MIT Biology

# Biology Undergraduate Research Symposium 2024

February 1st

12–4 pm

Location: 68-181

ALL ARE WELCOME!

Hosted by Professor Adam Martin and Professor Laurie Boyer

## Schedule

**12:00–1:05 PM**

**OPENING REMARKS**

**David Kwabi-Addo**
Church Lab

**Angela Gao**
Vos Lab

**Zoe Gotthold**
Lourido Lab

**Mudita Goyal**
Lourido Lab

**1:05 PM BREAK**

**1:10–1:55 PM**

**Maria Hernandez**
Griffith Lab

**Jonathan Huang**
Weng Lab
and Gehring Lab

**Katherine Kitzinger**
Martin Lab

**1:55 PM BREAK**

**2:00–3:00 PM**

**Yunbeen Bae**
Galloway Lab

**Pari Latawa**
Langer Lab
and Traverso Lab

**Kate Lu**
Lees Lab

**Ananth Shyamal**
Bhatia Lab

**3:00 PM BREAK**

**3:05–4:00 PM**

**Jessica Mann**
Vander Heiden Lab

**Erin Thompson**
Li Lab

**Anika Wadhera**
Wong Lab

**CLOSING REMARKS**

# Contents

## Computational Analysis Pipeline for tRNAs and tRNA Synthetases

**David Kwabi-Addo**, Felix Radford, George Church

Department of Genetics, Harvard Medical School, 77 Avenue Louis Pasteur, Boston, MA, 02115, USA

In the biological mechanism of translation, amino acids are added to a growing polypeptide chain by being transported to the ribosome via tRNAs (transfer RNAs). These tRNA molecules form connections with amino acids through the process of aminoacylation, which are catalyzed by aaRSs (Aminoacyl-tRNA synthetases). Each tRNA carries a specific amino acid, and each tRNA synthetase "charges" a specific type of tRNA. In this project, we have undertaken the development of a tRNA and aaRS analysis pipeline that performs a targeted exploration of the tRNA and aaRS sequences from multiple species. We first extract tRNA and aaRS sequences from online databases. We then map each tRNA that encodes a specific amino acid to its cognate aaRS for each organism in our dataset. Finally, we conduct pairwise alignments between corresponding tRNAs and aaRSs to observe similarities and differences in these molecules between organisms that are closely related and distantly related. As we progress, the insights gained from these analyses will lay the foundation for the subsequent stages of our project, where we aim to create Machine Learning (ML) models to predict features of tRNAs and aaRSs.

Faculty Supervisor: George Church

Postdoc Mentor: Felix Radford



## Structure of the LINE-1 ORF1-RNA Complex

**Angela Gao**, Seychelle Vos

HHMI, Dept. of Biology, MIT, Cambridge, MA 02139, USA

The LINE-1 retrotransposon (L1) is a mobile genetic element that constitutes an estimated 17% of the human genome–significantly greater than the portion of the genome that is composed of protein coding genes. While the majority of L1s are inactive due to truncations or other mutations, a few retain their ability to propagate and insert into new regions of the genome via a "copy-and-paste" mechanism. ORF1 is an RNA-binding protein encoded by the L1 element that plays an essential but unknown role in LINE-1 retrotransposition. In order to determine its function, we have expressed and purified the human ORF1 protein at high concentrations. Fluorescent anisotropy data indicates that ORF1 binds to single-stranded substrates with strong affinity, but associates with double-stranded DNA only at high concentrations. Additional investigations into the structure of ORF1 in complex with RNA using cryo-electron microscopy have yielded promising samples and may further shed light on the significance of these interactions.

Faculty Supervisor: Seychelle Vos

# Machine Learning can Predict Translation Efficiency in *Toxoplasma gondii*

**Zoe Gotthold**, Michelle Peters, Sebastian Lourido

Whitehead Institute, MIT, Cambridge, MA 02139, USA

*Toxoplasma gondii* is a ubiquitous parasite among warm-blooded animals that can cause both acute and chronic infections (toxoplasmosis). Symptoms can be particularly severe in immunocompromised individuals. After an acute infection, *Toxoplasma* can differentiate into long-lasting stages known as bradyzoites. Previous research has noted the importance of translational regulation in this *Toxoplasma* life cycle: in particular, BFD1, the master regulator of *Toxoplasma* differentiation, is translationally, rather than transcriptionally, controlled.

Our research focuses on understanding translational control in *Toxoplasma* through the creation of machine-learning models. Using ribosome profiling, we analyzed the specific RNAs bound by ribosomes, known as ribosome footprints. Normalizing the number of ribosome footprints to the total number of mRNAs provides a quantitative measure of translation efficiency for each gene.

Using a random-forest model trained on several parameters, including coding sequence length, upstream start codon data, GC content, and UTR lengths, we were able to generate a model of translation efficiency (on unseen data, R2=0.42, Pearson's correlation=0.65). Interestingly, this model is much less predictive in human data sets (R2=0.21) since a model trained on human fibroblasts places higher importance on GC content and 5' UTR length than the Toxoplasma model. This could indicate the unique role of the 5' UTRs in Toxoplasma, where specific UTR features might matter more than 5' UTR length.

We also trained several more unsupervised machine learning models on only the sequences of *Toxoplasma* transcripts. Classifying each gene as 'high' or 'low' in terms of translation efficiency, we were able to train an effective LSTM (long short-term memory) network on sequence and length data alone (AUC=0.76). These models will allow us to better understand the translational level of genetic regulation, a regulation which seems to be critical for parasite persistence in the host.

Faculty Supervisor: Sebastian Lourido

Graduate Student Mentor: Michelle Peters



# Characterization of Host-Parasite Interactions Required for *Toxoplasma* Invasion

**Mudita Goyal**, Dylan Valleau, Sebastian Lourido

Whitehead Institute for Biomedical Research, Cambridge, MA 02142, USA

Apicomplexans comprise a phylum of over 5,000 obligate parasites, including the human pathogens *Toxoplasma*, *Plasmodium*, and *Cryptosporidium spp.*, the causative agents of toxoplasmosis, malaria, and severe diarrhea, respectively. These obligate unicellular parasites actively invade and reside within host cells to grow and facilitate their parasitic life cycle. A key step in the apicomplexan invasion process is when specialized organelles known as rhoptries deliver effector proteins into the host cytosol, where they remodel host membranes and facilitate parasite invasion. While individual parasite species have evolved rhoptry secretion factors adapted to their host and tissue specificity, several core proteins are conserved and essential for rhoptry secretion among all apicomplexans. The CRMPs, MIC8, and CLAMP complex are three such factors, with varying levels of conservation across the evolutionary spectrum.

A recent unpublished host cell screen in the Lourido lab has identified host sugar transporters and transferases, N-glycan pathway biosynthesis, and cholesterol pathway components that are required for rhoptry secretion. However, the specific functions of these host factors in the rhoptry secretion process and their interplay with the CRMPs, MIC8, and CLAMP complex remained unknown. We have made human cell knockouts and used quantitative *T. gondii* rhoptry secretion assays to validate the top hits in the screen, confirming these host factors are essential for rhoptry discharge. We have found that host cell surface glycosylation, specifically galactosylation, is essential for host-parasite interactions during rhoptry secretion and invasion. Moreover, we have proven that certain saccharides are effective inhibitors of rhoptry secretion. We are employing a genetic and biochemical approach to define the interactions between these specific saccharides and conserved invasion proteins such as CRMPs and CLAMP. Our work will contribute to a deeper understanding of the mechanism of parasite invasion, offering potential avenues for therapeutic interventions.

Faculty Supervisor: Sebastian Lourido

Postdoc Mentor: Dylan Valleau

# Characterizing Progesterone Responsiveness for Engineering *In Vitro* Models of Endometriosis

**Maria Hernandez**, Ellen Kan, Jose Cadavid, Linda Griffith

CGR, Dept. of Biological Engineering, MIT, Cambridge, MA 02139, USA

Endometriosis, defined by growth of endometrial-like tissue outside the uterus, is a debilitating disease that affects 10% of women yet remains poorly understood. In the normal endometrium, elevated progesterone levels after ovulation suppress estrogen-induced proliferation and initiate decidualization, in which the endometrial stroma undergoes extensive remodeling in preparation for potential embryo implantation. However, some endometriosis patients experience "progesterone resistance," in which progesterone signaling is dysregulated, resulting in abnormal pathophysiological changes including impaired decidualization[1]. *In vitro* models of endometriosis hold potential for investigating patient-specific differences in disease phenotype and could elucidate mechanisms of progesterone resistance. To engineer such physiologically-relevant models, we are developing a protocol to characterize progesterone responsiveness from endometrial stromal cells (ESCs) derived from both control and endometriosis patients. Primary ESCs – isolated from 10 different donors – were encapsulated in a peptide-functionalized polyethylene glycol hydrogel that allows for recapitulation of key endometrial phenotypes[2]. The cells were stimulated to decidualize with progesterone and cyclic adenosine monophosphate over 6 days, with supernatant collected every 48 hours for downstream analyses. To simulate an inflamed microenvironment, exogenous IL-1β was added to some cultures as a positive control. Prolactin ELISAs were used to determine progesterone responsiveness of ESCs, as prolactin secretion is an established marker of stromal decidualization[1]. We observed a wide range of prolactin levels in the absence of IL-1β; notably, endometriosis-associated ESCs tended to produce less prolactin, highlighting the utility of this model for revealing disease-relevant variations among donors. We are in the process of optimizing immunofluorescence staining of key decidual markers as an additional characterization metric. These findings can help further investigate the downstream effects of progesterone resistance on disease progression, including effects on microvascular function and immune cell recruitment. Ultimately, models able to capture differences in progesterone responsiveness could enable improved clinical stratification of endometriosis patients and inform more targeted treatments.

[1]*MacLean, JA 2nd, Hayashi, K. (2022) Cells. 11,4 647*

[2]*Gnecco, JS et al. (2023) Med. 4, 8*

**Faculty Supervisor: Linda Griffith**

**Graduate Student Mentor: Ellen Kan**



# Optimizing Production of Withanolide Intermediates in Tobacco and Yeast

**Jonathan Huang**, Erin Reynolds, Jing-Ke Weng, Mary Gehring

Whitehead Institute, Dept. of Biology, MIT, Cambridge, MA 02139, USA

Withanolides are a class of steroidal lactone natural products synthesized by the medicinal plant *Withania somnifera*, among other species. The proposed biosynthetic pathway for withanolides begins with a previously identified sterol isomerase that acts on the substrate 24-methylenecholesterol[1], but the rest of the pathway is currently unknown. To elucidate further enzymes in the pathway, we used two methods to express candidate enzymes: (1) *Agrobacterium*-mediated transient expression in the model plant *Nicotiana benthamiana* and (2) genomic integration in the yeast *S. cerevisiae*. After identifying several key enzymes and intermediate compounds in the pathway, further progress was hindered by low yields of early withanolide intermediates, making detection of later intermediates difficult. Using the plant system, we aimed to increase withanolide production via transient expression of both a previously identified transcription factor shown to influence withanolide production in *W. somnifera*[2] and *tHMGR*, a truncated version of HMG-CoA reductase, the rate-limiting enzyme in the mevalonate pathway that produces sterols. Although *tHMGR* overexpression led to a significant increase in sterol precursors such as squalene, we observed only a small increase in withanolide intermediates. We attributed this to the complexity of the plant sterol biosynthesis pathway. Turning to the yeast system, we upregulated four pairs of enzymes in the native yeast mevalonate pathway. Although this also led to an increase in sterol precursors, we observed a decrease in withanolide intermediate production. We hypothesize this is due esterification of sterol products by native yeast enzymes such as ARE1 and ARE2, which would render pathway precursors and early intermediates unavailable to downstream enzymes. Direct knock-out of the esterification enzymes would likely be deleterious due to their important role during yeast growth, so we plan to engineer a system with repressible esterification enzymes and inducible esterase enzymes in an attempt to improve withanolide production in yeast.

*1. Knoch et al. (2018) PNAS. 115, 34, E8096-E8103*

*2. Singh et al., (2017) The New Phytologist. 215, 3, 1115-1131*

**Faculty Supervisor: Jing-Ke Weng, Mary Gehring**

**Graduate Student Mentor: Erin Reynolds**

## Perturbations of Rho Regulation Changes Actomyosin Organization and Force Generation During Ventral Furrow Formation

**Katherine Kitzinger**, Jonathan Jackson, Adam Martin

Dept. of Biology, MIT, Cambridge, MA 02139, USA

During development organisms undergo changes in shape to form increasingly complex structures through the process of morphogenesis. To create these structures, organisms' cells generate coordinated internal forces to reorganize themselves. A major generator of these forces is the activity of actin and myosin (collectively referred to as actomyosin), which is regulated by a GTPase switch RhoA. In *D. melanogaster,* one early actomyosin driven morphological change is the formation of the ventral furrow. During furrow formation, cells within the furrow region exhibit myosin pulses creating a ratcheted shrinking of the apical (outer) surface of the cells. When RhoA activation is perturbed by the over or underexpression of CGAP and RhoGEF2, the behavior of the myosin spots and the ability of the embryo to form a ventral furrow is changed. As CGAP and RhoGEF2 inactivate and activate RhoA, respectively, we anticipated that overexpressing both of them simultaneously would result in a similar phenotype as the control. Using *D. melanogaster* with GFP tagged myosin, we used confocal laser scanning microscopy to live image ventral furrow formation of embryos with five different perturbations of RhoA regulation to better understand how coordinated actomyosin activity drives ventral furrow formation. Noting differences in the size and patterning of the myosin spots, we measured the coverage fraction of the furrow using the machine learning program Ilastik. While the overall level of myosin was similar to the control, we found that the behavior of the myosin was altered. Our results suggest that RhoA signaling activity does not simply reflect a balance between activator and inhibior, but supports a more complex regulatory logic.

**Faculty Supervisor: Adam Martin**

**Graduate Student Mentor: Jonathan Jackson**



## Editing a Zinc Finger Inducible Promoter via Recombinases to Change Expression

**Yunbeen Bae**, Sneha Kabaria, Kate E. Galloway

Department of Chemical Engineering, MIT, Cambridge, MA 02139, USA

Engineering mammalian cells provides key insight into translatable research, but it requires precise control of gene expression to recapitulate and manipulate biology. Furthermore, natural systems often exhibit expression changes over time which can be difficult to precisely control with current synthetic biology tools. Toolkits like Composable Mammalian Elements of Transcription (COMET) provide design rules to vary the strength of a zinc finger (ZF) dependent promoter, but their features are often statically encoded.[1] Furthermore, current methods of gene expression control are binary or transient, limiting the extent to which we can engineer and recast biological systems. Therefore, we explore a new method that can introduce a stable change in gene expression between two non-zero levels upon a stimulus. To this end, we apply the COMET toolkit and the Cre-Lox recombinase system to edit the DNA length between the last ZF binding site and the TATA box to increase gene expression. In transient transfection, excising the spacer causes various fold increases in the downstream reporter gene expression. In addition to varying the spacer length, the level of ZF expression can modify the output gene expression level. This modularity of our design allows multiple ways to alter the reporter gene expression, which can enable multiplexing a variety of inputs to confer downstream changes. This work using recombinases to edit the DNA composition of a ZF-dependent promoter allows heritable changes to tune gene expression. Altogether, these designs can add to the synthetic biology toolkit by facilitating complex biological behaviors in mammalian cell engineering.

*1. Donahue, PS et al. (2020) Nature Communications. 11, 779*

**Faculty Supervisor: Kate E. Galloway**

**Graduate Student Mentor: Sneha Kabaria**

# Interfacing Domain-Specific Foundation Models with General Large Language Models for Accurate Proteomic Analysis

**Pari Latawa**, Alvin Chan, Giovanni Traverso, Robert Langer

Koch Institute for Integrative Cancer Research, MIT, Cambridge, MA 02139, USA

Department of Biological Engineering & Mechanical Engineering, MIT, Cambridge, MA 02139, USA

With the rapid advancement of machine learning and artificial intelligence techniques, there has been an increased exploration of the applications of these technologies in the fields of biology, drug discovery, proteomics, and computational chemistry. A notable area of high interest is the use of Large Language Models (LLMs). LLMs are deep-learning models with the ability to comprehend and generate language, and they offer huge opportunities when applied to domain-specific problems in biology. Alongside the general large-scale LLMs such as GPT-4, BERT, and Llama-2, biology-specific LLMs such as Evolutionary Scale Modeling (ESM) and deep-learning models such as AlphaFold-2 have demonstrated high accuracy and efficiency for protein structure prediction and molecule generation. Traditional techniques for training these models have involved first pretraining the models on initial datasets, and then fine-tuning the parameters of the models for specific downstream tasks—but this can be time-consuming and expensive since deep-learning models are composed of billions of parameters. As such, this project seeks to explore a novel interface that allows for the communication and integration of large language models such as Llama-2 with biology-specific foundation models such as ESM to allow access to rich protein knowledge through a text interface. The development of such an interface that allows general large deep learning models to coordinate information retrieval and processing through specialized models in biology holds tremendous potential for increasing the prediction power and coverage of these models.

Faculty Supervisor: Giovanni Traverso, Robert Langer

Postdoc Mentor: Alvin Chan



# Cohesin RAD21 Promotes Repair of Oncogenic Replication Stress-Induced Damage in Ewing Sarcoma

**Kate Lu**, Ruoxi W. Wang, Sofia Hu, Xiaofeng A. Su, Jacqueline A. Lees

Koch Institute for Integrative Cancer Research, Dept. of Biology, MIT, Cambridge, MA 02139

*EWS-FLI1*, the fusion oncogene that drives pediatric Ewing sarcoma, is a strong inducer of replication stress. It causes G1/S phase acceleration, R-loop accumulation and DNA damage, often leading to growth defects and senescence in primary cells. We previously demonstrated that gain of a copy of the cohesin subunit gene, *RAD21*, significantly mitigates EWS-FLI1-induced replication stress, promotes Ewing sarcomagenesis, and partially drives high recurrence of chromosome 8 gain in Ewing sarcoma.[1] Currently, we find that EWS-FLI1 expression in both normal and Ewing sarcoma cancer cells induces transcription-replication conflicts (TRCs) as indicated by significant increases in RNA polymerase II (PNAPII)-PCNA interacting foci by proximity ligation assay (PLA). Over-expression of RAD21 significantly reduces the TRCs caused by EWS-FLI1 expression in primary euploid cells, whereas reduction of RAD21 levels in a trisomy 8 cancer cell line increases TRCs. To determine the interaction between RAD21 and TRCs, we mapped genome-wide association between RAD21, DNA repair proteins and the transcription/replication machineries in Ewing sarcoma cancer cells using the CUT&RUN approach. We identified a large number of putative TRC regions indicated by convergent transcription pausing, DNA damage and replication stress signals in the presence of EWS-FLI1. Importantly, cohesin/RAD21 binding is highly enriched at these TRC regions and knocking down EWS-FLI1 drastically attenuates such binding, suggesting that RAD21 facilitates resolution of oncogene-induced TRCs. Furthermore, we discovered that expression of EWS-FLI1 impairs fork progression and causes stalled forks. Increased RAD21 levels significantly promote fork progression and reduce fork stalling in the presence of EWS-FLI1. Altogether, our findings suggest that increased RAD21 at the fork lesions resolves TRCs and mitigates replication stress in EWS-FLI1-expressing cells by promoting fork progression. These novel findings provide mechanistic evidence on how RAD21 promotes repair of oncogenic stress-induced DNA damage, and further suggests this cohesin subunit as a potential therapeutic target in treating Ewing sarcoma.

*1. Su, XA et al. (2021) Genes & Development. 35(7-8), 556-572*

Faculty Supervisor: Jacqueline A. Lees

Postdoc Mentor: Xiaofeng A. Su

## Conditionally Activatable Fusogenic Liposomes for Drug Delivery

**Ananth Shyamal**, Qian Zhong, Sangeeta Bhatia

Koch Institute for Integrative Cancer Research, MIT, Cambridge, MA, 02139, USA

Macromolecular drugs are rapidly being developed as therapeutics to act directly on intracellular targets. However, targeted drug delivery to the pathological microenvironment and subsequent drug transport to subcellular organelles are long-standing challenges limiting therapeutic efficacy. To address these issues, we engineered a class of conditional fusogenic liposomes (C-FLIPs) for efficient cytosolic delivery of susceptible biomacromolecules in a disease-specific manner. The C-FLIPs comprise fusion blockers, disease-specific protease substrates, and drug-loaded fusogenic liposomes. The C-FLIPs fuse with cells only when fusion blockers are removed via catalytic reactions of proteases that are dysregulated in the pathological microenvironment. This design allows payload release directly into the cytoplasm, bypassing the endosomes. We have demonstrated that the C-FLIPs enable highly efficient cytosolic delivery of membrane-impermeable drugs, including peptide-based proteolysis-targeting chimera, protein/enzyme therapeutics, and CRISPR-Cas9/guide RNA ribonucleoproteins. We further designed a matrix metalloproteinase-cleavable C-FLIP that enables targeted delivery of granzyme B (GzmB) into cancer cells in a perforin-independent manner. In lung and colorectal cancer mouse models, C-FLIP/GzmB treatment significantly suppressed tumor growth by inducing both apoptosis and gasdermin E-mediated pyroptosis. Furthermore, the C-FLIP/GzmB strongly synergized with immune checkpoint blockade (ICB). *In vitro*, C-FLIP/GzmB-treated cancer cells and conditional media upregulated the expression of activation markers on dendritic cells. *In vivo* immune profiling revealed that C-FLIP/GzmB treatment alone or in combination with ICB substantially increased the ratio of effector CD8 T cells to immunosuppressive regulatory T cells and reduced CD8 T cell exhaustion, suggesting that C-FLIP/GzmB can reprogram the tumor immune microenvironment. Therefore, the non-viral, cell-free C-FLIPs represent a promising platform that addresses both imprecise deliveries to target sites and inefficiencies in the cytosolic delivery of impermeable biomacromolecules and could enable new opportunities in tackling intracellular delivery of an extensive palette of macromolecular therapeutics for immunotherapy and gene editing.

Faculty Supervisor: Sangeeta Bhatia

Postdoc Mentor: Qian Zhong



## Metabolic Rewiring During Differentiation of the Pancreatic Epithelium in Standard and Physiologic Culture Conditions

**Jessica Mann**, Jeffrey Davis, Matthew Vander Heiden

Dept. of Biology, MIT, Cambridge, MA 02139, USA

Human embryonic stem (hES) cells are a powerful tool to model and treat diseases such as type 1 diabetes. The utility of these cells largely depends on their ability to recapitulate the metabolic pathways underlying tissue-specific physiology, including glucose-stimulated insulin secretion for diabetic patients. Yet, a significant limitation of hES cells is that functionality in culture conditions does not always match actual efficacy in more physiological conditions such as in patients after transplantation. Current protocols to differentiate stem cell-derived beta cells for diabetic patients produce cells with immature insulin secretion phenotypes, caused by defects in cellular metabolism. Therefore, understanding how the nutrient environment affects hES cell metabolism is essential to improving the development, differentiation efficiency, and functionality of stem cell-derived therapies. Here, we compare hES cell proliferation and differentiation into pancreatic progenitor cells using two different media conditions: mTeSR1, a commonly-used commercial growth medium, and flow-through adult bovine serum (ftABS), a serum-based culture medium containing near-completely physiological metabolite levels. We first show that hES cells proliferate similarly and maintain proper capacity for differentiation in both media conditions. Furthermore, physiologic oxygen and ftABS culture conditions allow for differentiation of pancreatic epithelium at the same efficiency as standard differentiation protocols. We also characterized how hES cell metabolism is rewired in these physiological and standard environments using stable isotope tracing and oxygen consumption measurements. Strikingly, it appears that the balance of lactate fermentation and the TCA cycle are shifted more toward mitochondrial metabolism in physiologic culture conditions despite decreased oxygen tensions. These results suggest that aerobic glycolysis is dictated by the nutrient environment much more strongly than oxygen tensions within physiologic ranges seen by the embryo in development.

Faculty Supervisor: Matthew Vander Heiden

Postdoc Mentor: Jeffrey Davis

# Characterization of ribosomal haploinsufficiency in human red blood cell development

**Erin Thompson**, Christine Goglia, Orville Kirkland, Hojun Li

Koch Institute for Integrative Cancer Research, MIT, Cambridge, MA 02139, USA

Diamond-Blackfan Anemia (DBA) is an inherited form of anemia where insufficient numbers of red blood cells are produced. Nearly all of the identified mutations associated with DBA cause haploinsufficiency in genes encoding ribosomal biogenesis proteins. The downstream effects of ribosomal haploinsufficiency in erythroid cells remain uncertain. My research aims to increase our understanding of how the red cell lineage is affected by ribosomal haploinsufficiency and the cellular consequences of such mutations. Haploinsufficiency of the large ribosomal subunit-associated protein 35A (RPL35A) is a known genetic cause of DBA, and as such we performed CRISPR engineering to model haploinsufficiency of the *RPL35A* gene in the erythroleukemic cell line, K562. After initial generation and screening of 96 clones targeted for haploinsufficient mutations using Cas9, I used next generation sequencing to validate three haploinsufficient clones for destructive frameshift mutations at the 5' end of the *RPL35A* gene. Given that extended culture of these clones would likely result in adaptation to the *RPL35A* haploinsufficiency, I performed molecular cloning to generate a lentiviral vector expressing *RPL35A* under the control of a tetracycline-inducible promoter. I transduced wild-type K562 cells with this vector and used western blotting to validate that doxycycline treatment results in RPL35A overexpression. I then transduced my *RPL35A* haploinsufficient engineered cell lines with the overexpression vector, and am currently pursuing western blot confirmation of inducible rescue of the haploinsufficiency in the mutated clones. These lentiviral-rescued *RPL35A* haploinsufficient K562 cells will be able to undergo extended culture, without adaptation, in the presence of doxycycline, and will also be able to undergo rapid induction of haploinsufficiency upon doxycycline withdrawal. My broader goal is to use this cell line for transcriptomic, proteomic, and metabolomic screening for cellular derangements from *RPL35A* haploinsufficiency, as well as CRISPR screening for novel mediators of cellular survival in the setting of ribosomal haploinsufficiency.

Faculty Supervisor: Hojun Li



# Exploring how quantitative differences in tissue injury alter local immune responses and susceptibility to tumorigenesis

**Anika Wadhera**,[1,2,3] Chris Skalnik,[1,3] Praveen Parasar,[1,3] Harikesh Wong[1,3]

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2 Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139,

3 Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Following tissue injury, damaged cells release inflammatory cues that promote the recruitment of innate and adaptive immune cells. While this accompanying immune response is essential for eliminating threats and fostering tissue repair, a trade-off emerges: under certain settings, it can initiate or accelerate tumorigenesis. To date, this context-dependency remains poorly understood. We hypothesized that differences in the magnitude of tissue injury might promote distinct immune responses, accounting for varying susceptibility to tumorigenesis. To begin to test this hypothesis, we employed high-resolution multiplexed microscopy to monitor innate and adaptive immune cells following quantitatively controlled injuries in murine skin. In response to a 1 mm diameter wound, we observed a spatially-uniform recruitment of neutrophils—critical innate immune cells that sterilize wounds—around the lesion border at 2 but not 7 days post-insult. By contrast, in response to a 2 mm diameter wound, we observed striking spatial asymmetry in neutrophil recruitment around the lesion border at 2 days post insult. At later timepoints, however, this polarized neutrophil response persisted, albeit to a lesser extent, and was accompanied by a spatially asymmetric recruitment of adaptive immune cells—predominantly CD4+ T cells and regulatory T cells (Tregs)—on opposing sides of the wound. These results highlight how relatively small changes in the extent of tissue injury can produce highly divergent shifts in the dynamics of local immune responses. This spatiotemporal variation may provide context, at least in part, for differing susceptibilities to tumorigenesis. Our future studies will employ different murine tumor models to explore this hypothesis in more depth.

Faculty Supervisor: Harikesh Wong

Graduate Mentor: Chris Skalnik

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