Biology Undergraduate Research Symposium 2012

MIT

All are welcome!

Thursday,
February 2, 2012
12:00–5:00 pm
Room 68-181
refreshments served

Schedule:

12:00 pm

Opening Remarks

12:10–1:10 pm

Ryan Alexander, Lodish Lab

Allison Alwan, Boyer Lab

Kateryna Kozyrytska, Drennan Lab

Victoria Lu, Sharp Lab

1:25–2:40 pm

Sabina Sood, Jacks Lab

Frances Chen, Jacks Lab

Hamsika Chandrasekar, Bhatia Lab

Mariya Samoylova, Amon Lab

Lauren Sless

2:55–3:55 pm

Kenneth Lin, Lodish Lab

Melissa Ko, Lodish Lab

Paul Jaffe, Yinxi Lin Lab

Akansh Murthy

4:10–4:55 pm

Sabine Schneider, Langer Lab

Christina Welsh, Nedivi Lab

Samuel Acquah, Yaffe Lab

4:55 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

Mir-365 Represses Osteogenesis during Brown Adipocyte Differentiation

Ryan Alexander, Lei Sun, Harvey Lodish
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Of the two types of body fat found in mammals, brown adipose tissue is specialized to dissipate excess dietary energy stored in intracellular lipid droplets through mitochondrial energy uncoupling as a defense against cold and obesity. Despite several overlapping morphological and functional features shared with white adipocytes, which serve primarily as chemical energy storage depots, brown adipocytes have a closer lineage relationship with skeletal myocytes, as the two are derived from a common Myf5 positive progenitor. [1] Additionally, recent studies have shown that brown adipocytes also have a close developmental relationship with osteoblasts, or bone forming cells. [2] Thus, the developmental basis of brown adipocyte maturation is complex and still poorly understood.

Previously, we showed that a co-transcribed microRNA cluster, miR-193b-365, is a robust brown adipocyte lineage determinant that is both essential for brown adipogenesis and represses the differentiation of C2C12 myoblasts [3]. Here, I propose an additional role of miR-365 in repressing osteogenesis, the process of bone cell maturation, during brown adipocyte differentiation. Repression of mir-365 in primary brown pre-adipocytes not only impairs adipogenesis but also causes a dramatic up-regulation of several osteogenic markers. Also, ectopic expression of mir-365 in MC3T3 osteoblasts is sufficient to down-regulate the expression of key pro-osteogenic transcription factors, Runx2 and Osterix, as well as bone sialoprotein, a late marker of extracellular matrix mineralization.

Lastly, mir-365 is computationally predicted to directly target and repress Igf-1, which has been shown to be essential for osteogenesis at least in part through promoting tight junction formation.

Faculty Supervisor: Harvey Lodish

Postdoc Mentor: Lei Sun

1. Seale P, Bjork B, Yang W, et al. PRDM16 controls a brown fat/ skeletal muscle switch. *Nature.* 2008;454(7207):961-7.
2. Sun L, Xie H, Mori MA, Alexander R, Yuan B, Hattangadi S, Liu Q, Kahn CR, Lodish H. Mir-193b-365 is essential for brown fat differentiation. *Nature cell biology.* 2011;13(8):958-65.
3. Calo E, Quintero-estades JA, Danielian PS, et al. Rb regulates fate choice and lineage commitment in vivo. *Nature.* 2010;466(August).

A Necessary Role for Histone Variant H2A.Z and its Deposition Complex SRCAP in Embryonic Stem Cell Differentiation

Allison Alwan, Vidya Subramanian, Laurie Boyer
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The packaging of DNA into chromatin has emerged as a key mechanism to regulate gene expression patterns and cell identity, but is still poorly understood. Embryonic stem (ES) cells provide an ideal system for investigating how chromatin influences cell fate since ES cells have the capacity to self-renew while maintaining the potential to differentiate into derivatives of all three germ layers. Incorporation of histone variants by ATP-dependent chromatin remodelers is an important mechanism by which chromatin structure can be regulated. The histone H2A type variant H2AZ has been broadly implicated in eukaryotic gene regulation and is essential for early development in all metazoans including mammals, suggesting that it has specialized functions in mediating cell fate transitions. Here, we investigated the role of the divergent acidic patch region of H2AZ compared to H2A using site directed mutagenesis and demonstrated that this structural feature is necessary for proper regulation of gene expression, chromatin dynamics, and ultimately cell fate determination. Additionally, depletion of Snf2-related CBP activator protein (Srcap), the catalytic subunit of the ATP-dependent remodeler implicated in the deposition of H2AZ, shows that this complex is important for ES cell differentiation. Together, these results underscore the role of divergent region of H2AZ and proper H2AZ incorporation during cell fate transitions. These studies provide important insights into the complex mechanisms that govern the changes in chromatin structure and composition during differentiation.

Faculty Supervisor: Laurie Boyer

Postdoc Mentor: Vidya Subramanian

Study of Substrate Specificity Control in Chlorinase SyrB2

Kateryna Kozyrytska, Michael Funk, Catherine Drennan
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While natural products, such as vancomycin and chlorotetracycline, are potential pharmaceutical agents or leads in drug design, the total synthesis of these complex, highly decorated molecules has presented a challenge. One of the mechanistically difficult reactions is substrate regiospecific halogenation of unactivated carbons, an example of which is chlorination of the methyl carbon of L-threonine by a tailoring enzyme SyrB2. SyrB2 is a non-ribosomal peptide synthesis chlorinase that works on L-threonine only when the amino acid is attached to the partner protein SyrB1 through a phosphopantetheinyl linker. SyrB2 shows no activity toward free amino acids, a property potentially controlled by a phenylalanine blocking passage of free molecules through a channel leading to the active site. To test this hypothesis and potentially re-engineer SyrB2 to act on free substrate, we have mutated the phenylalanine to alanine, crystallized the mutant protein, and solved the structures at high resolution. The apo protein crystallized with chloride and alpha-ketoglutarate in two forms: one very similar to the wild-type structure and the second with a striking rearrangement near the mutation site. When crystals were soaked with iron, both monomers in the asymmetric unit resembled the wild-type structure. However, in the absence of the phenylalanine, the channel might accommodate a free threonine. We co-crystallized the mutant SyrB2 with threonine in the absence of iron, but no substrate was observed in the active site. When iron was added to crystals containing threonine or a mimic of the phosphopantetheinyl-bound substrate, S-(L-threonine)-N-acetylcysteine, again, no bound substrate was found in the active site. However, in both structures containing iron and the substrate, the alpha-ketoglutarate co-substrate in the active site was replaced by a putative bicarbonate ion, a product of the natural reaction. We are currently setting up an activity assay to test whether the mutant SyrB2 can halogenate free threonine.

Faculty Supervisor: Catherine Drennan
Graduate Mentor: Michael Funk

Analysis of a Dicer Domain Mutated in Human Tumors

Victoria Lu, Allan M. Gurtan, Phillip A. Sharp
Koch Institute for Integrative Cancer Research,
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Dicer is an RNase III-family endoribonuclease essential for the biogenesis of miRNAs from hairpin precursor miRNAs (pre-miRNAs). MicroRNAs (miRNAs) are endogenous small RNAs (20–25 nts long) that post-transcriptionally regulate gene expression and affect a variety of significant biological processes, including oncogenesis, cell development, differentiation, and programmed cell death[1]. Dicer has two RNase III domains (A and B) that cleave pre-miRNAs to yield mature miRNAs, a PAZ domain that binds the free 5′ and 3′ ends of pre-miRNAs, and an ATPase/DExD helicase whose function in miRNA processing remains unclear. We carried out structure-function studies of human Dicer by introducing missense point mutations to key sites in each domain and re-expressing mutant Dicer constructs in somatic murine Dicer knockout (KO) cell lines. To characterize the activity of each mutant, we performed Northern blot analysis of let-7c and miR-22, two abundant miRNAs derived from the 5′ and 3′ arms, respectively, of pre-miRNAs. To examine changes in miRNA expression on a broader scale, we performed massively parallel deep sequencing of small RNAs (15–50 nts in length) on mutants that showed strong defects by Northern blot. Our results indicate that each RNase III domain can retain partial-to-complete activity *in vivo* when the other RNase III domain is inactivated. Furthermore, loading of pre-miRNA into hsDicer *in vivo* is directional, with the RNase IIIA and IIIB domains responsible for processing of the 3′ and 5′ arms of precursors, respectively. Residue D1709, a mutation which is associated with a subset of nonepithelial ovarian cancers2, is required for maturation of the tumor suppressive let-7 family3. Thus, mutation of this residue results in a uniquely miRNA-haploinsufficient state with preferential loss of a family of important tumor suppressor miRNAs.

Faculty Supervisor: Phillip A. Sharp
Postdoc Mentor: Allan M. Gurtan

1. He and Hannon (2004) *Nature Reviews Genetics*. 5, 522-531
2. Heravi-Moussavi, A. et al. (2011) *New England Journal of Medicine.*
3. Johnson, S. M. et al. (2005) *Cell*. 120, 635–647

Effective Therapeutic Delivery of Nanoparticles Containing MicroRNA, miR-34a, to Inhibit Lung Tumor Initiation and Progression

Sabina Sood, Wen Xue, Tyler E. Jacks
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The goal of my project is to investigate the biology of miR-34 in lung cancer development and therapy. MicroRNA miR-34a, b, and c are direct transcriptional targets of p53. MiR-34s are known to inhibit cell cycle genes and some oncogenes and assist in reinforcing the major p53-mediated anti-proliferation and anti-apoptosis pathways. We plan to use miR-34a as an example to study the function of this miRNA family in lung cancer to uncover the biological mechanisms that mediate tumor suppression by miR-34a and ascertain the therapeutic efficacy of miR-34a in human and mouse lung cancer models. In order to determine the effects of miR-34a on lung tumor initiation and progression, we will perform experiments with mouse lung cancer models by conditionally expressing miR-34a in lung tumors using a lentivirus strategy as well as conducting experiments using human lung cancer-derived cells. The data generated from these analogous experimental systems will help elucidate the mechanisms by which miR-34a inhibits lung tumor progression and identify new miR-34a target genes relevant to human lung cancer prognosis. The results will also assist in developing nanoparticles to therapeutically deliver miR-34a in vivo and monitor the tumor response through microCT, histology, and biomarkers. After establishing a set of genes that are upregulated in tumors and potential targets in cancer, we plan to combine multiple miRNAs/siRNAs into a successful nanoparticle for therapy.

Faculty Supervisor: Tyler E. Jacks
Postdoc Mentor: Wen Xue

Identifying Somatic Mutations Using High-throughput Sequencing of Mouse Lung Adenocarcinomas

Frances Chen, David McFadden, Tyler Jacks
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Recent advances in high-throughput sequencing technology (Illumina) have facilitated the description of human cancer genomes. Human tumors harbor a large number of somatic mutations, making identification of functionally important driver mutations challenging. We have recently applied high-throughput DNA sequencing techniques to genetically engineered mouse cancer models in order to identify conserved mutations acquired during tumor progression from benign to malignant disease. In this study, we utilize PCR and Sanger dideoxy sequencing to distinguish between true- and false-positive somatic single nucleotide variants (SNV). Based on these results, we tuned our analysis approach for DNA sequencing datasets through the development of bioinformatic filters. With this foundation for the identification of true-positive mutations, we can proceed with further characterization to identify conserved driver mutations using comparative DNA sequencing studies between mouse cancer models and human tumors. This work provides the evidence that Illumina sequencing technology can be used to identify genetic mutations.

Faculty Supervisor: Tyler Jacks
Postdoc Mentor: David McFadden

Visualizing Individual Genomes of Hepatitis C Virus Using Fluorescence *in situ* Hybridization

Hamsika Chandrasekar, Kartik Trehan, Sangeeta Bhatia
Department of Biology, MIT, Cambridge, MA 02139, USA

Hepatitis C virus (HCV) is a major public health problem, affecting nearly 200 million people worldwide and leading to end-stage liver diseases such as cirrhosis and hepatocellular carcinoma. There is currently no HCV vaccine available, and therapeutic options for HCV treatment are limited. A major historical hurdle in the development of HCV therapeutics has been the absence of cell culture systems and assays for studying HCV infection. While several culture models have recently been developed, however, tools for monitoring and characterizing infection have remained limited. Here we discuss the development of an imaging technology for visualizing individual genomes of HCV. By using a novel fluorescence *in situ* hybridization (FISH) method in combination with MATLAB-based analysis, we demonstrate the ability to characterize the single-cell stochasticity of HCV infection as well as the subcellular localization of viral genomes. This method is both highly sensitive and specific, and works in numerous cellular systems without modification to the viral genome. Ultimately, this technology will have direct applications in understanding HCV pathogenesis.

Faculty Supervisor: Sangeeta Bhatia
Graduate Student Mentor: Kartik Trehan

1 Ploss, A., Khetani, S.R., Jones, C.T.; Syder, A.J., Trehan, K, Gaysinskaya, V.A., Mu, K., Ritola, K., Rice, C.M., Bhatia, S. (2010). *Persistent hepatitis C virus infection in microscale primary human hepatocyte cultures.* Proc Natl Acad Sci USA 107, 3141-314.

IME2 Mediates a Network of Translational Control in *S. cerevisiae* Meiosis

Mariya Samoylova, Luke Berchowitz, Angelika Amon
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Translational control is an important and well-characterized process in many organisms, but it is not well explored in *S. cerevisiae* meiosis, where it is particularly simple to investigate. In this study, we propose that *IME2*, which encodes a highly conserved protein kinase homologous to human Cdk2, serves as a negative post-transcriptional regulatory element for a network of meiosis-specific genes. We demonstrate that stabilizing *IME2* results in the complete relief of the translational repression of *CLB3*, *GIP1*, *SPO20*, and *SPS1* that is usually seen in meiosis I. Because the translational control of *CLB3* is mediated by its 5'UTR, we propose that the network of translational repression involving *IME2* acts through a *trans*-factor binding the 5'UTR of the regulated genes. Localization of the necessary cis- element(s) within the 5'UTR and the discovery of an *IME2* phosphorylation target *trans*-acting factor will further illuminate a previously unobserved mechanism of translational repression in *S. cerevisiae* meiosis.

Faculty Supervisor: Angelika Amon
Postdoc Mentor: Luke Berchowitz

Ribosomal Protein SA and Laminin Regulation of Basal Constriction During Zebrafish MHBC Morphogenesis

Lauren Sless, Jennifer Gutzman, Isabel Brachmann, Hazel Sive
Whitehead Institute, Department of Biology, MIT, Cambridge, MA 02142, USA

Shaping of the vertebrate brain during development requires folding of the neuroepithelium to subdivide the brain and pack it into the skull. In the region of the midbrain-hindbrain boundary (MHB), a highly conserved fold occurs on the basal surface during development. The formation of the deepest point of this fold, the MHB constriction (MHBC), is driven by basal constriction of a small group of cells at the MHBC. Basal constriction is a little studied but essential process in the formation of the zebrafish MHBC. Prior studies have identified laminin, a signaling protein localized in the extracellular matrix, as a key regulator of basal constriction. This study examines whether or not the 37 kDa high affinity laminin receptor called Ribosomal Protein SA (RPSA) mediates the interaction between the neuroepithelium and laminin to regulate basal constriction. RPSA loss-of-function studies were performed by selectively targeting RPSA using microinjection of a customized antisense morpholino oligonucleotide (MO) into single-cell wild-type embryos. At 24 hours post fertilization (hpf) the MHBC in MO injected embryos was observed using brightfield and confocal microscopy. Overall brain morphogenesis and basal constriction of individual cell shapes at the MHBC were assayed. Similar loss-of-function studies were performed using various MO concentrations. The data showed that with high concentrations of MO an RPSA loss-of-function does result in a basal constriction defect. In addition, results from a double loss-of-function experiment using MOs targeting both laminin and RPSA demonstrated a more severe basal constriction defect at the MHBC, and therefore possible synergy between the two proteins. Data strongly suggest that while RPSA and laminin both function in regulating basal constriction during MHBC morphogenesis, they may function in parallel regulatory pathways as opposed to acting in concert as part of the same pathway.

Faculty Supervisor: Hazel Sive
Postdoc Mentors: Jennifer Gutzman and Isabel Brachmann

Ypel4: Highly Conserved Gene Required for Terminal Differentiation and Enucleation of Murine Erythroid Cells

Kenneth Lin, Wenqian Hu, Harvey Lodish
Whitehead Institute, Department of Biology, MIT, Cambridge, MA 02142, USA

The *Ypel4* (Yippee-like 4) gene is a member of the highly conserved *Ypel* gene family that encodes putative zinc-finger-containing proteins present in diverse eukaryotic organisms. Although several of the *Ypel* family members are implicated to be involved in cell cycle regulation, the function of *Ypel4* is still largely unknown. Here, we establish *Ypel4* as an essential player in hematopoietic development, playing a necessary role in terminal differentiation of red blood cells. In mice, *Ypel4* is expressed specifically in terminally differentiating erythroid cells in both fetal liver and bone marrow, and is significantly upregulated as terminal erythropoiesis proceeds. Knockdown of *Ypel4* by shRNA revealed that it is required for at least two critical events of erythropoiesis, including induction of Ter119 antigen (a surface marker of terminal differentiation) and subsequent enucleation (loss of the nucleus necessary for becoming a mature erythrocyte). To further characterize how *Ypel4* regulates this developmental process, we are currently performing yeast two-hybrid and microarray analyses. Our present findings suggest that *Ypel4* is necessary for normal development of red blood cells and further indicate the *Ypel* gene family as important regulators of eukaryotic life.

Faculty Supervisor: Harvey Lodish, Ph.D.
Postdoc Mentor: Wenqian Hu, Ph.D.

Isolating PKCA and PPP1R14D, Intracellular Regulators of TGFalpha Ectodomain Cleavage by ADAMs

Melissa Ko, Andreas Herrlich, Harvey Lodish
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Cell signaling through epidermal growth factor (EGF)-like ligands such as transforming growth factor alpha (TGFalpha) results in excessive cell proliferation and tumor growth when improperly regulated. In order to develop more specific medical treatments, it is necessary to understand how initiation of these signaling pathways is controlled through regulation of TGFalpha cleavage by ADAM (**a d**isintegrin and **m**etalloprotease) enzymes in response to stimuli. Using a FACS-based assay to quantitatively measure cleavage of TGFalpha in single living cells, we performed an shRNA knockdown and overexpression screen to identify phosphatases and kinases involved in induced ADAM-mediated cleavage. We then demonstrate that protein kinase C alpha (PKCA) and the PKC-regulated protein phosphatase 1 regulatory subunit 14D (PPP1R14D) play a role in the TGFalpha ectodomain cleavage pathway by ADAMs. In cells stimulated with TPA, a phorbol ester that activates PKC enzymes, we found that shRNA knockdown of PPP1R14D and PKCA led to a maximum decrease of 25% and 37% respectively in the extent of TGFalpha cleavage after 30 minutes of stimulation compared to the shRNA control cells. Similarly, overexpression of PPP1R14D led to an increase in ectodomain cleavage of 27% compared to wildtype cells over the same time frame; thus, we hypothesize that PKCA and PPP1R14D interact to promote TGFalpha-specific cleavage in TPA-activated ectodomain cleavage. These results corroborated with microarray data from hypertension mouse models, where high expression levels of PPP1R14D and PKCA show a significant positive correlation with a disease commonly attributed to improper TGFalpha signaling. Deeper understanding of the pathway underlying TPA-induced cleavage by ADAMs and the molecular signaling partners involved could provide drug targets for aberrant TGFalpha signaling.

Faculty Supervisor: Harvey Lodish
Postdoc Mentor: Andreas Herrlich

Absence of Homeostatic Compensation by Inhibitory Synapses after Prolonged Increases in Neuronal Activity

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Homeostatic plasticity elicited by prolonged changes in electrical activity is thought to preserve the informational storage capacity of the brain. While substantial progress has been made toward understanding the molecular mechanisms of homeostasis at excitatory synapses, little is known about the regulators of homeostatic plasticity at inhibitory synapses. Using the whole-cell patch clamp technique, we examined how deletion of Npas4, an activity-responsive transcription factor known to regulate inhibitory synapses, affects the response of inhibitory synapses to prolonged increases in activity. In wild-type hippocampal neurons, we observed that 24 h stimulation with bicuculline did not affect the amplitude, inter-event interval, rise time, or decay time of miniature inhibitory synaptic currents (mIPSCs). Under conditions of basal activity, we found that deletion of Npas4 had no effect on parameters of mIPSCs. Finally, we observed that in neurons in which Npas4 was deleted, 24 h stimulation with bicuculline did not affect parameters of mIPSCs. In sum, we demonstrate that under certain culture conditions, inhibitory synapses may not compensate homeostatically for prolonged increases in activity. Further, we demonstrate that deletion of Npas4 does not affect regulation of inhibitory synapses in our culture system.

Faculty Supervisor: Yingxi Lin

In Vivo Electrophysiological Characterization of Sleep and Spindle Oscillations in a Mouse Model of Schizophrenia

Akansh Murthy, Michael M. Halassa, Matthew Wilson
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Department of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139, USA

Spindles are waveforms that are generated from the thalamus and occur primarily during non-rapid eye movement sleep. These 10-15 Hz oscillations are thought to be important for memory consolidation and modulation of sensory information. Recent literature indicates that spindles are attenuated in schizophrenia, suggesting that spindles might be a marker for this illness. Using *in vivo* electrophysiology with implants that received electroencephalographic and electromyographic data, we characterized the novel PV-Cre/NR1f/f mouse, which represents a newly developed schizophrenia model. More specifically, the PV-Cre/NR1f/f mouse has a disruption of N-methyl-D-aspartate (NMDA) receptor activity in fast-spiking parvalbumin interneurons, resulting in altered gamma rhythms and cognitive impairments, typical of psychiatric disorders such as schizophrenia. Our preliminary findings show that spindles were significantly reduced in number and altered in morphology in the PV-Cre/NR1f/f mice compared to age-matched control mice. Surprisingly, the amount of time spent in each in NREM sleep was decreased in the PV-Cre/NR1f/f mice compared to the controls. The results indicate that the mice with the NMDA deletion have spindle alterations and perhaps consequent malfunctions within thalamic circuitry. Overall, we present one of the first phenotypic characterizations of spindles and sleep in a mouse model of schizophrenia. We are pursuing our findings with more investigations including auditory experiments, thalamic recordings, and optogenetic stimulations.

Faculty Supervisor: Matthew Wilson

Postdoc Mentor: Michael M. Halassa

Efficient Generation of Protein-induced Pluripotent Stem Cells from Somatic Cells via Microfluidic Delivery

Sabine Schneider, Kaila Deiorio-Haggar, Janeta Zoldan, and Robert Langer
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Department of Biology, MIT, Cambridge, MA 02139, USA

Diseases associated with degenerative cell loss, like Muscular Dystrophy and Diabetes Type I, might potentially be cured with therapies based on induced pluripotent stem cell (iPSC) transplants. However, to realize these therapeutic goals, first an efficient and non-tumorigenic generation of iPSCs is required, which has not been given in the past. In this study, a novel microfluidic device was used to transfect neonatal fibroblasts with the reprogramming factors Oct4, Klf4, Sox2 and c-Myc in proteinaceous form. The delivery of proteinaceous reprogramming factors removes the risk of genomic alterations and insertional mutagenesis, a risk associated with chemical and viral delivery methods, respectively. However, up to now the efficiency of non-viral delivery mechanisms and especially protein-based reprogramming has been too low to be of practical use. The reprogramming efficiency of microfluidic delivery was determined and contrasted with two more traditional non-viral methods. The two methods, Nucleofection and polymer-based delivery of recombinant plasmids, resulted in reprogramming efficiencies comparable to that of previous studies, ranging between 0.003% and 0.125% ‡. The efficiency of microfluidic delivery, with 1.7%, was one order of magnitude greater than those found in published literature. This novel method to generate iPSCs thus has the potential to greatly advance research focused on stem cell-based therapies due to its high efficiency and safety.

Faculty Supervisor: Robert Langer

Postdoc Mentor: Janet Zoldan

‡ Okita, K et al. (2007) *Nature.* 448, 313-317
Zhou, H et al. (2009) *Cell Stem Cel*l. 4, 381-384
Stadtfeld, M et al.(2008) *Science.* 322, 945-949
Jia, F et al. (2010) *Nature Methods*. 7, 197-199

The Synaptic Plasticity Protein CPG2 Binds to an Endocytic Machinery Component

Christina Welsh, Sven Loebrich, Elly Nedivi
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The ability of the brain to remodel in response to external stimuli is crucial for learning and memory. One important aspect of neuronal plasticity is the ability of synapses, the connecting points between neurons, to increase and decrease in strength. Synapses function through the release of neurotransmitters from the presynaptic terminal of one neuron; the neurotransmitters subsequently bind to receptors on the postsynaptic site of the receiving neuron, eliciting a response in that cell. Synaptic strength can therefore be modulated by changing the number of neurotransmitter receptors in the synapse. The protein CPG2 (candidate plasticity gene 2) helps regulate glutamate receptor internalization, implicating it in synaptic plasticity†. Genome-wide association studies have linked SNPs in CPG2 to bipolar disorder, suggesting that CPG2 dysfunction could underlie the elevated risk for bipolar disorder and hence point toward a possible synaptic phenotype as part of the basis for neuropsychiatric disease. Immunoprecipitation assays reveal that CPG2 is associated with nine proteins. We have used a yeast two-hybrid system to probe whether CPG2 binds directly to any of these proteins. Strongest binding is displayed by AP2-ß, a protein previously known to link transmembrane proteins destined for internalization to clathrin coated vesicles. We are proceeding to delineate the minimal region of interaction between AP2-ß and CPG2 by employing a collection of CPG2 truncation constructs, which differ in size by as little as 20 amino acids. Thanks to this fine resolution, it may be possible in the future to develop interfering peptides or screen for small molecule modulators capable of disrupting or enhancing the interaction. Our research hence has potential clinical applications for bipolar disorder.

Faculty Supervisor: Elly Nedivi
Postdoc Mentor: Sven Loebrich

† Cottrell, JR et al. (2004) *Neuron*. 44, 677-690.

An Integrated Pharmacological, Structural, and Genetic Analysis of Pathological ROS Production in Neutrophils

Samuel Acquah, Christian Ellson, Michael B. Yaffe
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Department of Biology, MIT, Cambridge, MA 02139, USA

While NADPH-oxidase dependent intracellular ROS production is essential for effective host defense against microorganisms, aberrant extracellular ROS production by neutrophils has been implicated in a wide range of pathogenic inflammatory diseases including chronic granulomatous disease, acute lung injury, and multiple organ dysfunction syndrome. In this study, we identified PI3K and PLD as major signaling pathways that preferentially regulate plasma-membrane-localized (extracellular) ROS production. The enzymes p110b and PLD1 generate lipid products PtdIns(3,4)P_2 and PA respectively, at the site of plasma-membrane-localized NADPH oxidase cytochrome activity. The PX domain of p47phox, an important subunit of the NADPH oxidase, binds these products synergistically and integrates the PI3K and PLD signaling pathways. A critical amino acid residue in the p47phox PX domain, R43 in humans and K43 in mice, ligates the 3-phosphate of PtdIns(3,4)P_2. A targeted mutation of this site in mouse neutrophils results in defective extracellular ROS production and increased dependence on the PLD signaling pathway, but has no effects on intracellular ROS production or bacterial killing. Our results suggest that the p47phox PX domain may be an attractive drug target for preventing diseases caused by excessive extracellular ROS production.

Faculty Supervisor: Michael B. Yaffe
Postdoc Mentor: Christian Ellson