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Characterization of a Novel Mechanism for Cell Division Inhibition in *Caulobacter crescentus*

Alex Hopkins, Joshua W. Modell, Michael Laub
Department of Biology, MIT, Cambridge, MA 02139, USA

In response to DNA damage, all cells must stall cell division to allow time for DNA repair. In bacteria, DNA damage typically induces a transcriptional program known as the 'SOS response', which is mediated by cleavage of the SOS repressor LexA. While SOS-induced DNA repair genes are relatively well conserved, the mechanisms of cell division inhibition are poorly understood. In *E. coli*, the SOS division inhibitor SulA interferes with polymerization of the cytokinetic scaffold FtsZ. However, many bacteria, including Caulobacter crescentus, do not contain sulA orthologs, indicating that novel mechanisms must exist for inhibiting cell division after DNA damage. In this study, we characterized a new SOS gene in C. crescentus called sidA, which inhibits cell division when overexpressed. Deleting sidA suppressed the cell division defect of a ΔlexA strain, indicating that sidA is the primary division inhibitor within the Caulobacter SOS regulon. In contrast to SulA, SidA does not affect the localization of FtsZ or other components of the division machinery. To identify SidA targets, we performed a genetic screen for mutations that suppress the lethality of overproducing SidA. We isolated 5 suppressors, 3 of which mapped to the late-arriving cell division gene ftsW. This result was corroborated by bacterial two-hybrid experiments showing that FtsW and SidA interact directly in vivo. FtsW inhibition thus represents a novel mechanism by which bacteria inhibit cell division after DNA damage, and suggest that FtsW is a focal point for cell division regulation.

Faculty Supervisor: Michael Laub
Postdoctoral Mentor: Joshua W. Modell

Unbiased Discovery of Genes Relevant for Breast Cancer Metastasis

Amelia Chang, Scott Valastyan, Robert A. Weinberg
Whitehead Institute for Biomedical Research,
Department of Biology, MIT, Cambridge, MA 02139, USA

The acquisition of a motile phenotype is a crucial prerequisite for tumor cells to initiate the invasion-metastasis cascade, a stepwise process culminating in the formation of macroscopic metastases that are frequently fatal. In this study, we propose an unbiased approach to identify genes involved in breast cancer metastasis, using a novel experimental system that preserves the genetic heterogeneity pre-existing within tumor cell populations, minimizes selection bias, and is capable of identifying genes involved in a specific step of the invasion-metastasis cascade. Using this approach, we identify RhoJ as a candidate gene that regulates tumor cell motility in vitro. RhoJ is a Cdc-42-like Rho GTPase whose role in cancer has not yet been described. Transcript levels of RhoJ are significantly downregulated in cells exhibiting a high motility phenotype. Restoring RhoJ expression inhibits the migration of certain high motility cell populations, whereas knocking down RhoJ expression increases the migration of low motility cell populations. The convergence of results from these complementary gain-of-function and loss-of-function experiments strongly suggests that RhoJ is a bona fide regulator of cell motility. Suppression of RhoJ protein expression therefore constitutes a putative mechanism by which some cancer cell types acquire the ability to metastasize. Further investigations providing greater insight into RhoJ-mediated pathways will be invaluable for enhancing our fragmentary understanding of metastasis, and may represent a step towards making this complex process amenable to therapeutic intervention.

Faculty Supervisor: Bob Weinberg
Graduate Student Mentor: Scott Valastyan

Temporal and Concentration Requirements of Klf4 for Reprogramming Mouse Embryonic Fibroblasts to Pluripotency

Ezgi Hacisuleyman, John Cassady, Rudolf Jaenisch
Whitehead Institute for Biomedical Research,
Department of Biology, MIT, Cambridge, MA 02139, USA

Generation of induced pluripotent stem (iPS) cells from differentiated cells has greatly impacted the fields of genetics, epigenetics, and regenerative medicine; however, obtaining these cells is still very inefficient. Klf4 is among the four transcription factors, Oct4, Sox2, cMyc, and Klf4, which are required for inducing pluripotency in differentiated cells. The mechanism of reprogramming has been explored through identifying genetic markers during transition to pluripotent stage (Stadtfeld *et al.*, 2008), analysis of time-course of stage-specific embryonic antigen activation (Brambrink et al., 2008), and investigation into the genome-wide histone modification, methylation, and X-chromosome inactivation during reprogramming (Maherali *et al.*, 2007). After trying to characterize the mechanistic steps of reprogramming and seeing the varying amounts of markers, researchers have hypothesized that each Yamanaka factor might be needed at different times in different quantities. Therefore, to address the problem of inefficiency, we decided to focus on the role of Klf4 and developed an inducible system for Klf4 expression. The system allowed us to analyze when and how much Klf4 should be present in the cells for most efficient reprogramming: Klf4 was required in low concentrations during the first week but in high concentrations throughout the rest of reprogramming. We found that Klf4 was indispensable at every phase of this step-wise process: its absence was detrimental, and early cell fate determination without Klf4 hindered re-establishment of pluripotency. Our findings contribute to the revision of iPS cell protocols to determine a more common and efficient method for iPS cell generation, and open new avenues for therapeutic applications.

Faculty Supervisor: Rudolf Jaenisch
Graduate Student Mentor: John Cassady

A Mechanistic Model for Binding of Exogenous Peptides to Cell Surface MHC Class I Proteins

Helen Xun Hou, Herman N. Eisen
Koch Institute for Integrative Cancer Research,
Department of Biology, MIT, Cambridge, MA 02139, USA

The recognition by T cell receptors of antigens on other cells plays a central role in the adaptive immune system. The antigens are non-covalent complexes of peptides bound to proteins encoded by genes in the major histocompatibility complex (MHC). The dynamics of interactions between MHC proteins and intracellular (endogenous) peptides have been extensively characterized but the binding of extracellular (exogenous) peptides to MHC proteins is not well understood. We aimed to develop and evaluate a model that predicts the number of pep-MHC complexes formed on cells after exposure to an exogenous peptide. In this model, there are two sources of cell surface MHC proteins with unoccupied peptide-binding sites ("empty" MHC): newly synthesized MHC that had not been loaded with an endogenous peptide, or had been loaded with an endogenous peptide that dissociated to leave a vacant groove. These empty MHC molecules either bind exogenous peptides to form pep-MHC complexes, or undergo irreversible denaturation. Analyses of the model show that the number of cell surface complexes at a specific time depends on the four parameters, k_{on}, k_{off}, k_{den} and the rate at which empty MHC appear (dM_0/dt). To test the model, we measured the binding of two synthetic peptides: SIINFEKL and SIYRYYGL to Kb, a mouse MHC-I molecule, on RMA-s cells and DC2.4 cells. The results showed that the numbers of predicted complexes value are consistent with the experimentally measured values. The model works well for the full range of time and concentration points sampled.

Faculty Supervisor: Herman N. Eisen

Inhibition of Epithelial-Mesenchymal Transition (EMT) through BMP Signaling in Breast Cancer Cells

Sophia Hsin-Jung Li, Christina Scheel, Robert A. Weinberg
Whitehead Institute for Biomedical Research
Department of Biology, MIT, Cambridge, MA 02139, USA

When re-activated in cancer cells, a normally latent transcriptional circuitry is capable of inducing epithelial- mesenchymal transition (EMT). EMT reconfigures epithelial cells with mesenchymal attributes, and induces motility and self-renewal ability. During embryonic development, EMT programs are activated during gastrulation to induce mesoderm formation. In cancer cells, EMT is a necessary process for metastasis. The expression of several distinct transcription factors induces an EMT in embryonic and carcinoma cells. However, it remains unknown how these EMT-related transcription factors are activated during tumor progression. We hypothesized that, analogous to development, the EMT circuitry might be induced by paracrine factors. Further, we speculated that EMT-associated functional traits, such as motility, might be maintained by autocrine signaling loops. Here, we show that Wnt and TGF-beta signaling are active in immortalized mammary epithelial cells (HMLE) that have passed through an EMT. This is demonstrated by an increase of beta-catenin transcriptional activity assessed by luciferase reporter assay and by constitutive phosphorylation of smad-2 detected by immunoblotting. Furthermore, RT-PCR revealed all BMP ligands to be downregulated in HMLE cells after EMT while secreted BMP inhibitors, notably Gremlin, were upregulated. We hypothesized that the EMT signaling circuitry is maintained by autocrine Wnt and TGF-beta loops. Indeed, by adding the secreted Wnt antagonists Dkk-1 and SFRP-1 or BMP-4 to HMLE cells, the EMT program can be perturbed. We are currently investigating the mechanism of EMT inhibition by BMP-4. Our preliminary data suggest that this might occur through the crosstalk of BMP signaling with Wnt and TGF-beta pathways.

Faculty Supervisor: Robert A. Weinberg
Postdoctoral Mentor: Christina Scheel

An inexpensive, Open-source Method for Image-based Cell Sorting

Ylaine Gerardin, Joseph Kovac, and Joel Voldman
Research Laboratory of Electronics, Department of Electrical Engineering and Computer Science, MIT, Cambridge, MA 02139, USA

Currently, flow cytometry is the dominant method for sorting cells, but it can only distinguish cells based on their total fluorescent signals. While micromanipulators can isolate more complex phenotypes, they are prohibitively expensive. The Voldman lab has developed a novel method, called photo-polymer activated cell sorting (PACS), that can isolate cells of any image-based phenotype, such as cell shape or the localization of a fluorescent signal, and takes advantage of standard laboratory equipment. PACS relies on a light-sensitive pre-polymer solution that crosslinks to form a hydrogel upon exposure to UV radiation. Adherent cells growing in liquid media are imaged and cells to be isolated are selected by the user with the aid of custom-written software. UV radiation is patterned to encapsulate unwanted cells in the polymer, and the remaining un-encapsulated cells are enzymatically collected. The composition of the pre-polymer solution and the UV exposure time were adjusted to optimize cell health. Successful separation by PACS has been demonstrated in several murine and human cell lines (mESC D3, MCF7 and HeLa S3) with purity of up to 73% and enrichment of a minority population of up to 65-fold.

Faculty Supervisor: Joel Voldman
Graduate Student Mentor: Joseph Kovac

Introducing Hydrolytic Activity into *C. roseus* Strictosidine Synthase

Jenna Caldwell, Lesley-Ann Giddings, Summer Thyme, Peter Bernhardt, and Sarah E. O'Connor
Department of Chemistry, MIT, Cambridge, MA 02139, USA

The beta-propeller structural family encompasses enzymes with a variety of catalytic activities, including the calcium-dependent human serum paraoxonase (PON1), which is a hydrolase with broad specificity, and the cofactor-independent *Catharanthus roseus* strictosidine synthase (CrSTS), which catalyzes the Pictet-Spengler condensation of tryptamine and secologanin, a key step in the synthesis of monoterpene indole alkaloids. This variety of native catalytic activities suggests that the beta-propeller architecture may be able to serve as a flexible scaffold for switching catalytic activity. To test this hypothesis, we attempted to introduce hydrolytic activity into the CrSTS scaffold. Based on sequence alignments and structural comparisons of CrSTS, its *Vitis vinifera* homologues, and PON1, thirty-seven key residues were identified and assigned to five spheres based on their degree of conservation and their anticipated effect on catalytic activity. Five constructs were made from a CrSTS template by mutating residues in successive spheres using a gene assembly method in which dozens of overlapping oligonucleotides (collectively comprising the entire sequence of the desired construct) self-assemble in a modification of standard PCR. Spectrophotometric assays indicate that large-sphere constructs are able to hydrolyze para-nitrophenylacetate (V_{max}= 1.3-1.6 μmol mg^{-1} min^{-1}; K_m = 7-9 mM). In the presence of EDTA, this activity is significantly reduced, suggesting that the mutated construct, unlike wild-type CrSTS, is dependent on a metal (possibly calcium). In order to pinpoint the residues that are essential for the introduction of metal-binding and hydrolytic activity, we plan to conduct further assays on constructs with new mutations within the appropriate sphere. These data highlight the plasticity of this beta-propeller enzyme scaffold and also suggest that the specialized Pictet-Spenglerase may have evolved from a hydrolase enzyme.

Faculty Supervisor: Sarah O'Connor
Graduate Student Mentor: Lesley-Ann Giddings

Reduced TrkB-PSD-95 Spine Colocalization by Inhibition of BDNF-TrkB Pathway, *in vivo*

Jihye Kim, Akira Yoshii, Martha Constantine-Paton
Department of Biology & Department of Brain and Cognitive Sciences
MIT, Cambridge, MA 02139, USA

Long-term potentiation (LTP) plays a crucial biological role in strengthening and improving important connections in the brain that control essential every-day biological mechanisms. LTP has been shown to be regulated by a number of essential postsynaptic neuron components, including the N-methyl-D-aspartate receptor (NMDAR), brain derived neurotrophic factor (BDNF), tyrosine kinase B (TrkB), postsynaptic density-95 (PSD-95), and phosphatidylinositol 3-kinase (PI3K). Recently, a comprehensive positive feedback signaling pathway *in vitro* relating the NMDAR-induced BDNF-TrkB signaling pathway and the PSD-95-PI3K trafficking pathway has been proposed. This study demonstrated that the BDNF-TrkB-PSD-95 relationship is the critical bridge to understand NMDAR-induced LTP; however, the validity of this pathway *in vivo* has yet to be characterized. Using TrkB knock-in transgenic mice that allow for spatially and temporally controlled inhibition of TrkB, we showed that the inhibition of TrkB *in vivo* leads to significantly decreased PSD-95 trafficking to the dendritic spines, which is where most excitatory synapses are formed. In addition to decreased PSD-95 distribution, these neurons with inhibited TrkB function show a significantly smaller cell soma size and sparser dendritic arborization. These results provide another pathway to explore in order to increase synapse stabilization. Therefore, the manipulation of the BDNF-TrkB-induced delivery of PSD-95 through the PI3K/Akt pathway has numerous clinical implications for diseases with synaptic dysfunction.

Faculty Supervisor: Martha Constantine-Paton
Postdoctoral Mentor: Akira Yoshii

Patterned Multi-site Stimulation in Conjunction with Multi-site Calcium imaging: *a new experimental system for investigating function and plasticity in neuronal networks*

Sherry Yan, Nathan Wilson, Mriganka Sur
Picower Institute for Learning and Memory, MIT, Cambridge, MA 02139, USA

Although much about synaptic transmission in neurons has been elucidated through experimentally induced synaptic changes at single cells, not much has been investigated about the representation of information across populations of identified cells, yet it is the coordination among groups of neurons that is thought to establish higher cognitive functions. Our lab is interested in bridging the gap between an understanding of single synapse properties and network processing and plasticity. We have developed a multielectrode array (MEA) with sixty electrodes, each controlled by custom-made MATLAB program. A pattern of stimulation was delivered, via selected electrodes, to populations of cortical neurons in an acute slice of mouse brain. Calcium imaging was used in conjunction to monitor the induced responses in groups of neurons. The calcium imaging technique offers not only a way of monitoring of large population of neurons but also several advantages over previously described recording methods. We used this experimental setup to study learning in neuronal networks. Under 40x magnification, responses from individual cells were detected. Under 2.5x magnification, responses from populations of neurons were detected. In one experiment in which sequence stimulation had been applied to two areas of the cortical slice, an enhanced response from the second area was observed when the two groups of neurons were stimulated with an offset of half a second. This observation suggested that stimulating two populations of neurons in sequence might strengthen communication between the two areas of the brain slice. This finding is important for understanding neuronal network communication. By applying calcium imaging in conjunction with the multi-site stimulation, we hope to monitor structural, chemical, and electrical changes in a network of neurons curing patterned stimulation to an intact preparation of brain circuit.

Faculty Supervisor: Mriganka Sur
Postdoctoral Mentor: Nathan Wilson

The Loss of ATR and Chk1 Sensitizes p53-deficient Cells Following DNA Replication Stress

Stephanie Wu, Hai Jiang, Michael Hemann
Koch Institute for Integrative Cancer Research
Department of Biology, MIT, Cambridge, MA 02139, USA

In response to various chemotherapeutics, p53 acts as the essential inducer of cell death. However, many tumors exhibit a loss of p53, which render them intrinsically resistant to many chemotherapeutic drugs. Using RNA interference technologies with B-lymphoma cells, we show that depletion of the ATR and Chk1 kinases sensitizes p53-deficient cells following DNA replication stress. DNA replication stress is a source of endogenous DNA damage that arises from stalled replication forks. Cells with suppressed expression of ATR and Chk1 did not effectively respond to replication stress that was induced by DNA cross-linking agents (cisplatin, carmustine, chlorambucil, oxaliplatin, and melphalan) and anti-metabolites (methotrexate, gemcitabine, and hydroxyurea). Furthermore, p53-deficient B-lymphoma cells with depleted TopBP1, an ATR activator, were particularly sensitized following replication stress. Cells with reduced expression of TopBP1 were found to be 63.1% to 76.1% less likely than control cells to survive treatment with DNA cross-linking agents. p53-deficient B-lymphoma cells with depleted TopBP1 were 64.0% to 66.0% less likely than control cells to survive treatment with anti-metabolites. Thus, the ATR/Chk1 pathway is not only important in the cellular response to single-strand DNA breaks, but is also crucial to cell survival following DNA replication stress, given that TopBP1 is strictly expressed at DNA replication forks.

Faculty Supervisor: Michael Hemann
Postdoctoral Mentor: Hai Jiang

Using Nanoparticle-chaperoned Peptide Libraries to Profile Cancer and Clotting Proteases

Steven Mo, Sangeeta Bhatia

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

Cancer cells are known to excessively produce different proteases which are involved in tumor processes such as metastasis, angiogenesis, avoidance of apoptosis, and cancer cell proliferation. Fluorescence techniques and biopsy, the current methods of *in vivo* protease assessment, are limited by their invasiveness, low multiplexing, and non-ideal environmental sampling. Here, we present a new technique with the potential to simultaneously probe hundreds of enzymesubstrate activities in vivo using a system of nanoparticle-chaperoned, mass-encoded peptide libraries. Specifically, small peptides, which would otherwise be rapidly cleared renally, are conjugated to long-circulating nanomaterials to improve their exposure to proteases *in vivo* and direct their clearance through the reticuloendothelial organs. In the event that peptides get proteolytically cleaved *in vivo*, we hypothesized that free peptide fragments would be released from the particle chaperone to serve as mass-encoded, renally-cleared "messengers" of protease activity, allowing HPLC-MS urine analysis to reveal a "barcode" of protease activity *in vivo*. In an effort to evaluate this hypothesis, we initially synthesized 43 unique protease substratecarrying, magnetofluorescent iron oxide nanoparticles. These particles were rapidly screened *in vitro* for cleavage by a panel of proteases, including coagulation factors activated during vascular injury (thrombin, tissue factor, factor Xa, and cathepsin B) and enzymes associated with cancer invasion (MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, and MMP-14). Highly active nanoparticle-chaperoned substrate conjugates were subsequently scaled up and evaluated for protease-triggered urinary clearance *in vivo* using two proteolytic disease models: vascular injury and cancer. In vascular injury tests *in vivo*, our results show that there was statistically significant increase in the peptide signals found in the urine of injured mice compared with normal mice. Further, the testing of nanoparticle-chaperones optimized for detection of tumor proteases also shown statistically significant increase in urinary peptide signals. Therefore, we are now able to monitor progression and regression of both tumor and internally injury. The success of the proposed innovation may offer a unique window into proteolytic processes of diseases such as cancer, inflammation, infection, and injury.

Faculty Supervisor: Sangeeta Bhatia

A Screen of the Complete Set of Known *Drosophila* miRNAs Identifies miRNAs with Tumor-suppressor Andoncogenic Activity in Adult Gut Stem Cells

William Yee, Bonnie Berger

Department of Mathematics, MIT, Cambridge, MA 02139, USA

Cancer is the second most common cause of death in the United States. Current therapeutic methods target fast dividing cells but fail to correct the genetic changes that cause cancer. A reasonable approach is to correct genes that cause development of tumors such as those mutated oncogenes. However, the approach of inhibiting oncogenes is limited because some of these genes are also essential for cells to maintain normal functions. Another approach is to target genes that are not oncogenes themselves but vital for tumor maintenance. Recent studies have been conducted in vitro to find these genes using human tumor cell lines by RNA interference (RNAi) to knock down all genes in the human genome individually to access each gene's function in maintaining tumor cells. However, similar screens haven't been conducted in animal tumor models due to high consumption of resources. Here we report the first screen for these genes in vivo using the insect *Drosophila melanogaster*. To rapidly screen the whole *Drosophila* genome, instead of using RNAi to knock down each gene individually, we overexpress individual microRNAs (miRNA) in flies carrying a RAF-gain-of-function hyperproliferation mutation on their intestinal stem cells. In theory, each miRNA represents a group of RNAi and the effect of each miRNA is accessed quantitatively using a luciferase assay that reports stem cell number. Of the approximate 150 miRNA screened, 10% of them were found to be able to supress cell growth in this hyperproliferation model. Each miRNA will be followed up by computational analysis to identify the actual targeted genes of inhibition, and the effects of inhibiting these genes will be confirmed by RNAi.

Faculty Supervisor: Bonnie Berger

Effect of Histone H1 on PBAE-mediated Gene Delivery in HepG2 Cells

Jay Rajan, Ahmed Eltoukhy, Robert Langer
Department of Chemical Engineering, MIT, Cambridge, MA 02139, USA

Poly(β-amino esters), or PBAEs, are a class of cationic polymers being developed for Hemophilia B gene therapy. PBAEs are able to form electrostatic complexes with DNA that can be delivered into cells for subsequent expression. Because histone proteins have been previously shown to improve nonviral gene delivery, they are predicted to improve PBAE-mediated transfection efficiency. To test this prediction, H1 histones were incubated with luciferase DNA in H1:DNA weight ratios ranging from 1:2 to 10:1. HepG2 cells were transfected by this H1-DNA mixture and PBAEs C32-117 and C32-122. The addition of H1 increased polymeric DNA delivery by approximately 20% in C32-117 and by 1000% in C32-122, compared to control transfections without histone. Increasing the ratio of H1:DNA also increased luciferase expression intensity until maximum transfection efficiency was reached, after which luciferase expression decreased with further ratio increases. This maximum efficiency was achieved at ratios of 2:1 H1:DNA for C32-117 and 1:1 for C32-122. The addition of histone to DNA prior to complexation with PBAEs may serve to dramatically improve DNA delivery, thereby increasing the potential of these polymers for gene therapy applications.

Faculty Supervisor: Robert Langer
Graduate Student Mentor: Ahmed Eltoukhy