

Biology Undergraduate Research Symposium

January 30th | 1–4:30 pm | 68-181

1:00 pm Opening Remarks

1:10–2:10 pm

Amy Fan
Weinberg Lab

Andrea Kriz
Sharp Lab

Kaitlin Allen
Bhatia Lab

Prakriti Paul
Sharp Lab

2:25–3:10 pm

Cynthia Chen
Lodish Lab

Laura McCulloch
Housman Lab

Quynh Nguyen
Saeji Lab

3:40–4:10 pm

Rebecca Shi
Xu Lab

Robert Williams
Wittrup Lab

Vivian Liu
Vander Heiden Lab

4:15 pm Closing Remarks

Organized by:
The Biology Undergraduate Committee
Biology Undergraduate Student Association (BUSA)
Biology Education Office
Sponsored by the Howard Hughes Medical Institute

Contents

2. **Amy Fan**, PLA2G16 Upregulation in Mesenchymal Cancer Cells Contributes to EMT through Multiple Pathways
3. **Andrea Kriz**, Cat and Mouse: Localization of the Exosome Complex Helps Control Promoter Directionality
4. **Kaitlin Allen**, *In vivo* Application of Attenuated *Salmonella typhimurium* for Targeted Anticancer Therapy
5. **Prakriti Paul**, Chromatin-Dependent Alternative Splicing Regulation by RbFox2
6. **Cynthia Chen**, E2F Binding Partner TFDP2 Regulates Terminal Erythroid Differentiation by Modulating Cell Cycle Progression
7. **Laura McCulloch**, Concatemerization of Spinach Aptamers Holds Promise for RNA Visualization *in vivo*
8. **Quynh Nguyen**, *Toxoplasma gondii* Inhibits IFN γ -induced STAT1 Transcriptional Activity by Retaining Phosphorylated STAT1 in the Nucleus
9. **Rebecca Shi**, Shank Proteins Regulate Hippocampal Synaptic Transmission
10. **Robert Williams**, Production of TA99 and 237 Mouse Bispecific T-Cell Engagers and Transcriptome Analysis of BiTE-Activated Human T cells
11. **Vivian Liu**, Effects of Cancer-Associated Mutations on Pyruvate Kinase M2 Enzyme Function

PLA2G16 Upregulation in Mesenchymal Cancer Cells Contributes to EMT through Multiple Pathways

Amy Fan, Jordan Krall, Robert Weinberg

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In the epithelial-mesenchymal transition (EMT), epithelial cells gain mesenchymal traits. In the context of cancer progression, this enables cancers to metastasize. Recent research has shown that shifts in fatty acid (FA) metabolism may play a role in this process. Our studies show that PLA2G16 is highly upregulated in mesenchymal cancer cells relative to epithelial cells. We also show that PPAR γ , a lipid-responsive transcription factor potentially downstream of PLA2G16, is highly upregulated. Increased mRNA levels of CPT1A and FABP3, proteins involved in FA transport, further suggest that fatty acid transport and oxidation (FAO) are upregulated. We propose that PLA2G16 is upregulated in mesenchymal cancer cells, allowing FAs to be released and used in FAO and other cellular processes that enable the cell to gain and maintain mesenchymal characteristics. Further study of PLA2G16 and FA metabolism may implicate PLA2G16 in other roles contributing to EMT and lead to therapeutic targets for cancer.

Faculty Supervisor: Robert Weinberg

Postdoc Mentor: Jordan Krall

Cat and Mouse: Localization of the Exosome Complex Helps Control Promoter Directionality

Andrea Kriz, Xuebing Wu, Phillip A. Sharp

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Divergent transcription is pervasive in mammals, but even though mRNAs and upstream antisense RNAs (uaRNAs) are processed similarly, mRNAs are long and stable while uaRNAs are short and quickly degraded by the exosome, a protein complex with 3' to 5' exoribonuclease activity. Noting that the shortness of uaRNAs leaves their 3' ends much closer to promoters than the 3' ends of mRNAs, we used Chromatin Immunoprecipitation and Sequencing (ChIP-seq) to determine if the exosome was localized to active promoters genome-wide, a position which would enable the complex to degrade uaRNAs from the 3' end while allowing mRNAs to remain stable. We show that genomic regions of exosome localization indeed do significantly overlap and are aligned with Transcription Start Sites but not Transcription End Sites, and are furthermore aligned with markers of active promoters but not a marker of active gene bodies. In addition to uaRNA degradation, these ChIP-seq data provide the first genome-wide localization data for the exosome in mammalian cells, and so will likely be relevant to other RNA processing pathways involving the exosome as well.

Faculty Supervisor: Philip Sharp

Graduate Student Mentor: Xuebing Wu

In vivo Application of Attenuated *Salmonella typhimurium* for Targeted Anticancer Therapy

Kaitlin Allen, Tal Danino, Sangeeta Bhatia

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Bacterial strains which preferentially colonize tumor tissue over healthy body tissue provide an opportunity to engineer directed anticancer therapies. These therapies could alleviate many of the harsh side effects of current chemotherapies while also targeting tumors from the inside out. In this study, the therapeutic efficacy of an engineered strain of attenuated *Salmonella typhimurium* was characterized through the assessment of two luminescent reporter systems. The luxCDABE cassette was included on a plasmid in the experimental therapeutic strain, allowing for visualization of bacterial presence. Tumor cells constitutively expressed firefly luciferase, allowing for the assessment of tumor activity and growth. The experimental strain was designed with a plasmid containing lysis genes under the control of a quorum sensing promoter. This construct was developed with the intent that colonization could yield a density sufficient for the triggering of synchronized bacterial autolysis. Bacterial lysis would release cytolysin proteins which permeate mammalian cells, leading to apoptosis. The test strain was administered intravenously to mice with bilateral hind flank tumors; colonization levels and tumor size were quantified in the time course following. Imaging studies showed peak colonization between Days 2 and 4 post-injection, which coincided with a decrease of one to five orders of magnitude in the luminescence signal corresponding to tumor cell activity. These results suggest possible tumor cell death upon lysis. Further quantification is necessary to reliably determine the precise therapeutic effects observed, but future work will allow for a more sensitively tuned system for the determination of dosage and frequency of therapeutic administration.

Faculty Supervisor: Sangeeta Bhatia

Postdoc Mentor: Tal Danino

Chromatin-Dependent Alternative Splicing Regulation by RbFox2

Prakriti Paul, Mohini Jangi, Philip Sharp

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While it was previously believed that splicing was a post-transcriptional process, recent evidence suggests that splicing may occur co-transcriptionally. Two models that explain this co-transcriptionality are the “recruitment” model, and the “kinetic” model. In the first model, splicing machinery is recruited to introns and exons (often via the C-terminal domain of RNA polymerase) as they are transcribed; while splicing catalysis may be complete before or after transcription, commitment to splicing occurs before the nascent transcript is released. In the second model, the rate of splicing is dependent on the elongation rate of RNA polymerase, in which slower elongation rates have been shown to promote inclusion of alternate exons. Rbfox2 is an RNA-binding protein that binds the motif UGCAUG and regulates various alternative splicing events. From co-IP and ChIP experiments, we observe that Rbfox2 interacts with RNA polymerase and binds gene promoters and enhancers in mouse embryonic stem cells, which are characterized by specific histone marks, such as H3K4 methylation and H3K27 acetylation. We have also identified and validated several Rbfox2-dependent splicing events where the Rbfox2 binding sites have marks characteristic of transcriptional enhancer marks. Given the emerging evidence of chromatin-dependent effects on alternative splicing regulation, I am interested in studying the effect of enhancing or repressing these chromatin marks on alternative splicing patterns regulated by RbFox2. To this end, we tested a variety of small-molecule inhibitors of histone modifying enzymes that globally change the levels of histone marks and assayed inclusion levels of both Fox-enhanced and Fox-repressed exons.

We identified several exons that changed in splicing significantly upon treatment with the HDAC inhibitor Sodium Butyrate, and also with the drugs TCP and Pargyline (both have shown to affect chromatin states). Through these experiments, we are testing the recruitment versus the kinetic model. While the ChIP signal argues against the kinetic model, we will conduct experiments that will specifically test whether altering histone marks affects the kinetics of splicing through altering elongation rates. Thus, we aim to better understand the mechanism by which chromatin state affects the co-transcriptionality of alternative splicing.

Faculty supervisor: Philip Sharp

Postdoctoral mentor: Mohini Jangi

E2F Binding Partner TFDP2 Regulates Terminal Erythroid Differentiation by Modulating Cell Cycle Progression

Cynthia Chen, Harvey Lodish

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Proper coordination of the erythroid cell cycle with its differentiation program is essential for formation of functional mature erythrocytes. Here we identify TFDP2, an E2F-binding partner, as a critical regulator of cell cycle control in terminally differentiating murine erythroid progenitors. Both Tfdp2 and E2f2 expression are upregulated by erythroid-specific transcription factors GATA1 and TAL1 during terminal differentiation, whereas many E2F2 target cell cycle genes are concomitantly downregulated. Knockdown of Tfdp2 expression results in significantly reduced rates of proliferation, and reduced upregulation of many erythroid-important genes. TFDP2 co-localizes with E2F2 in the nucleus of erythroid precursors, and loss of Tfdp2 also globally inhibits the normal downregulation of many E2F2 target cell cycle genes, causing cells to accumulate in S phase and resulting in increased erythrocyte size. These findings highlight the importance of TFDP2 in modulating expression of cell cycle genes repressed during differentiation by E2F2 and provide a unifying molecular mechanism for coupling erythroid cell cycle control with terminal differentiation.

Faculty Supervisor: Harvey Lodish

Concatemerization of Spinach Aptamers Holds Promise for RNA Visualization *in vivo*

Laura McCulloch, Eric Wang, David Housman

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Visualization of single molecules of RNA within living cells is an important but challenging problem. One promising new system for RNA imaging is Spinach, an RNA aptamer that binds the GFP-mimic, 3,5-difluoro-4-hydroxybenzylidene imidazolinone (DFHBI).

1) While neither molecule fluoresces on its own, the complex produces a GFP-like signal, and by attaching Spinach to RNAs of interest, individual RNAs can in principle be visualized fluorescently. However, the fluorescence produced by a single Spinach-DFHBI complex is insufficient to visualize an individual RNA. To strengthen the Spinach signal, we have concatemerized several Spinach aptamers, each with a unique "stem" (hairpin sequence) to prevent sequence-repetition-based misfolding. We have demonstrated near-linear increases in brightness *in vitro* when using longer Spinach concatemers. Despite these improvements in Spinach visualization, we have been unable to produce sufficient signal to effectively see individual RNAs *in vivo*. Recently, it has been hypothesized that low Spinach signal may result in part from the aptamer's low T_m of 34°C; a substantial proportion of Spinach molecules may therefore be unfolded at 37°C, reducing Spinach-DFHBI interactions and consequently decreasing fluorescence. To improve thermal stability and folding, a modified version of Spinach with a T_m of 38°C, coined Spinach2, has been created.

2) We have produced concatemers of Spinach2, using the same unique stem approach we used with Spinach. We will evaluate our concatemers *in vitro* and *in vivo* to assess the efficacy of the system as an RNA-visualization tool. Being able to observe single molecules of RNA using Spinach2 will prove to be of great utility in many studies of RNA localization and particularly inform our study of RNA-based human diseases..

Faculty Supervisor: David Housman

Postdoc Mentor: Eric Wang

***Toxoplasma gondii* Inhibits IFN γ -induced STAT1 Transcriptional Activity by Retaining Phosphorylated STAT1 in the Nucleus**

Quynh Nguyen, Ana Camejo, Jeroen Saeij

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

The interferon gamma (IFN γ) pathway triggers potent host defense mechanisms to inhibit pathogen growth and survival. IFN γ binding to a host cell surface receptor leads to phosphorylation of the transcription factor STAT1, which translocates to the nucleus and activates transcription. *Toxoplasma gondii*, an opportunistic pathogen which chronically infects about 30% of the world population, causes life-threatening disease in immunosuppressed individuals and the developing fetus. Previous studies have shown that *Toxoplasma* infection blocks IFN γ -induced gene expression and STAT1 transcriptional activity, although the mechanism by which this inhibition occurs remains unknown. In this study, through quantification of nuclear phospho-STAT1 in immunofluorescence assays, we find that over a 24-hour period, phosphorylated STAT1 accumulates and is retained in the nucleus of IFN γ -stimulated cells infected with a type I, II, or III strain of *Toxoplasma*. The levels of nuclear phospho-STAT1 in infected, stimulated cells are 2- to 6-fold higher than those levels in uninfected, stimulated cells, and increase between 6 and 24 hours. This parasite-induced prolonged nuclear accumulation is not due to the activity of the parasite rhopty kinase ROP16 and is also observed when cells are stimulated with IFN β , another cytokine involved in the immune response. Overall, we conclude that *Toxoplasma gondii* is inhibiting gene expression by keeping STAT1 bound to DNA, thus preventing its reactivation and further rounds of STAT1-mediated transcriptional activity.

Faculty Supervisor: Jeroen Saeij

Postdoc Mentor: Ana Camejo

Shank Proteins Regulate Hippocampal Synaptic Transmission

Rebecca Shi, Weifeng Xu

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The postsynaptic density (PSD) of excitatory glutamatergic synapses contains many PDZ-proteins that assemble into a large molecular scaffold. These proteins include the Shank (SH3 and multiple ankyrin repeat domains) family proteins, encoded by three genes (Shank1, Shank2, and Shank3). Shank1 has been proposed to be a master regulator of the postsynaptic density, and Shank2 and Shank3 have been implicated in Autism Spectrum Disorders (ASDs). This project seeks to determine the relative contribution of Shanks to regulation of glutamatergic synaptic function in mature neurons and to examine functional differences among different family members. Quantitative Western blot showed that the expression of Shank family proteins increased throughout development, correlating with synapse development. Shank3 expression showed a potential delay of onset compared to those of Shank1 and Shank2. To analyze the functional role of Shank proteins, we used lentivirus-mediated expression of small hairpin RNAs (shRNAs) to knockdown endogenous Shank proteins in hippocampal CA1 neurons by targeted injection. Simultaneous dual whole cell recording was then used to measure AMPA and NMDA receptor-mediated excitatory postsynaptic currents (AMPA and NMDAR EPSCs) in these infected hippocampal slices. Knockdown of Shank1 or Shank2 decreased AMPAR evoked EPSCs (eEPSCs) with no effect on NMDAR eEPSCs, primarily due to a decrease in the number of AMPAR-containing synapses. By superinfecting two viruses at once, we simultaneously knocked down Shank1 and Shank2, which decreased both AMPAR and NMDAR eEPSCs, and the number and unitary response size of AMPAR-containing synapses. Knockdown of Shank3 individually did not have significant effects on synaptic transmission, possibly due to the relatively low expression of Shank3 at early developmental stages in hippocampal CA1 neurons. Future studies will focus on how neural activity and developmental stages may influence the relative contribution of different Shank family proteins in regulating glutamatergic synaptic transmission.

Faculty Supervisor: Weifeng Xu

Production of TA99 and 237 Mouse Bispecific T-Cell Engagers and Transcriptome Analysis of BiTE-Activated Human T cells

Robert T. Williams, Cary F. Opel, Li-Lun Ho, K. Dane Wittrup,

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Bispecific antibodies, capable of binding to two unique epitopes, have shown promise in the field of targeted immunotherapy for cancer. Fusing the antigen binding domains of monoclonal antibodies against CD3, a co-receptor of the T-cell receptor involved in T-cell activation, and against a tumor-specific antigen yields a protein capable of linking T-cells to tumor cells. These bi-specific T-cell engagers (BiTEs) direct T-cell cytotoxic activity against target cells. Here, we reproduced a 237-BiTE against a murine cell line (Ag104A) that contains a mutation in the molecular chaperone Cosmc, causing the development of a tumor-specific glycosylation antigen on the transmembrane protein OTS8. We observed specific binding of the 237-BiTE to both murine T cells and Ag104A cells. We also designed a BiTE targeting tyrosinase-related protein 1 (TRP1), an enzyme involved in melanin synthesis and expressed in melanomas, using the monoclonal antibody TA99. Binding of the TA99-BiTE to either murine T cells or TRP1-expressing murine melanoma B16 cells was not observed. Finally, we investigated the transcriptional impact of BiTEs in human CD8+ T cells activated against carcinoembryonic antigen-expressing (CEA) cells by a CEA-BiTE. Preliminary analysis of the T cell transcriptome shows upregulation of proliferation markers, granzymes, and IFN- γ in BiTE-activated CD8+ T cells.

Faculty Supervisor: K. Dane Wittrup

Postdoc Mentor: Cary F. Opel

Effects of Cancer-Associated Mutations on Pyruvate Kinase M2 Enzyme Function

Vivian Liu, William Israelson, Matthew Vander Heiden

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Pyruvate kinase catalyzes the final step of glycolysis and regulation of its activity is important for control of central carbon metabolism. In cancer cells, the M2 isoform of pyruvate kinase (PKM2) is preferentially expressed over the constitutively active M1 isoform. Regulation of PKM2 activity impacts cell proliferation, and PKM2 mutations are found in human cancer patients. The role of PKM2 in cancer can be better understood by analyzing the functional effects of these mutations. Kinetic parameters (V_{max} , K_m , Hill coefficient) were obtained for each mutant PKM2 (P117L, R246S, G415R, and R455Q). Overall, these mutants exhibited either lowered maximal activity or reduced affinity for substrate compared to wild-type PKM2. In addition, when compared to wild-type, these mutations either lowered or abolished binding by the key allosteric activator fructose-1,6-bisphosphate (FBP). These mutations thus have significant effects on the activity and regulation of PKM2, which is consistent with the hypothesis that down-regulation of enzyme activity is important for proliferative metabolism.

Faculty Supervisor: Matthew Vander Heiden

Postdoc Mentor: William Israelson