MIT Biology 2013 Undergraduate Research Symposium

Thursday, January 31st
12:00–4:30 pm
Room 68-181
Refreshments Served

All are welcome!

Invited Speakers:

12:00
Opening Remarks

12:10–1:10 pm
Matthew Skalak, Bhatia Lab
Tatiana Mamaliga, Dedon Lab
Erica Pino, Desimone Lab
Jaime Reyes, Guarente Lab

1:25–2:10 pm
Natanya Kerper, Jacks Lab
Elliot Akama-Garren, Jacks Lab
Jacob Austin-Breneman, Sive Lab

2:25–3:10 pm
Angela Zhang, Ribbeck Lab
Eric Boyer, Langer Lab
Catherine Koch, Sabatini Lab

3:25– 4:10 pm
Meme Tran, King Lab
Max Wu, Walker Lab
Tuyen Phung, Laub Lab

4:10 pm
Closing Remarks

MIT DEPARTMENT OF BIOLOGY

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

Contents

In Vivo Testing of a Bacterial Cancer Diagnostic

Matthew Skalak, Tal Danino, Sangeeta Bhatia
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Engineering bacteria to act as a cancer diagnostic tool could be an alterative solution to the problem of invasive diagnosis techniques. Bacteria have been shown to selectively colonize, and grow, and express plasmids in tumors. In the present study, *Escherichia coli* with the pTKWlacZ plasmid were IV injected into mice having either hind flank tumors or orally administered to mice with liver metastasis tumors, then induced with IPTG. Cleavage of 6-O-β-galactopyranosyl luciferin (lu-gal) increased over time in nude mice containing both flank tumors and bacteria, but not significantly. Control mice containing tumors but no bacteria had a significantly higher rate of cleavage than other controls (no tumors no bacteria, no tumors with bacteria). Real time imaging of bacterial colonization was done using IVIS Luminescent imaging in hind flank tumors while liver tumors were imaged after the final time point and subsequent dissection from the mouse. Colonization was observed for all tumors. This study showed that the pTKWlacZ plasmid is capable of expression *in vivo.* Although significant detection was not observed in the subcutaneous model, the liver metastasis model showed statistically significantly higher lacZ activity when liver tumors were sufficiently colonized. Control of induction of the pTKWlacZ plasmid was of concern, as well as background cleavage by the tumors. Improvements include injecting each mouse separately with IPTG to control induction and using competitive substrate to reduce background cleavage. In the future more tests are necessary to produce more robust, tunable results.

Faculty Supervisor: Sangeeta Bhatia

Postdoc Mentor: Tal Danino

Defining the role of 5-Hydroxycytidine in 23S rRNA in Oxidative Stress

Tatiana Mamaliga, Chen Gu, Peter Dedon
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Non-coding RNA in prokaryotic and eukaryotic cells is decorated with dozens of post-transcriptionally modified ribonucleosides in addition to the canonical C, U, A, and G. One of these modifications, 5-hydroxycytidine (ho5C), is located in the 23S ribosomal RNA (rRNA) of *E. coli* and its low reduction potential suggests a possible role in the bacterial oxidative stress response. However, the role of rRNA degradation in prokaryotic oxidative stress is not as clearly established as it has in eukaryotic cells in which there is a selective degradation of 28S rRNA. To test this model, we defined the correlation between the oxidation of ho5C and the degradation of 23S rRNA in *E. coli* subjected to oxidative stress. Using a chromatographic method to quantify rRNA species and mass spectrometry to quantify ho5C, we observed that, in *E. coli* exposed to H_2O_2, ho5C decreases in parallel with a selective loss of 23S rRNA. We next tested the hypothesis that reactive oxygen species arising during antibiotic treatment would similarly affect ho5C and 23S rRNA. However, dose-response studies for *E. coli* exposed to equitoxic doses of bactericidal antibiotics revealed only a small decrease in the 23S:16S rRNA ratio with ampicillin treatment and an increase in the ratio with norfloxacin. Our results point to a potential role for ho5C as a redox sensor in the oxidative stress response mechanism of *E. coli*. Furthermore, the comparison of H_2O_2 and antibiotics suggests that the preferential degradation of 23S rRNA in *E. coli* is specific to certain types or levels of reactive oxygen species. That this role for ho5C is not unique to *E. coli* is supported by our observation of ho5C in the 23S rRNA of *Helicobacter pylori* and *Mycobacterium smegmatis*, which suggests that this modified ribonucleoside might be a component of 23S rRNA of many prokaryotes.

Faculty Supervisor: Peter Dedon

Graduate Student Mentor: Chen Gu

In vivo Measurements of Light Propagation and Brain Heating: Implications for Optogenetics

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Currently, there are multiple light propagation models characterizing light *in vitro* which show that light irradiance (i.e., power density) decreases in the brain with distance from the illuminator, due to scattering in tissue, as well as geometric effects. However, when measured *in vivo*, other factors, such as the absorption of light by blood, may modulate the light power that can reach a neural target. Such absorption processes can also result in tissue heating, an important factor to consider in optogenetic experiment design. Therefore, we sought characterize light propagation and tissue heating in the living mouse brain. We measured absolute powers of red (635 nm) and green (532 nm) light at different distances (0.5-2.5 mm) from a penetrating optical fiber in the anesthetized mouse brain with an isotropic light probe. We also implemented a broadly emitting illuminator, which allows for illumination from the brain surface, and measured light powers and induced temperatures as a function of depth from the surface of the brain. Tested light power densities included 1- 50 mW/mm2 for the broadly emitting illuminator and 1-100 mW/mm2 for the penetrating optical fiber, over pulse durations commonly used for *in vivo* mammalian optogenetics. It was found that, at certain depths, red light propagates up to five times the distance as green light at equivalent densities. It was also found that too high of a laser duty cycle does not allow adequate time for the brain tissue to return to its baseline temperature, thus resulting in additive heating with each light pulse.

Faculty Supervisor: Robert Desimone

Mentors: Leah Acker and Edward Boyden

Caloric Restriction-like Regulation of the Neuroendocrine Axis by SirT1 as Evidenced in Pituitary-specific SirT1 Knock Out Mice

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There is mounting evidence suggesting a strong role for SirT1 in mediating the effects of calorie restriction (CR). Modulation of the neuroendocrine axis is a common trait in most long-lived mouse mutants and mice on calorie restriction. Normal mice on CR have suppressed growth and reproductive hormonal axes, compared to *ad libitum* (AL) controls. CR mice have reduced body size, pituitary and testis weights, sperm counts, and serum IGF-1, LH, and FSH. Importantly, CR mice have reduced pituitary SirT1 protein levels when compared to AL controls. Therefore, we developed pituitary-specific SirT1 knockout (PitSKO) mice. PitSKO mice are dwarfed with reductions in pituitary size. Further analysis of PitSKO mice found reductions in pituitary stores of GH, LH, FSH and serum levels of IGF-1, LH, and FSH, similar to mice on CR. We hypothesize that CR reduces pituitary SirT1 and suppresses the neuroendocrine axis.

To further elucidate this mechanism, we analyzed pituitary transcripts using RT-PCR. We found that PitSKO mice have reduced GH, PRL, LH and FSH mRNA compared to littermate controls. Interestingly, these transcripts are also reduced in the long-lived Ames and Snell dwarf mice, which harbor mutations in their Pit1 and Prop1 genes. Next, we found that pituitary SirT1 co-precipitates with Pit1 *in vitro* and in *vivo*. Using luciferase reporter assays, we've determined that SirT1 is a Pit1 co-activator. We also observe that Pit1 is differentially acetylated in cell lines using Pit1 and SirT1 expressing plasmids. Analysis of those samples using mass spectrometry revealed that Pit1 acetylates at specific lysine residues in the absence of SirT1. Finally using 90 mice in a pooled experiment, we found that PitSKO mice have hyperacetylated Pit1. These data suggest that increased SirT1 drives growth and reproduction under AL conditions, and mice on CR have decreased SirT1 and resulting Pit1 activity, leading to a smaller less-fertile mouse.

We plan on further testing residues identified my mass spectrometry to determine specific SirT1/Pit1 interactions that result in changes in Pit1 and downstream Pit1 target activity.

Faculty supervisor: Leonard Guarente

Postdoctoral mentor: Michael Bonkowski

Wnt Signaling Promotes Cancer Cell Self-renewal in Mouse Lung Adenocarcinoma

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The Wnt/β-catenin signaling pathway is crucial in regulating embryonic development and maintaining adult stem cells. Abnormal Wnt pathway activation is known to promote cancer formation in tissues such as the colon, ovary, liver, and skin. Recent studies have elucidated the role of the Lgr receptor family (Lgr4, Lgr5, Lgr6) in promoting Wnt signaling through the binding of R-spondin ligands (Rspo1, Rspo2, Rspo3, Rspo4), which act as facultative Wnt pathway agonists. Lgr5 and Lgr6 are expressed exclusively in normal stem cells of the colon and skin, respectively, and may also be expressed in a subpopulation of cancer cells with stem-like properties. In this study we examined the role of Wnt signaling, focusing particularly on Rspo/Lgr signaling, in a mouse model of non-small cell lung cancer (NSCLC) driven by a constitutively active oncogenic KrasG12D allele and deletion of the tumor suppressor p53. Stimulation of mouse NSCLC cell lines with recombinant Wnt3a or Rspo1 significantly increased the formation of tumor spheroids in a 3D self-renewal assay, whereas inhibition of Wnt using either a Wnt response inhibitor or a Wnt secretion inhibitor decreased the ability of cells to form spheroids. Lgr4 and Lgr5 transcripts were significantly upregulated in mouse NSCLC tumors, and spheroid-formation and response to Rspo1 were significantly inhibited by shRNA targeting Lgr4 or Lgr5, suggesting that these receptors collaborate to promote self-renewal of NSCLC cancer cells. These results implicate Lgr/Rspo signaling as a novel target for pharmaceutical intervention. Current efforts are directed at generating tools to evaluate cellular hierarchy in 3D cultures and in tumors *in vivo*. By elucidating the mechanisms that contribute to establishment of cellular hierarchy in cancer we seek to gain new insight into novel treatment strategies for human NSCLC.

Faculty Supervisor: Tyler Jacks

Postdoc Mentor: Tuomas Tammela

Hiding Inducible T Cell Target Peptide Antigens in the Mouse Genome

Elliot H Akama-Garren, Nikhil S Joshi, Tyler Jacks
Koch Institute for Integrative Cancer Research,
Dept. of Biology, MIT, Cambridge, MA 02139, USA

Tumors are critically influenced by immune cells in their microenvironment, particularly tumor-specific T cells. "Inducible" genetically-engineered mouse cancer models are ideal for studying how these cells influence tumor development as tumors arise from previously normal tissues, with defined kinetics, similar to human tumors. However, adapting these models to study tumor-specific T cell responses has been difficult. Ideally, induced tumors would express defined immunogenic T cell target antigens and this expression would be limited just to tumor cells. But attempts to regulate genome-encoded antigens using inducible or tissue-specific promoters have suffered from leaky expression in non-tumor tissues, altering development of antigen-specific T cells. Therefore, a major challenge has been silencing tumor-associated antigens prior to induction. To this end, we are developing a Rosa26 knock-in allele called inversion-inducible joined antigen-GFP (ninja-GFP), in which the DNA sequences encoding a known T cell target antigen (LCMV Glycoprotein 33-41) are physically unable to produce antigen in the "OFF" state because they are discontinuous. Antigen induction requires FlpO recombinase-mediated DNA inversion, which is linked to GFP expression. The ninja-GFP allele also encodes FlpO, which is regulated at three levels, requiring Cre-mediated recombination and subsequent Doxycycline (Dox) and Tamoxifen (Tam)-mediated induction for FlpO activity. Here, we tested KP lung tumor cells containing the Rosa26$^{ninja\text{-}GFP}$ allele (KP$^{ninja\text{-}GFP}$). KP$^{ninja\text{-}GFP}$ cells were GFP-, but infection with FlpO-expressing adenoviruses induced GFP. In contrast, infection with Cre-expressing adenoviruses (Ad-Cre) alone did not induce GFP until cells were subsequently treated with Tam+Dox, even if infection was separated from Tam+Dox treatment by several weeks. This demonstrates tight regulation of the Rosa26$^{ninja\text{-}GFP}$ allele. Moreover, GFP+ KP$^{ninja\text{-}GFP}$ cells were immunogenic, but GFP- cells were not, showing that the Rosa26$^{ninja\text{-}GFP}$ allele was truly silent in the OFF state. Current efforts focus on studying T cell responses against KP$^{ninja\text{-}GFP}$ cells *in vivo* and generating *Rosa26$^{ninja\text{-}GFP}$* mice.

Faculty Supervisor: Tyler Jacks

Postdoc Mentor: Nikhil Joshi

Using the Zebrafish to Study Autism: Use of an Optokinetic Assay

Jacob Austin-Breneman, Alicia Blaker-Lee, Hazel Sive
Whitehead Institute, Dept. of Biology, MIT, Cambridge, MA 02139, USA

Autism Spectrum Disorders (ASD) are widespread, affecting one in 88 children in the United States. Copy number variants (CNVs), where a genomic region is duplicated or deleted, are often associated with ASD, and the 16p11.2 CNV is most frequently altered in patients. Studies by the Sive lab, using the zebrafish, have shown that most 16p11.2 genes are required for normal brain development. Initial assays analyzed brain and body morphology, and axon tract formation. In order to extend analyses of 16p11.2 gene function, a behavioral assay, the optokinetic response, was employed, which assays whether the fish responds to movement. While zebrafish do not develop ASD per se, core symptoms in ASD patients include deficits in eye movements, such as an averting gaze or fixation on an object. The 16p11.2 test gene in this study encoded the Major Vault Protein (*mvp*) that is necessary for normal brain morphology. Using RNAi, which the Sive lab has pioneered as a loss of function (LOF) method in zebrafish, two shRNA hairpins were tested, under control of either the *β-actin* or *miR92b* promoter, using the Tol2 transposase method in an F0 transgenic assay. These hairpins resulted in a whole body or brain morphology phenotype. The optokinetic response was monitored at 6 days-post-fertilization, which is equivalent to a human toddler. The assay employs moving vertical stripes, and tracks eye movements of the fish. In *mvp* LOF larvae, animals were unable to track moving stripes effectively, indicating a abnormal optokinetic response and a behavioral defect. In contrast, the "startle response" to touch was normal, demonstrating selective abnormality after *mvp* LOF. These results demonstrate a connection between an ASD risk gene and an abnormal behavior.

Faculty Supervisor: Hazel Sive

Postdoc Mentor: Alicia Blaker-Lee

Mucins Inhibit *Candida albicans* Adhesion and Filamentation

Angela Zhang, Nicole Kavanaugh, Katharina Ribbeck
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The opportunistic fungal pathogen *Candida albicans* causes epithelial infections evidenced as vaginal, mouth, esophageal, and bloodstream infections that may spread to the brain, kidneys, heart, liver, and lungs. The current model for *C. albicans* infection involves the adhesion of yeast-form cells, followed by maturation to form hyphae and penetration into the epithelia and bloodstream. Mucus, composed of antimicrobial molecules and mucin glycoproteins, is secreted by cells lining the intestinal tract and protects the host from infection. The present study aims to elucidate the mechanism by which mucins inhibit infection. Microarrays were used to screen for genes affected by the presence of mucins, and quantitative PCR (qPCR) was used to more precisely measure the expression of these genes. Microscopy was then used to observe the effect of mucins on *C. albicans* cell morphology and adhesion to silicone, as well as to elaborate upon how mucins change expression of the ZAP1 gene. qPCR indicated that the expression of genes involved in cell adhesion (ALS1, ALS3), hyphae formation (ALS3, ECE1, HWP1), virulence (SAP5), and biofilm formation (ZAP1) appeared to be significantly downregulated by native porcine gastric mucins (PGM). In the presence of mucins, *C. albicans* exhibited decreased adhesion to silicone and decreased filamentation. Growth assays with a ZAP1Δ/Δ strain, which cannot grow on low-zinc medium, showed growth on low-zinc medium supplemented with mucins, suggesting that mucin samples may contain zinc. These findings indicate that mucins inhibit infection primarily by suppressing adhesion and hyphae formation, but may also act on later stages of infection; this provides insight into the role of mucus in protecting the host from infection and suggests the possibility of using mucus in treatments and preventative measures for candidal infections.

Faculty Supervisor: Katharina Ribbeck

Graduate Student Mentor: Nicole Kavanaugh

Novel Topical siRNA Delivery System Utilizing Staphylococcal Exfoliative Toxin A and Lipidoids Nanoparticles

Eric Boyer, Thanh-Nga Tran, David Fisher, Robert Langer
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Active biomolecules such as small interfering RNA (siRNA) is a promising therapeutic for combating disorders where protein expression anomalies cause undesirable phenotypes. However, effective topical delivery of these large and often highly-charged molecules remain one of the most difficult challenges in dermatology and the field of transdermal drug delivery due to the formidable stratum corneum (SC) permeability barrier. Here, we present a method to deliver siRNA intracutaneously into epidermal and dermal cells for the treatment of skin disorders using the biologically active molecule, Staphylococcal Exfoliative Toxin A (SEA). SEA, a protease that cleaves extracellular portion of desmoglein-1 (Dsg1), was optimized to desquamate the stratum corneum barrier after 4 hours. We then showed that lipid-like molecules known as lipidoids allow endocytosis of the siRNA into the target epithelial cells. SiRNA at a concentration of 10nM, is able to reduce its target gene expression by ~100% while causing no decrease in cell viability. This intracutaneous system was validated in tissue culture, a human explant model and a mouse model with human skin xenografts. Particularly, siRNA nanoparticles were shown to effectively inhibit phosphodiesterase degradation of cAMP, enhancing pigmentation production in our human skin explant model. Our study establishes that SEA and lipidoids have the potential to impact therapeutic delivery and ultimately applications in many arenas of drug delivery.

Faculty Supervisors: David Fisher and Robert Langer

Postdoc Mentor: Thanh-Nga Tran

MCT1-mediated Transport of a Toxic Molecule is an Effective Strategy for Targeting Glycolytic Tumors

Catherine Koch, Kivanc Birsoy, David Sabatini
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Oncogenic transformation modifies the metabolic program of cells. One consequence of this transformation is a shift of dependence from oxidative phosphorylation for energy production to glycolysis despite the presence of oxygen—a phenomenon known as the Warburg effect. Alterations in cancerous cells, such as the upregulation of glycolysis, create potential therapeutic targets for anticancer therapies. Inhibition of glycolysis is one such target. A possible manner of inhibiting glycolysis, currently under study as a cancer treatment therapy, is use of the toxic molecule 3-Bromopyruvate (3-BrPA). We conducted a genome-wide haploid genetic screen to identify resistance mechanisms to 3-BrPA. The screen identified loss of the *SLC16A1* gene product, monocarboxylate transporter 1 (MCT-1), as the main mechanism of resistance to 3-BrPA. In addition, MCT-1 is both necessary and sufficient for uptake of 3-BrPA into cells. One manner in which 3-BrPA induces cell death is through glycolytic inhibition by directly inhibiting the GAPDH enzyme in the glycolytic pathway. Tumor xenografts were sensitized to 3-BrPA treatment *in vivo* through forced expression of MCT-1 in cancer cells previously resistant to treatment with 3-BrPA. In addition, the levels of *SLC16A1* mRNA are upregulated in glycolytic tumors and are a good predictor of sensitivity of cancer cells to treatment with 3-BrPA. Our study identifies MCT-1 as a potential biomarker for treatment with 3-BrPA and ultimately illustrates that selectivity of cancer expressed transporters can be exploited for the specific delivery of toxic molecules to tumors.

Faculty Supervisor: David Sabatini

Postdoc Mentor: Kivanc Birsoy

Mutation in TRiC Subunit May Cause Protein Misfolding: Implications for Hereditary Sensory Neuropathies

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Hereditary Sensory Neuropathies (HSN) are a rare group of disorders caused by various genetic mutations and drugs, resulting in a wide range of phenotypes. Some clinical observations include skin infections, ulcers, and loss of sensation to the hands and feet. Genetic testing of HSN patients has led to the discovery of two point mutations in the human chaperone called tailless complex polypeptide-1 (TCP-1) ring complex (TRiC). Due to the difficulty of purifying and expressing all 8 subunits of TRiC, we expressed the human CCT5 subunit individually in *E. coli*, which forms TRiC like ring conformations. We report here that, as observed with transmission electron microscopy, the wildtype and mutant single-subunit CCT5 formed similar sized and shaped double-barrel rings, and sedimented at the same position in a 5–40% sucrose gradient, suggesting that both proteins were the same size and had the same conformations. Both wildtype and mutant CCT5 suppressed initial aggregation of and refolded a model substrate protein, human γ-D crystallin. Although we saw no drastic differences between wildtype and mutant CCT5, more studies are needed to see if a subtle difference is present with different substrates and assays.

Faculty Supervisor: Jonathan King

Graduate Student Mentor: Oksana Sergeeva

Cell Cycle Synchronization of *Sinorhizobium meliloti* and Assessment of Global Gene Expression

CA Max Wu, Nicole De Nisco, Graham Walker
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Rhizobia are α-proteobacteria with the ability to establish a nitrogen-fixing symbiosis with compatible legume hosts. The symbiosis can be viewed as a chronic infection comprised of multiple developmental stages, in which bacteria coordinate their cell proliferation with the development of the host plant cells. Sinorhizobium meliloti progresses through a dramatic terminal differentiation process that includes enlargement of the cell body and endoreduplication of the genome. It has been shown that host factors are largely responsible for bacteroid differentiation, though specific molecular mechanisms are unknown, due in part to the lack thus far of a means of cell synchronization. Here, we describe a method for synchronization of S. meliloti using nutrient deprivation, which triggers cell cycle arrest at the G1 phase. Cells released from starvation replicate and divide synchronously for the duration of approximately one cell cycle. Through RNA microarray analysis we have identified functional clusters of genes involved in responses to nutrient stress and regulation of distinct phases of the cell cycle. Preliminary findings will be presented.

Faculty Supervisor: Graham Walker

Graduate Mentor: Nicole De Nisco

A Novel Type II Toxin-antitoxin System in *Caulobacter crescentus* Blocks Replication Progression through a Direction Interaction with DnaN

Tuyen Phung, Chris Aakre, Michael Laub
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Toxin-antitoxin systems are widely present on bacterial chromosomes, and inhibit growth by many of the same mechanisms as antibiotics. Here, we identified a novel type II toxin-antitoxin in *Caulobacter crescentus*, in which expression of the toxin, SocB, is toxic to the cell by blocking replication progression. We performed a suppressor screen for mutations that can bypass the lethality of *socB* expression, and identified two mutations in *dnaN*, which encodes the replicative beta clamp. Using the bacterial two-hybrid system, we demonstrated that SocB interacts directly with DnaN, and that this interaction is abolished by suppressor mutations in *dnaN*. The two mutations identified reside within the same conserved groove on DnaN that is required for its interaction with HdaA and DNA polymerase III, suggesting that SocB binds DnaN in a similar fashion and sequesters it from its normal protein partners. Given the high degree of overlap between the targets of antibiotics and toxins, we hypothesize that this conserved groove on DnaN may represent a promising target for the development of future antibiotics.

Faculty advisor: Michael Laub

Graduate student mentor: Chris Aakre