% sheep's blood, 90 genes to be essential in M9, nine genes to be essential in SCFM, and 74 genes to be essential in urine. These results identify key genes that may be useful targets for anti-pseudomonal drug discovery in both general and site-specific infections.

Faculty Supervisor: Deb Hung

Postdoc Mentor: Brad Poulsen

Identifying Key Genes in the 16p11.2 Copy Number Variant Region

Xiao Chen, Jasmine McCammon, Hazel Sive

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Copy number variation (CNV) refers to the deletion or duplication of a genomic DNA segment. The 16p11.2 CNV is associated with multiple symptoms including intellectual disability, autism spectrum disorders, schizophrenia, seizures and obesity. Human genetic data indicates that more than one gene is responsible for 16p11.2 symptoms. The Sive lab hypothesized that specific gene pairs in the 16p11.2 CNV region are associated with specific symptoms. Using the zebrafish as a model, the lab carried out an initial screen for interacting 16p11.2 homologs by knocking down gene expression with antisense oligonucleotides. To further explore gene combinations found in this screen I used gene editing to construct ten zebrafish F1 lines carrying mutations in 16p11.2 homologs. In screening F2 embryos for phenotypes reflective of symptoms in 16p11.2 deletion patients, we observed increased propensity for seizure activity in *cdipt* and *fam57ba* double heterozygotes. These data suggest one key gene combination involved in directing 16p11.2 deletion pathology.

Faculty Supervisor: Hazel Sive

Postdoc Mentor: Jasmine McCammon

Elucidating the Role of mRNA Mislocalization in Huntington's Disease

Alina Li, Theresa Wasylenko, David Housman

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Huntington's Disease (HD) is a fatal neurodegenerative disorder characterized by involuntary movement and psychological and cognitive decline. The disease is caused by an expanded CAG repeat in exon 1 of the huntington gene (*HTT*). Normally, this repeat number ranges from 10 to 36, while 36 to 39 repeats leads to incomplete penetrance. HD symptoms manifest once the repeat number expands beyond 39. *HTT* encodes the large HTT protein, whose normal function facilitates transport of mRNA transcripts by associating with microtubule-dependent motor proteins in neurons. Proper localization of mRNA transcripts in neurons allows for spatial and temporal translational regulation and is integral for synaptic plasticity and health.1 Previous studies have demonstrated that downregulation of wildtype Htt disturbs β-actin mRNA localization in cortical rat neurons[2]. This study hypothesizes that proper mRNA localization may be altered due to mutant Htt dysfunction in HD. We established a protocol to cleanly fractionate mouse brain tissue into three fractions: homogenate, cytosol, and synaptosome. From each of these fractions, we successfully isolated highly intact RNA. When we fractionated brain tissue from wildtype mice, we identified approximately 4,000 transcripts that were localized to synaptosomes. Of those transcripts, over 200 were mislocalized in mutant mouse brain. Further examination of these dysregulated transcripts in HD mice could illuminate the development of poor synaptic health observed in patients with this devastating disease.

1 Martin, K. C., & Ephrussi, A. (2009). Cell, 136(4), 719–730.
2 Savas, J. N., Ma, B. et al. (2010). Journal of Biological Chemistry, 285(17), 13142–13153.

Faculty Supervisor: David Housman

Postdoc Mentor: Theresa Wasylenko