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Crystallographic Investigations of the Genetic Regulator NikR in Helicobacter pylori

Andrew VanBenschoten, Sarah Bowman, Catherine Drennan
Dept. of Biology, MIT, Cambridge, MA 02139, USA

The Gram-negative bacterium Helicobacter pylori, which inhabits the human stomach, has been implicated as a significant risk factor for stomach cancer. In order to survive in the harsh gastric environment, H. pylori synthesizes the nickel-dependent enzyme urease to neutralize its cytoplasmic pH. The critical process of cellular nickel uptake is controlled by the transcription factor HpNikR, a pleiotropic regulator. In this study we crystallized apo-HpNikR as a first step towards finding the protein's molecular structure via X-ray crystallography. More specifically, we employed the hanging-drop diffusion technique to screen various chemical solutions for their ability to assist HpNikR crystal formation. It was discovered that a solution of 0.3M tri-Na citrate and 20% Polyethylene Glycol 3350 could be used to create crystals with approximate dimensions of 400mm x 70mm x 70mm. X-ray diffraction images of these crystals were collected to a maximum resolution of 9Å. We plan to continue optimizing these crystals through additional chemical screening. Ultimately, this will enable us to collect higher-resolution data and more accurately determine the structure of the apo-HpNikR protein.

Faculty Supervisor: Catherine Drennan

Postdoc Mentor: Sarah Bowman

Downregulation of Nkx2.1 by Oncogenic Kras and Inflammatory Cytokine TNF-α is Mediated by Mek/Erk Signaling

Diana Wang, Eric Snyder, Tyler Jacks
MIT Koch Institute, Cambridge, MA 02139, USA

Nkx2.1 is a master regulator of lung differentiation and has a role in lung tumorigenesis that is not well understood. Clinically, Nkx2.1 has been used widely as a marker for the diagnosis of primary and metastatic lung cancer. While several studies have shown positive correlation between Nkx2.1 expression and lung cancer patient survival, the reasons for the close association between Nkx2.1 expression and survival rates is not well understood.

Understanding the pathways that regulate Nkx2.1 may help elucidate the role Nkx2.1 has in normal lung development and lung tumorigenesis. Previous studies have shown that oncogenic Ras is a repressor of Nkx2.1 activity. Here, we use short hairpin RNAs and inhibitor drugs to investigate which Ras downstream pathways are involved in Nkx2.1 regulation. We find that the Raf/Mek/Erk pathway suppresses Nkx2.1 expression. Other pathways such as the Ral, Akt, and Jnk pathways seem to have no role in regulating Nkx2.1. Additionally, we confirm Nkx2.1 activity is affected by the inflammatory response. We find that inflammatory cytokine TNF-α suppresses Nkx2.1 and further propose that TNF-α suppresses Nkx2.1 through activation of Erk. To investigate the molecular mechanism behind Erk repression of Nkx2.1, we plan to mutate the three known Erk phophorylation sites on Nkx2.1. With these mutant forms of Nkx2.1 we will determine if and which Erk phosphorylation sites are important for Nkx2.1 regulation by Ras and TNF-α.

Faculty Supervisor: Tyler Jacks

Postdoc Mentor: Eric Snyder

Studying Poly(ADP-ribose) (pADPr) Using the pADPr-binding Zinc Finger Motif

Yun-Han Huang, Paul Chang
Koch Institute for Integrative Cancer Research, Dept. of Biology, MIT, Cambridge, MA 02139, USA

Poly(ADP-ribose) (pADPr) is a post-translational modification and dynamic protein-binding scaffold. The pADPr biopolymer is known to play an important role in cellular processes that are often impaired during cancer progression, such as DNA damage repair, the cytoplasmic stress response, and mitotic regulation. The structures of pADPr synthesized during each of these processes are distinct, likely resulting in differences in protein binding specificities. In order to better understand the role of pADPr in different processes, we isolated a protein motif known to bind pADPr, called the pADPr-binding zinc finger (PBZ) motif, and used it to purify pADPr from human somatic cell extracts. The PBZ motif is a 25 amino acid motif found in DNA damage response proteins. We showed that the PBZ motif is able to isolate pADPr from cellular extracts generated from both normal and stress-induced cells. Interestingly, our results demonstrate that the PBZ domain binds specific sets of proteins in DNA damaged cells, in addition to proteins that also bind from cells that are cytoplasmically stressed or arrested in mitosis. These results suggest that the PBZ motif shows specificity for pADPr structures synthesized during DNA damage.

Faculty Supervisor: Paul Chang

A Gold Nanoparticle Platform for DNA Anticoagulants

Helen D'Couto, Dept. of Biological Engineering, MIT, Cambridge, MA 02139, USA

Complications with the predominant anticoagulant therapeutic create a need for a therapeutic to control blood coagulation with decreased toxicity, improved reversibility, and improved localization. We are working to design a system in which laser irradiation of gold nanoparticles (AuNP) at their longitudinal surface plasmon resonsance can selectively release an anticoagulant and its antidote. Using Activated Partial Thromboplastin Tests (APTTs), we demonstrated the ability of a DNA aptamer, Thrombin Binding Aptamer (TBA) to inhibit thrombin and the TBA complement to act as an antidote to anticoagulation. However, illumination of the AuNPs with laser pulses of 3.33E 6W/pulse did not release surface conjugated or hybridized aptamer. While we were unable to achieve laser-induced release of aptamer, our results clarify some of the parameters for selective release of aptamers from a AuNP platform and demonstrate the potential for TBA to act as a reversible anticoagulant.

Faculty Advisor: Prof. Kimberly Hamad-Schifferli

Post-doctorate Mentor: Dr. Salmaan Baxamusa

High Throughput Screen for Small Molecules that Reactivate Mutant p53

Kamena Kostova, David Feldser, Tyler Jacks
Dept. of Biology, MIT, Cambridge, MA 02139, USA

Mutations of the p53 tumor suppressor occur in a majority of human tumors and contribute to cancer initiation, progression and chemotherapeutic resistance. Somatic point mutation leading to expression of non-functional p53 is the most common genetic alteration in human cancer. That tumors depend on sustained inactivation of the p53 pathway is supported by multiple studies in which restoration of wild type p53 expression in established tumors ***in vivo*** led to a decrease in the tumor burden. Therefore, pharmacological reactivation of mutant p53 could be a potent strategy to eliminate cancer cells. Here, we present a gene expression-based high throughput screen for identifying small molecules that can reactivate mutant forms of p53. To identify a gene expression signature of p53 action and to model pharmacological reactivation of mutant p53, we performed conventional microarray analysis in sarcoma cell lines that harbor a tamoxifen-dependent restorable wild type p53 allele and a constitutively expressed point mutant allele of p53 that is commonly found in human cancer. We defined a p53-restoration signature encompassing 76 up-regulated, 13 down-regulated and 4 control genes. We used a ligation mediated amplification (LMA) assay for the rapid detection and quantification of the restoration signature. The methods described herein allow high throughput screening of large libraries of compounds for p53 restoration activity and have the potential of identifying small molecules with a profound clinical impact.

Faculty Supervisor: Tyler Jacks

Postdoc Mentor: David Feldser

EGF Ligand Release by Substrate-specific ADAM Metalloproteases Involves Different PKC Isoenzymes Depending on the Stimulus

Michelle Dang, Andreas Herrlich, Harvey Lodish
Dept. of Biology, MIT, Cambridge, MA 02139, USA

The dysregulation of EGF family ligand cleavage has severe consequences for the developing as well as the adult organism. Therefore their production is highly regulated. The limiting step is the ectodomain cleavage of membrane-bound precursors by one of several ADAM metalloproteases and understanding the regulation of cleavage is an important goal of current research. We have previously reported that in mouse lung epithelial (MLE) cells, the pro-EGF ligands TGF-alpha, NRG and HB-EGF are differentially cleaved depending on the cleavage stimulus‡. In our current study in mouse embryonic fibroblasts (MEFs) that lack different ADAMs, we show that induced cleavage of EGF ligands can involve the same substrate-specific metalloprotease but does require different stimulus-dependent signaling pathways. Cleavage was stimulated by phorbol ester (TPA; a mimic of diacylglycerol and PKC activator), hypertonic stress, lysophosphatidic acid (LPA)-induced GPCR activation, or by ionomycin-induced intracellular calcium release (IM). Although ADAMs showed substrate preference (ADAM17: TGFalpha, HB-EGF; ADAM9: NRG), substrate cleavage differed substantially with the stimulus and cleavage of the same substrate depended on the presence of different, sometimes multiple, PKC isoforms. For instance, classical PKC was required for TPA-induced, but not hypertonic stress-induced cleavage of all EGF family ligands. Inhibition of PKCzeta enhanced NRG release upon TPA stimulation, but blocked NRG release in response to hypertonic stress. Our results suggest a model in which substantial regulation of ectodomain cleavage occurs not only on the metalloprotease level but also on the level of the substrate or of a third protein.

‡ Herrlich et al. (2008) *FASEB J.* 22

Faculty Supervisor: Harvey Lodish

Postdoc Mentor: Andreas Herrlich

Connecting Genetic and Transcriptional Information to Reveal Cellular Response Pathways in Pancreatic Cancer

Oana Ursu, Shao-shan Carol Huang, Ernest Fraenkel
Dept. of Biological Engineering, MIT, Cambridge, MA 02139, USA

In response to perturbations, cell signaling and regulatory pathways are rewired. These rewired response pathways can be interrogated at multiple levels: genetic library screens find genes whose knock-down alters the response to the perturbation; mRNA profiling identifies differentially expressed genes due to the perturbation. However, biases in these experiments make the biological interpretation of the pathways difficult for two reasons. First, since the two types of experiments focus on different aspects of the response, their results show little overlap. Second, some of the genes involved in the response pathway escape experimental detection. To integrate these disjoint data into coherent pathways explaining the response, we use a computational method[1], which relies on molecular interactions. Our approach reconstructs a compact network, linking the genes found in the two experiments through additional interactions with genes not detected experimentally, but likely involved in the pathway.

We use this approach to study mechanisms by which pancreatic cancer PANC-1 cells display resistance to gemcitabine, the most frequently used chemotherapeutic agent for this cancer. Despite its wide use, gemcitabine shows limited efficacy, by mechanisms that are unclear. To study the response to gemcitabine, we integrate data from knock-down screen[2] and mRNA profiling[3], and recover pathways likely responsible for resistance to the drug. Among these is epithelial to mesenchymal transition, previously observed to be a distinguishing feature in gene expression between gemcitabine resistant and sensitive pancreatic cancer cell lines. These results are testable hypotheses for the mechanisms of gemcitabine resistance, which can be used to make this drug more effective, as well as design complementary therapies for pancreatic cancer.

1 E. Yeger-Lotem et al. (2009). *Nat. Genet.* 41, 316-323
2 Dr. Vikram Bhattacharjee, Tim Yen Lab, Fox Chase Cancer Center, PA, unpublished data
3 T. Shimasaki et al. (2007). *Journal of the Japan Pancreas Society.* 22, 14-20

Faculty Supervisor: Ernest Fraenkel

Graduate Student Mentor: Shao-shan Carol Huang

Investigation of the Mechanism and Reactivities of the Halogenase SyrB2

Sarah Whiteside[2], Mishtu Dey[1,3], Catherine Drennan[1,2,3]
HHMI[1], Depts. of Biology[2] and Chemistry[3], MIT, Cambridge, MA 02139, USA

Customized natural product molecules that have a significant impact in certain areas of medicine and drug development can be produced in several ways. Here, we focus on altering the structure and reactivities of an enzyme which helps build a natural product, eventually leading to an altered product molecule. Syringomycin biosynthesis enzyme II (SyrB2) is a key enzyme involved in the biosynthesis of the natural product Syringomycin E, an antibiotic specific to fungi. SyrB2 is a non-heme iron halogenase that is responsible for the essential conversion of L-Thr to 4-Cl-Thr. This reaction is special because it occurs at the site of an inactivated carbon. Using site-directed mutagenesis, we altered SyrB2 to create mutants with larger active sites that will be assayed for their ability to employ halogens such as bromine and iodine instead of chlorine. We then crystallized the A118G mutant in order to examine this novel active site structure. If a mutant SyrB2 that is capable of performing an iodination reaction is created, it will be the first enzyme in its super-family with the ability to add iodine to a substrate molecule. Additionally, the mutant SyrB2 enzymes could be further used to halogenate non-natural substrate molecules. These combined effects will advance our ability to custom-design natural products such as novel antibiotics, thereby increasing their usefulness in the fields of medicine and biochemistry.

Faculty Supervisor: Catherine Drennan

Postdoc Mentor: Mishtu Dey

Identification of DNA Damage Response Genes in *Caulobacter crescentus*

Tracy Kambara, Michael Laub, Dennis Kim
Dept. of Biology, MIT, Cambridge, MA 02139, USA

The wide range of bacterial responses to DNA damage, from the induction of repair pathways to the inhibition of cell division, requires the action of numerous genes. Previous studies have identified damage-inducible genes in major pathways such as the SOS response. However, less is known about the genes involved in the DNA damage response that are constitutively expressed or found in other pathways. In this study, we identified genes involved in the functional response to DNA damage. To do this, we created mutants in ***Caulobacter crescentus*** using transposon mutagenesis and an overexpression library and screened for altered responses to mitomycin C-induced DNA damage. Among the twenty-three genes identified are those that encode known DNA repair proteins as well as transcriptional regulators not associated with the SOS response. Our findings suggest that bacteria use many other pathways in addition to the classical SOS response to overcome DNA damage, and that constitutively expressed genes may be just as important as those that are damage-inducible.

Faculty Supervisors: Mike Laub and Dennis Kim