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POSTER SESSION ABSTRACTS

ODD NUMBERS 1-71
Regulation of pluripotency by DNA methylation in F9 Embryonal carcinoma

Lama AlAbdi¹, Stephen MacCune¹, Brice, H. Spears¹, Mohd Saleem Dar¹, Smriti Hoda¹,
Humaira Gowher¹,²

¹Department of Biochemistry, ²Purdue University Center for Cancer Research,
Purdue University, West Lafayette, Indiana 47907
*Corresponding author: hgowher@purdue.edu

During embryonic stem cell (ESC) differentiation, pluripotency genes must be silenced. Our previous studies delineated the mechanism leading to pluripotency gene repression through the targeted methylation of their enhancers in differentiating ESCs. Several reports detected the expression of pluripotency genes in the undifferentiated cancer stem cell populations in tumors to which tumor initiation, metastasis, and resistance to cancer therapies have been attributed. Due to their small population size and difficulty in isolation, the mechanism by which they escape the repression of their pluripotency program is not fully addressed. We use F9 Embryonal carcinoma cells (ECs) as a model to study cancer stem cells, because of their embryonic origin and potential to generate tumors. Similar to ESCs, F9 ECs can differentiate into germ layers and express all repression factors contributing to early mouse embryogenesis. However, unlike ESCs, when F9 ECs are induced to differentiate they fail to gain DNA methylation at pluripotency gene enhancers leading to incomplete repression of their pluripotency program. Our work investigating the mechanisms leading to incomplete repression of pluripotency genes during F9 ECs differentiation is paramount for our understanding of how cancer stem cells rise and persist as well as the development of differentiation-mediated cancer therapies.
Candida glabrata, the second highest cause of invasive candidiasis, is naturally resistant to azole drugs, drugs that are typically used as the first line of defense in treating systemic infections. Azole antifungals work by inhibiting Erg11, an enzyme necessary for ergosterol synthesis. One mechanism of azole resistance is the overexpression of genes that promote drug resistance such as ABC transporters or ergosterol biosynthesis genes such as ERG11 and/or ERG3. In S. cerevisiae, we have determined that deletion of SET1 results in a growth defect compared to WT cells when treated with azoles. In addition, histone H3K4R mutations are also sensitive to azole drug treatment suggesting Set1-mediated histone modification is important for WT drug resistance. Interestingly, when SET1 was deleted from C. glabrata all H3K4 methylation was abolished and an azole sensitive phenotype was observed similar to what was seen in S. cerevisiae. To determine the role of Set1 in altering azole sensitivity in C. glabrata, we performed RNA-sequencing analysis comparing WT to a set1Δ strain with and without fluconazole treatment. We have confirmed that transcript levels of genes encoding for cell wall proteins and multidrug transporters are significantly lower in the set1Δ strain than in WT. To determine if the set1Δ strain could alter drug resistance in vivo, we used the G. mellonella larvae infection model. Larvae were injected with WT or set1Δ strains and were subsequently treated with fluconazole or vehicle. Surprisingly, no significant difference in larvae viability was detected with the different strains or treatments. Because C. glabrata can uptake exogenous sterols from the host, we hypothesize that set1Δ strains are no longer azole sensitive when grown in the presence of sterols. Our data demonstrate a new and unexplored role of a Set1 and H3K4 methylation in altering azole drug sensitivity in S. cerevisiae and C. glabrata.
About 50 million years ago, an \textit{Hsmar1} transposon invaded an early primate genome and inserted itself downstream of a SET methyltransferase gene, leading to the birth of a new chimeric protein now called SETMAR. While all other \textit{Hsmar1} sequences in the human genome have suffered inactivating mutational damage, the transposase domain of SETMAR has remained remarkably intact, suggesting that it has gained a novel, evolutionarily advantageous function. While SETMAR can no longer transpose itself throughout the genome, it has retained its ancestral sequence-specific DNA binding activity, the importance of which is currently unknown.

To investigate this, we solved the crystal structure of DNA-bound SETMAR and performed ChIP-seq to examine SETMAR binding in the human genome. We also utilized RNA-sequencing to assess the effect of SETMAR on transcription and analyzed its transposase-derived chromatin-looping ability using chromosome-conformation-capture-on-ChIP (4C).

The crystal structure of DNA-bound SETMAR confirmed sequence-specific recognition of \textit{Hsmar1} terminal inverted repeat (TIR) sequences through seven nucleobase-specific interactions. ChIP-seq showed that SETMAR binds extensively throughout the genome, amassing 7457 binding sites, 94\% of which include a TIR sequence. RNA-seq indicated 177 genes that are differentially regulated by SETMAR, including repression of 17 histone genes, suggesting a possible role in chromatin dynamics. 4C analysis revealed numerous chromatin loops that form both intrachromosomal and interchromosomal connections.

As previous studies have shown that SETMAR prevents chromosomal translocations, our findings raise the possibility of a novel mechanism whereby SETMAR influences the stability and 3-dimensional positioning of chromosomes in the nucleus. The prevalence of SETMAR binding in the human genome combined with its dimeric structure and DNA looping capacity suggest a novel role for SETMAR as a chromatin organizing factor.
Targeting PRMT5 as a novel approach for the treatment of castration-resistant prostate cancer

Elena Beketova, Xuehong Deng, Jake Owens, Chang-Deng Hu

Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University College of Pharmacy, West Lafayette, IN 47906

Androgen receptor (AR) reactivation is a major cause of metastatic prostate cancer resistance to the first-line treatment option androgen deprivation therapy (ADT). Following ADT, the disease inevitably progresses within 18-24 months to a lethal end stage castration resistant prostate cancer (CRPC). In general, AR reactivation occurs through multiple mechanisms such as AR gene amplification, expression of androgen-independent mutants, expression of ligand-independent splice variants or intratumoral androgen synthesis. Current CRPC therapies are not curative and only prolong survival by 4-5 months. Thus, development of novel therapeutic approaches for CRPC treatment is urgently needed. Recently our group demonstrated that protein arginine methyltransferase 5 (PRMT5), an emerging oncogene in various cancers that symmetrically dimethylates arginine residues of histone and non-histone substrates, regulates hormone-naïve prostate cancer (HNPC) cell growth in AR-dependent manner. Mechanistically, it was demonstrated that PRMT5 epigenetically activates AR transcription and regulates growth of HNPC cells. To further expand these findings and determine whether PRMT5 regulates the growth of CRPC via AR activation, we implemented two CRPC cell lines: C4-2 expressing androgen-independent AR, and CWR22Rv1 expressing both full length and androgen-independent AR splice variant V7. Using short hairpins RNA (shRNA) knockdown and small-molecule inhibitor BLL3.3, we found that PRMT5 targeting inhibited proliferation and downregulated AR expression in both cell lines. Notably, AR full length and V7 were downregulated upon PRMT5 targeting in CWR22Rv1 at the protein and mRNA levels. We confirmed PRMT5 binding and methylation of H4R3 at the AR proximal promoter. Taken together, these results suggest that PRMT5 acts as epigenetic activator for both full length and spliced variants of AR in CRPC cells. Basing on these findings, we hypothesized that targeting PRMT5 may present a novel treatment approach for CRPC via eliminating the expression of AR and its splice variants.
Deciphering the role of N-terminal methylation in modulating yeast protein function including the multitasking stress response protein, Hsp31

Panyue Chen, Guangping Dong, Rong Huang and Tony R. Hazbun

Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN

The first cytosolic N-terminal methyltransferase identified in yeast, Tae1, recognizes a protein N-terminal motif sequence M-X-P-K. This conserved sequence motif is found in multiple proteins including ribosomal proteins, histones and small heat shock proteins across species. The human homolog of Tae1, N-terminal methyltransferase 1 (NTMT1/NRMT1) has important functions in cell division by regulating RCC1 and centromere proteins such as CENP-A and is implicated as a cancer target.

Hsp31 was predicted as one of the substrates of Tae1 based on protein sequence in addition to 44 other yeast proteins. The yeast chaperone, Hsp31, is a multitasking protein that adopts a homodimer conformation and has functional similarities to Parkinson’s disease protein, DJ-1. Hsp31 is involved in multiple cellular functions including oxidative stress sensing, protein folding, proteasome degradation, and deglycase enzyme activity. We have previously demonstrated that Hsp31 expression is induced by oxidative stress and αSyn mediated proteotoxic stress. However, it remains unclear how Hsp31 function is regulated post-transcriptionally.

Yeast is an ideal model to investigate the function of N-terminal methyltransferase activity and function. Thus, we are investigating the N-terminal methylation of Hsp31 and its regulation in Hsp31 function. We have discovered the first evidence of mono- and dimethylation of Hsp31 using mass spectrometry. Further studies are aimed at investigating the functional aspects of n-terminal methylation of Hsp31 and its effect on chaperone and enzyme activities. We are expanding the characterization and identification of substrates of Tae1 including the histone linker protein, H1. We demonstrated that decreased methylation occurs in Tae1-deficient yeast using antibodies specific for mono-, di-, or tri- methylated peptides. Further identification of additional substrates of Tae1 in this manner will be the most comprehensive investigation of the N-terminal methylome in a model organism. These studies will be the foundation for understanding this enigmatic post-translational modification on a global level.
Polycomb signaling as a therapeutic axis for enhancing epigenetic therapy in colorectal cancer

Alison A. Chomiak¹, Rochelle L. Tiedemann¹, Xiangqian Kong², Stephen B. Baylin², Scott B. Rothbart¹

¹ Center for Epigenetics, Van Andel Research Institute, Grand Rapids, Michigan 49503, USA.
² Department of Oncology, the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21287, USA.

DNA methylation and PRC2-mediated tri-methylation of lysine 27 on histone H3 (H3K27me3) are mutually exclusive epigenetic modifications appreciated for their developmental and pathologic roles in gene silencing. Through a cell-based chemical screen for regulators of tumor suppressor gene silencing in colorectal cancer, we paradoxically reveal that combining the DNA demethylating agent 5-aza-2'-deoxycytidine (decitabine) with the PRC2 inhibitor EPZ6438 (tazemetostat) – two clinically applied epigenetic agents – synergizes to alleviate repression of tumor suppressor genes, negative regulators of WNT signaling, and immune response genes. Notably, we reveal that tazemetostat has high potency and low toxicity compared to other EZH2 inhibitors, a combination that enhances the efficacy of nucleoside analogs that require DNA replication for therapeutic activity. Mechanistically, we reveal that tazemetostat does not enhance the DNA demethylating effects of decitabine. Rather, our data suggest the combination works by blocking an appreciated epigenetic switch between DNA methylation and H3K27me3 that maintains repressive chromatin environments when one or the other is lost. Our studies reveal new insights into molecular mechanisms of epigenetic crosstalk and suggest a rational drug combination to maximize the molecular and clinical response to current epigenetic therapies.
POSTER #13

Elucidating the role of the CBX8 chromodomain as a therapeutic target

Katelyn Connelly1*, Tyler Weaver2*, Kyle Denton1, Sijie Wang1, Aktan Alpsoy1, Casey Krusemark1, Catherine Musselman2, Emily Dykhuizen1

1Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN 47907; 2Department of Biochemistry, University of Iowa, IA 52242

*These authors contributed equally

The transcriptional repressor Polycomb Repressive Complex 1 (PRC1) is critical for stem cell maintenance and lineage specification. PRC1, via its chromodomain, binds trimethylated lysine 27 on histone H3 (H3K27me3) to monoubiquitinate and compact chromatin. Interestingly, the CBX chromodomain-containing subunit has five mutually exclusive paralogs (CBX2,4,6,7,8), which are misregulated in a variety of cancers, such as glioblastoma multiforme (GBM), where the CBX8 paralog is overexpressed in over half of patients. Our work demonstrates that CBX8 and its chromodomain are necessary for GBM viability, suggesting it may be a good drug target. Previous in vitro peptide studies with the CBX8 chromodomain (CD8) demonstrate negligible H3K27me3 binding, unlike its counterparts, raising questions as to how CBX8 is targeted to chromatin and how to best inhibit this interaction. Our analysis of genome-wide data indeed reveals a high correlation between H3K27me3 and CBX8 localization, and assessment of bulk chromatin demonstrates tight binding relative to the other paralogs. Surprisingly, our in vitro gel shift assays with unmodified nucleosome and linear DNA demonstrate that CD8 non-specifically binds DNA with higher affinity than H3K27me3 peptides. Furthermore, depletion of H3K27me3 in vivo is not sufficient to abrogate CBX8 chromatin binding. Taken together, our data highlights key considerations regarding CBX8 inhibitor development, most prominently, whether the inhibition of methyl binding alone will be sufficient to disrupt CBX8 activity. To address this, we have used a DNA-encoded library to identify the first high affinity ligand for CD8. Initial in vitro characterization of this ligand demonstrates selectivity for CBX8 over the highly-studied CBX7, and ongoing studies will define the effects of this ligand on CBX8 binding and activity in vivo. Our studies provide a foundation to better understand paralog-specific function of CBX8 and the contribution of chromodomain binding to CBX8 activity.
Chromatin remodeler Brahma is necessary for age dependent cell survival and visual behavior in *Drosophila* photoreceptors

Spencer Escobedo\(^1\), Hana Hall, PhD\(^1\), Vikki Weake, PhD\(^1\)

Department of Biochemistry, Purdue University, West Lafayette IN

Age is a major risk factor for ocular disease. The goal of our lab is to understand the gene expression changes that occur with age. Our previous work shows that there is a visual senescence, the decrease in visual function with age, in *Drosophila melanogaster*. We have also shown that this age-dependent decrease in visual function correlates with changes in gene expression. Epigenetic factors have been proposed to play a key role in maintaining ocular function with age. However, the epigenetic mechanisms that are involved in age-dependent gene expression changes have yet to be identified. To address these questions we use *Drosophila* photoreceptor neurons as a model of the aging eye.
Chromatin structure is a key regulator of gene expression. The basic subunit of chromatin, the nucleosome, consists of an octamer of histones wrapped by ~147 base-pairs of DNA. Protruding from each nucleosome are unstructured histone tails that can be post-translationally modified in a bevy of ways, playing a key role in directing the association and function of chromatin regulators. These regulatory complexes recognize histone tails through effector domains, which are often referred to as reader domains. The interactions between histone tails and effector domains have been widely investigated. However, these studies have largely been conducted using peptide fragments representative of the histone tails, leaving a large gap in our knowledge of how effector domains recognize their substrate in the proper context of the nucleosome. Recently, our lab has shown that the conformation of the histone H3 tail with respect to the nucleosome core inhibits binding of the BPTF PHD finger to H3K4me3 (Morrison et al., eLife, 2018). Thus, confirming the importance of investigating effector domain binding in the context of the entire nucleosome. Here, we present results on the conformation of the H4 tail in the context of the nucleosome and our investigation into the effect of this conformation on the interaction between the BPTF bromodomain and the acetylated H4 tail. Utilizing NMR spectroscopy, we find that H4K16ac alters histone tail conformational dynamics and may alter the specificity for the H4 tail in the context of the nucleosome.
Bromodomains are chromatin interaction modules of ~110 amino acids that bind acetyl-lysine residues on histones and other nuclear proteins. Bromodomains target transcriptional complexes to chromatin, and thus influence transcriptional activity of associated genes. Polybromo-1 (PBRM1), contains six bromodomains and is a subunit of the Polybromo-1-BRG-Associated-Factors (PBAF) chromatin remodeling complex. The bromodomains of PBRM1 target PBAF to specific gene loci, thereby guiding the influence of PBAF on chromatin structure and gene transcription. PBRM1 is implicated in many cancer phenotypes, where loss of PBRM1 function is associated with oncogenesis; in particular, mutations in PBRM1 bromodomains are especially common in renal clear cell carcinoma (ccRCC), the most lethal subtype of renal cancer where PBRM1 is mutated in ~40% of patients. Although PBRM1 is commonly regarded as a tumor suppressor, a 2018 study by Pan et al. showed that inactivation of PBRM1 in patients with ccRCC resulted in increased response to immunotherapeutic treatment. This followed a 2017 study by Porter and Dykhuizen that showed the bromodomains of PBRM1 are essential to its tumor suppressor function, the second bromodomain of PBRM1 (PBRM1-BD2) being especially vital. Despite the importance PBRM1 bromodomains towards PBAF function, only one small molecule exists that inhibits a PBRM1 bromodomain (PFI-3; PBRM1-BD5). Thus, inhibitors that specifically target individual PBRM1 bromodomains are sorely needed for biological study and drug development. Here, we performed fragment-based screening of PBRM1-BD2 against ~2000 drug-like fragments by protein-detected NMR. We identified 13 hits with binding affinities of 150-400 µM. These hits are currently being validated by secondary assay techniques including isothermal titration calorimetry and thermal shift assays. Future studies will optimize these fragments to improve $K_d$ values via structure-activity relationship and medicinal chemistry approaches. A potent ligand for PBRM1-BD2 may exhibit synergistic effects with immunotherapeutic interventions, leading to increased efficacy in the treatment of ccRCC and other cancers.
Dissecting the maintenance of *MuDR* transposon silencing in maize

Wei Guo and Damon Lisch

Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907

Transposons make up a substantial portion of most plant genomes. Due to their mutagenic potential, most organisms have evolved mechanisms to silence them. *MuDR* is the autonomous member of *Mutator* family of transposons. *Mu killer*, a naturally occurring derivative of *MuDR*, can heritably silence *MuDR element*, a process that is associated with methylation of the terminal inverted repeats (TIRs) surrounding *mudrA*, the transpose-encoding gene carried by *MuDR*. Our previous work demonstrated that *Mediator of Paramutation1 (MOP1)*, a putative RNA-dependent RNA polymerase-encoding gene, is required for the maintenance but not the initiation of *MuDR* silencing. However, silenced *MuDR* elements can be progressively reactivated after multiple generations in a *mop1* mutant background. This leads us to ask a fundamental question: what are the means by which silenced transposons are maintained in a silenced state and what other factors can result in a reversal of that state? To address this question, we performed a bisulfite sequencing assay to determine DNA methylation status at the TIR region of *MuDR in a mop1* mutant and wild type plants. We find that DNA methylation is lost at *MuDR in mop1* mutants. Despite this, *MuDR* remains transcriptionally silenced. We hypothesize that histone H3 lysine-9 methylation (H3mK9) and H3mK27 methylation are necessary to maintain the silencing status of *MuDR* transposon in the absence of DNA methylation in a *mop1* mutant background. To test this hypothesis, histone H3 modification status will be examined in plants that carry silenced *MuDR* elements in *mop1* mutant and wild type backgrounds. In addition to our observation that methylation is not required to maintain silencing of *mudrA*, we also find that that heat stress can reactive silenced *MuDR* in a *mop1* mutant background and this activity is maintained in new emerging leaves following that heat stress. Given this, it will be interesting to determine which changes in chromatin are associated with heat reactivation, whether or not this active state can be transmitted to the next generation.
Defining the mechanisms for R-loop formation in yeast

Youssef A. Hegazy\textsuperscript{1}, Sara C. Cloutier\textsuperscript{1}, Zheng Xing\textsuperscript{1}, Elizabeth J. Tran\textsuperscript{1,2}

\textsuperscript{1}Department of Biochemistry, Purdue University, West Lafayette, IN 47907, USA
\textsuperscript{2}Purdue University Center for Cancer Research, Purdue University, West Lafayette, IN 47907, USA

R-loops are nucleic-acid structures composed of an RNA:DNA hybrid and a displaced single-stranded DNA. R-loops occur more frequently in the genome and have greater physiological importance than was previously predicted. They play vital roles in regulating gene expression, DNA replication, and DNA and histone modifications. Paradoxically, while they do play essential positive functions, they also contribute to DNA damage and genome instability. Recent evidence shows that R-loops are involved in a number of human diseases, including neurological disorders and cancer. Despite the growing interest in R-loop regulation, there are currently few techniques available to identify and characterize R-loop structures in vivo. We are developing new strategies for mapping R-loops genome wide. We will present progress on establishing these techniques to map R-loops in \textit{Saccharomyces cerevisiae}. We are also using reporter assay and the budding yeast genetic system to identify factors that may promote formation of R-loops formed by IncRNAs (an abundant class of non-coding RNAs that have been implicated in gene regulation). Taken together, we hope to provide a detailed understanding of molecular interactions that promote formation of biologically beneficial R-loops.
Pre-replicative complex protein ORCA/LRWD1 regulates homologous recombination at ALT-telomeres by modulating RPA binding.

Rosaline Hsu¹, Yo-Chuen Lin¹, Deepak Singh¹, Yating Wang¹, Vasudha Aggarwal², Jaba Mitra², Abhijith Matur¹, Taekjip Ha²,³, Kannanganattu V. Prasanth¹ and Supriya G. Prasanth¹

¹Department of Cell and Developmental Biology, University of Illinois at Urbana-Champaign, 601S Goodwin Avenue, Urbana, IL 61801 USA; ²Biophysics and Biophysical Chemistry, Johns Hopkins University, Baltimore USA; ³Howard Hughes Medical Institute, Johns Hopkins University.

Telomeres are repetitive sequences at the ends of linear chromosomes. In mammalian cells, telomeres are protected by the Shelterin complex that coordinates end protection with telomere replication. Dysfunctional telomeres result in genomic instability and cancer development. Cancer cells achieve immortalization by acquiring a telomere maintenance mechanism. Approximately 10~15% of human tumors, mostly mesenchymal origin, utilize a telomerase-independent mechanism termed Alternative Lengthening of Telomeres (ALT), which involves a homologous-recombination-mediated DNA replication. We have identified that the human Origin Recognition Complex-associated protein (ORCA/LRWD1) as well as several components of ORC are enriched at ALT-telomeres throughout the cell cycle. The extent of ORCA enrichment at ALT-telomeres directly correlates with the levels of the Shelterin components. The loss of ORCA induces the formation of ALT-associated PML bodies (APBs), shows elevated levels of RAD51 and robust accumulation of RPA at ALT-telomeres, and increased telomere sister chromatid exchange (T-SCE). Furthermore, cells lacking ORCA show increased frequency of global sister chromatid exchange, suggesting that ORCA plays a role in the inhibition of homologous recombination. The depletion of ORCA causes decreased H3K9me3 at the telomeres, increased TERRA RNA, and overall chromatin decondensation. Furthermore, ORCA directly interacts with the single stranded DNA binding protein RPA and modulates its binding to ssDNA. In summary, our findings suggest that ORCA represses unwanted homologous recombination.
POSTER #27

Mismatch-repair signature mutations modulate gene enhancer activity across colorectal cancer epigenomes

Stevephen Hung1, Alina Saiakhova1, Devin Neu1, Zachary Faber1, Cynthia Bartels1, Ian Bayles1, Gursimran Dhillon1, Ellen Hong1, Matthew Kalady3, Sanford Markowitz1,2, & Peter C. Scacheri1

Department of Genetics and Genome Sciences1, Department of Medicine2, Case Western Reserve University, Cleveland, Ohio
Lerner Research Institute3, Cleveland Clinic, Cleveland, Ohio

The search for cancer driver mutations has largely focused on the 2% of the human genome that codes for genes. Commonly mutated genes have been found for many cancers, but far less is known about the prevalence of mutations in cis regulatory elements. We leveraged an approach that exploits gains in enhancer activity in tumor versus normal in combination with mutation detection from H3K27ac ChIP-seq data to pinpoint potential activating mutations in enhancer elements in colorectal cancer (CRC). Analysis of CRC specimens from all clinical stages revealed that samples of MSI subtype have a particularly high rate of indel mutations in active enhancers. In support of a functional role, enhancers with indels show evidence of positive selection and their target genes show elevated expression. Moreover, a subset of the enhancer indels is highly recurrent. The indels arise in short homopolymer tracts of A/T’s and generate sequences that closely resemble consensus motifs for the FOX family of pioneer transcription factors. We demonstrate the capacity of the noncoding indels to modulate enhancer activity through CRISPR/Cas9 inactivation of the MLH1 gene followed by H3K27ac ChIP-seq studies. Our results suggest that indel mutations in noncoding poly (A/T) sequences, previously presumed benign, frequently augment enhancer activity in the epigenomes of MMR-deficient CRC tumors and provide a selective advantage for tumor growth.
BEND3 regulates pluripotency by p21 mediated pathway

Mohammad Kamran¹, Sun Qinyu¹, Christophe E. Redon², Yating Wang¹, Abid Khan¹, Mirit I. Aladjem², Kannanganattu V Pransanth¹ and Supriya G Prasanth¹

1. Department of Cell and Developmental Biology, School of Molecular and Cellular Biology, University of Illinois at Urbana-Champaign, IL, 61801.
2. Developmental Therapeutics Branch Center for Cancer Research National Cancer Institute Building 37, Room 5068A Bethesda, MD 20892-4264.

Transcriptional regulation of genes is controlled by transcription factors, coregulators as well as by histone and DNA modifications. Histone and DNA Methylation at CpG position is associated with transcriptional repression. HP1 alpha is a heterochromatin-associated protein, which is recruited to the methylated histone 3 at lysine 9 position (H3K9me3) for the formation and propagation of heterochromatin. BEND3 is a quadruple BEN domain containing protein, which interacts with H3K9me3 and HP1 alpha to form heterochromatin and works as a transcriptional repressor. We have previously demonstrated that tethering of BEND3 at gene promoter causes repression and BEND3-bound genes also show inability in recruitment of RNA Polymerase II at the promoters. Further, overexpression of BEND3 causes hyper-heterochromatinization and cell cycle arrest. BEND3 also associates with rDNA clusters and represses the transcription of rDNA by stabilizing nucleolar-remodeling complex (NoRC) at the promoters. We find that BEND3 is highly expressed in the pluripotent carcinoma cell line NTERa2 as well as in embryonic stem cells. Retinoic Acid-mediated differentiation of NTera2 caused downregulation of BEND3. Loss of BEND3 resulted in cells exhibiting a differentiated phenotype as evident by reduction in pluripotency markers including Oct4 and Nanog and a concomitant increase in HoxA1 expression. Genome-wide ChIP-seq analysis revealed that BEND3 occupies gene promoters with potential G-quadruplex motifs. GO analyses demonstrated that MAPK and p53 pathway were the most affected pathways in cells lacking BEND3. Intersection of RNA-seq and ChIP-seq results revealed that BEND3 binds to CDKN1A promoter and inhibits the expression of p21 in order to maintain the pluripotency in NTera2 cells.
Covalent modifications to both the DNA and the histone proteins allow chromatin to act as a dynamic information hub that integrates diverse biochemical stimuli to regulate genomic DNA access to the transcription machinery and ultimately establish and maintain cellular phenotypes. Moreover, there is increasing appreciation that chromatin alterations per se, including DNA and histone modifications, are involved in the pathogenesis of cancer. Nowhere is this better supported than with the groundbreaking discoveries of high-frequency, somatic mutations in histones that are drivers of oncogenesis. These mutations (collectively called "oncohistones") cause amino acid substitutions that localize to conserved residues in the N-terminal tail of histone H3 and all seem to be linked, either directly or indirectly, to disruption of normal levels and distribution of histone H3 methylation and thus genomic regulation. Specifically, oncohistones directly or indirectly promote aberrant genome-wide distribution of lysine 27 methylation on histone H3. H3K27 methylation, catalyzed by the Polycomb Repressive Complex 2 (PRC2), is mechanistically linked to establishment and maintenance of gene repression. We are currently using a combination of biochemical and genomic approaches to investigate how oncohistone-driven changes in histone H3 K27 methylation lead to altered chromatin states that profoundly influence gene expression patterns. I will discuss some of our recent mechanistic and functional work on various oncohistone mutations.
PCNA-mediated stabilization of E3 ligase RFWD3 at the replication fork is essential for DNA replication

Yo-Chuen Lin+, Yating Wang+, Sumanprava Giri, Susan Wopat, Arindam Chakraborty, Kannanganattu V. Prasanth, and Supriya G. Prasanth*

Department of Cell and Developmental Biology, University of Illinois at Urbana-Champaign 601S Goodwin Avenue, Urbana, IL 61801 USA

RFWD3 is an E3 ligase, known to facilitate homologous recombination by removing RPA and RAD51 from DNA damage sites. Further, RPA-mediated recruitment of RFWD3 to stalled replication forks is essential for interstrand crosslink repair. Here we report that in unperturbed human cells, RFWD3 localizes at replication forks, and associates with Proliferating Cell Nuclear Antigen (PCNA) via its PCNA-interacting (PIP) motif. PCNA association is critical for the stability of RFWD3 and for accurate S-phase progression. Cells lacking RFWD3 show slower fork progression, a prolonged S-phase, and an increase in the loading of several replication fork components on the chromatin. All these point to increased frequency of stalled forks in the absence of RFWD3. The S-phase defect is rescued by WTRFWD3 but not by the PIP-mutant, suggesting that the interaction of RFWD3 with PCNA is critical for DNA replication. Finally, we observe reduced ubiquitination of RPA in cells lacking RFWD3. Our results suggest that the stabilization of RFWD3 by PCNA at the replication fork enables the polyubiquitination of RPA and its subsequent degradation for accurate fork progression.
Plk1 inhibition enhances the efficacy of BET epigenetic reader blockade in castration-resistant prostate cancer.

Fengyi Mao, Jie Li, Qian Luo, Ruixin Wang, Yifan Kong, Colin Carlock, Zian Liu, Bennet D. Elzey, and Xiaoqi Liu

1 Department of Biochemistry, Purdue University, West Lafayette, IN 47907
2 Department of Animal Sciences, Purdue University, West Lafayette, IN 47907
3 Department of Comparative Pathobiology, Purdue University, West Lafayette, IN 47907
4 Center for Cancer Research, Purdue University, West Lafayette, IN 47907

Polo-like kinase 1 (Plk1), a crucial regulator of cell cycle progression, is overexpressed in multiple types of cancers, and has been proven to be a potent and promising target for cancer treatment. In case of prostate cancer, we once showed that anti-neoplastic activity of Plk1 inhibitor is largely due to inhibition of androgen receptor (AR) signaling. However, we also discovered that Plk1 inhibition causes activation of the β-catenin pathway and increased expression of c-Myc, eventually resulting in resistance to Plk1 inhibition. JQ1, a selective small molecule inhibitor targeting the amino-terminal bromodomains of BRD4, has been shown to dramatically inhibit c-Myc expression and AR signaling, exhibiting anti-proliferative effects in a range of cancers. Since c-Myc and AR signaling are essential for prostate cancer initiation and progression, we aim to test whether targeting Plk1 and BRD4 at the same time is an effective approach to treat castration resistant prostate cancer (CRPC). Herein, we show that a combination of Plk1 inhibitor GSK461364A and BRD4 inhibitor JQ1 had a strong synergistic effect on CRPC cell lines in vitro, as well as in CRPC xenograft models in vivo. The combination treatment led to inhibition of cell growth, a massive increase in apoptosis, and a concurrent drop in glycolysis. Mechanistically, the synergistic effect is likely due to two reasons: 1) Plk1 inhibition results in the accumulation of β-catenin in the nucleus, thus elevation of c-Myc expression, whereas JQ1 treatment directly suppresses c-Myc transcription. 2) Plk1 and BRD4 dual inhibition acts synergistically in inhibition of AR signaling.
FOXQ1 interacts with the KMT2/MLL core complex to promote transcriptional activation of the epithelial to mesenchymal transition (EMT) program

Allison Mitchell¹,², Benjamin Kidder Ph.D.¹,², Zhe Yang Ph.D.³ and Guojun Wu Ph.D.¹,²

¹Department of Oncology, Wayne State University School of Medicine, ²Molecular Therapeutics Program, Karmanos Cancer Institute, ³Department of Microbiology, Immunology and Biochemistry, Wayne State University School of Medicine

Epithelial to mesenchymal transition (EMT) is an essential process during development characterized by the reversible phenotypic switch of polarized epithelial cells towards a mesenchymal cell state. Aberrant activation of EMT has become an evident mechanism of acquired metastatic competence and drug resistance in epithelial cancer types. Our published studies identified forkhead box transcription factor FOXQ1 as a potent driver of the EMT program in breast cancer. However, the epigenetic mechanisms regulating FOXQ1 activity remain elusive. To address this problem, we identified FOXQ1-binding proteins using tandem affinity proteomics. Three proteins (RbBP5, WDR5, ASH2L) that define the core complex of mixed lineage leukemia (MLL/KMT2) family of histone methyltransferases, were identified as highly abundant FOXQ1-interacting proteins. These three proteins (RbBP5, WDR5, ASH2L) complex with an MLL enzyme to facilitate the histone-3 lysine-4 trimethylation (H3K4me3) within promoters of actively transcribed genes. We hypothesize that FOXQ1 binding to the MLL core complex is required for activation of critical downstream genes that facilitate EMT and promote tumor progression. Human mammary epithelial cells with ectopic FOXQ1 (HMLE/FOXQ1) were utilized as a model of breast EMT for chromatin immunoprecipitation sequencing (ChIP-seq) and differential expression by RNA-seq. Analysis of chromatin localization of FOXQ1 and the MLL core complex, utilizing RbBP5 as a marker, revealed 92% of the FOXQ1 activated promoters were also occupied by RbBP5. These downstream gene targets are specifically enriched functions in EMT signaling pathways and contain well-characterized EMT transcription factors. In addition, shRNA downregulation of RbBP5 in HMLE/FOXQ1 cells reduced transcript abundance of the targets with FOXQ1 and RbBP5 co-bound promoters and diminished the acquired stem-like phenotype, assessed by mammosphere formation and stem cell surface markers. These data support cooperation of FOXQ1 and the MLL core complex is critical for regulating the EMT transcriptional program.
Enhancer co-option on extrachromosomal oncogenic amplifications

Andrew R. Morton¹, Stephen C. Mack², Xiuxing Wang³, Megan Piazza⁴, Shashirekha Shetty⁴, Fredrick R. Schumacher⁵, Jeremy N. Rich³, Peter C. Scacheri¹

¹Department of Genetics and Genome Sciences, Case Western Reserve University, Cleveland, OH
²Department of Pediatrics, Baylor College of Medicine, Houston, TX
³Department of Medicine, Division of Regenerative Medicine, University of California - San Diego School of Medicine, La Jolla, CA
⁴Center for Human Genetics Laboratory, University Hospitals, Cleveland, OH
⁵Department of Population and Quantitative Health Sciences, Case Western Reserve University, Cleveland, OH

Focal amplifications of oncogenes are frequent driver events across many solid tumors. These amplifications have been characterized extensively at the genetic level, but few groups to date have done paired genetic and epigenetic analyses necessary to delineate the role, if any, of cis-regulatory elements on amplicons. We performed ChIP-seq of H3K27ac, a histone mark of active promoters and enhancers, in human glioblastoma models harboring amplifications of EGFR and/or MYC. Using paired-end sequencing reads from the ChIP-seq and input control, we reconstructed the genetic architecture of the EGFR and MYC amplifications and mapped the accompanying landscape of active cis-regulatory elements. We found that EGFR and MYC amplifications most often occur on double minutes, circularized extrachromosomal fragments of DNA propagated at very high copy number. Remarkably, genes incorporated on the double minutes are virtually always accompanied by their regulatory elements, indicating that there is a strong selective pressure for not only the oncogene, but also the elements that spatiotemporally control their expression. In support of their function, regulatory elements on the double minutes undergo pervasive transcription, and the expression of their gene targets scales linearly with copy number. A broader analysis of 170 EGFR-amplifications in glioblastoma shows that EGFR is non-randomly positioned on amplicons. Specifically, EGFR amplifications display a 5-prime “skew”, in which non-coding regions upstream of EGFR are included on amplicons more often than expected by random chance ($p < 1 \times 10^{-7}$). This upstream region contains two active enhancer elements that are nearly ubiquitously co-amplified with EGFR. Focally-amplified oncogenes in other solid tumors also show amplicon skewing indicative of enhancer selection. Collectively, our studies indicate that enhancer elements undergo strong positive selection during oncogene amplification and that co-amplification of oncogenes and enhancers may be a determinant of amplicon size in solid tumors.
POSTER #41

Early Aging Phenotypes Associated With Haploinsufficiency Of Hat1 In Mice

Prabakaran Nagarajan, Chitra Iyer*, William Arnold* and Mark Parthun

Department of Biological Chemistry and Pharmacology, *Department of Neurology, The Ohio State University Wexner Medical Center, Columbus, OH 43210.

The acetylation of newly synthesized histones is catalyzed by enzymes known as type B histone acetyltransferases. Histone acetyltransferse 1 (Hat1) is one of a type B histone acetyltransferase for the acetylation of lysines 5 and 12 on histone H4 during chromatin assembly. In order to understand the biological role of Histone acetyltransferase 1, we have generated a conditional mouse knockout model of the enzyme. We previously reported that Hat1 is necessary for mammalian development; genome stability and the processing of newly synthesized histones. In this study, we show that haploinsufficiency of Hat1 results in defects consistent with an early aging phenotype. These defects include shortened life span, lordokyphosis (hunchback), muscle atrophy, minor growth retardation, reduced skin fat deposition, cancer, paralysis and loss of vitality. At cellular level, fibroblasts from Hat1 haploinsufficient embryos undergo early senescence and accumulate high levels of p21. Hat1 +/- MEFs accumulate increased levels of endogenous DNA damage and display a delay in DNA damage repair in response to DNA double strand break agents. Thus, Hat1 haploinsufficient mice provide a useful model for mechanistic investigation of the role of chromatin structure and DNA repair in mammalian aging.
Epigenomic landscape, spatial conformation and function of regulatory elements of the PDGFRA-KIT locus in gastrointestinal stromal tumor (GIST) cells

Fei Gao\textsuperscript{1}, Sabriya A. Syed\textsuperscript{1}, Jeong-Heon Lee\textsuperscript{1}, Chuanhe Yu\textsuperscript{1}, Sergii M. Kvasha\textsuperscript{1}, Gabriella B. Gajdos\textsuperscript{1}, Siva Arumugam Saravanaperumal\textsuperscript{1}, Natalie G. Tran\textsuperscript{1}, Liang Cheng\textsuperscript{1}, Jagneet Kaur\textsuperscript{1}, Aditya Bhagwate\textsuperscript{1}, Zhifu Sun\textsuperscript{1}, Krutika S. Gaonkar\textsuperscript{1}, Zhenqing Ye\textsuperscript{1}, Huihuang Yan\textsuperscript{1}, Dong Fang\textsuperscript{2}, Zhiguo Zhang\textsuperscript{2}, Ying Peng\textsuperscript{1}, Yi Guo\textsuperscript{1}, Yujiro Hayashi\textsuperscript{1}, Tamas Ordog\textsuperscript{1}

\textsuperscript{1}Mayo Clinic, Rochester, MN, USA; \textsuperscript{2}Columbia University, New York, NY, USA

GISTs, the most common human sarcoma, most often arise from activating mutations in the receptor tyrosine kinase KIT. Most GISTs also express wild-type PDGFRA, a closely related gene located ~420 kb upstream of KIT. KIT-PDGFRA co-expression is rare in interstitial cells of Cajal (ICC), the normal counterpart of GIST. Thus, KIT and PDGFRA transcription may be co-regulated in a disease-specific manner. To understand the underlying mechanisms, we studied the epigenetic states (by chromatin immunoprecipitation-sequencing (ChIP-seq) targeting H3K4me1/3, H3K27ac/me3 and CTCF), conformation (by 3C and Hi-C) and function (by CRISPR-(d)Cas9-aided genome and epigenome editing) of the regulatory elements of the PDGFRA-KIT locus in mouse ICC and human GIST cells. In FACS-isolated, KIT\textsuperscript{+}PDGFRA\textsuperscript{−} mouse ICC, ChIP-seq revealed an active Kit and a bivalent Pdgfra promoter, as well as an intergenic and an intra-Kit super-enhancer. The enhancers were enlarged in KIT\textsuperscript{+}PDGFRA\textsuperscript{−} GIST882 and GIST-T1 cells and much reduced in KIT\textsuperscript{−}PDGFRA\textsuperscript{−} GIST-T1-10R and GIST48B cells. In all GIST cell lines, interaction between the PDGFRA promoter and an enhancer 134 kb downstream of KIT enclosed the entire locus in a loop. The PDGFRA and KIT promoters variably interacted with enhancers located within -25 and +202 kb and -449 and -79 kb, respectively, with KIT\textsuperscript{−}PDGFRA\textsuperscript{−} cells showing fewer loops. Genomic deletion of any one of the 4 intergenic super-enhancers nearly completely abolished GIST-T1 clonogenicity. The 2 surviving clones (out of 3150) displayed ~50\% reduced KIT protein, increased apoptosis and reduced proliferation, clonogenicity and migration. In GIST882 cells, targeting the same super-enhancers with dCas9-EZH2 and 4-10 single guide RNAs reduced KIT and PDGFRA expression to 22-25\% and 48-58\% of dCas9 controls, respectively. We conclude that enhancers of the PDGFRA-KIT locus are critical for PDGFRA and KIT expression in the ICC-GIST lineage and malignant behavior. Disrupting these enhancers may represent a novel approach to the treatment of GIST.
POSTER #45

Aberrantly expressed microRNAs drive the development of acquired Erlotinib-resistance in Non-Small Cell Lung Cancer (NSCLC)

Arpita Pal¹²³, Alejandra Agredo¹², Manvir Bains¹, Kayla Gates¹, Dr. Andrea Kasinski²³. Purdue University Interdisciplinary Life Science Program (PULSe)¹, Department of Biological Sciences², Purdue Center for Cancer Research³, Purdue University, West Lafayette, IN 47907.

Lung cancer is the third most prevalent cancer, and yet the leading cause of cancer-related deaths worldwide, signifying the necessity for better treatment strategies. The outcome of my thesis project may advance an innovative therapeutic agent to treat Non-Small Cell Lung Cancer (NSCLC) patients, the subtype of lung cancer that accounts for ~85% of cases. NSCLC patients frequently harbor mutated causal genes, of which ~10-35% of cases express an activated form of the epidermal growth factor receptor (EGFR) resulting in uncontrollable growth and proliferation. The treatment for such patients is Erlotinib, a tyrosine kinase inhibitor, although most develop Erlotinib resistance (ER) within a year post treatment. Alternative pathways and unknown mechanisms are implicated in the development of ER, but the role of microRNAs (miRNAs) as drivers of ER in NSCLC remain largely unexplored.

MiRNAs are small RNA molecules that regulate cellular homeostasis, and are often dysregulated in diseases such as cancer. Loss or gain of miRNAs drive multiple cancer hallmarks, however their role as drivers of drug-resistance is not well defined. Therefore, to identify miRNAs as inducers of ER, a two-prong study has been conducted: 1) an overexpression analysis, and 2) a knock-out screen. For the overexpression analysis and subsequent validation, two Erlotinib-sensitive NSCLC cell lines were utilized, that led to the identification of five candidate miRNAs that drive ER. Currently, the candidates are being validated. In parallel, a CRISPR-Cas9-mediated knocked-out screen was also conducted in the presence of Erlotinib. We hypothesize that loss of critical Erlotinib responsive genes will promote ER. Importantly, candidate genes from both approaches are being validated in patient biopsies acquired pre- and post-ER. Successful completion of this project will identify miRNAs and/or protein-coding genes promoting ER, and advance the field towards development of efficient targeted therapeutics for NSCLC.
The ubiquitin-proteasome system (UPS) is a regulator of proteins involved in the regulation of cellular events. However, a full compendium of substrates of the UPS does not exist. As a protein complex, the proteasome can serve as a model for the analysis of protein complex dynamics in the presence of protein sequence variants such as missense mutations. Analysis of the impact of missense mutations on protein stability is currently intractable at a high-throughput scale. Yeast strains containing missense mutations in individual proteasome subunits were used to analyze the effects of mutations on global protein abundance and stability via quantitative mass spectrometry. In the mutants analyzed to date, over 1,200 proteins increased in abundance as compared to WT. Next, we were interested in examining whether mutations in individual subunits would impact the thermal stability of the proteasome. We developed a new application of the cellular thermal shift assay (CETSA) to profile the impact of mutations in a single protein on the full proteome. This approach enables analysis of the effects of amino acid sequence changes in specific subunits of the proteasome on the proteasome as a multi-subunit complex. The addition of CETSA led to the identification of a more specific set of proteins changing in the mutants relative to WT, with many of the changes being within the proteasome itself. Gene Ontology analysis of proteins that increased in the proteasome mutants showed an enrichment for proteins involved in transcription. Using the data gathered from these experiments as a base, we will repeat these experiments with both genetic and biochemical disruptions in transcription along with mutations in the proteasome to measure transcription dependent protein accumulation. These experiments will better our understanding of the role of the proteasome in the regulation of transcription and may provide insight for future therapeutics
Polymono-1 (PBRM1) is a member of the PBRM1-Brg-Associating Factors (PBAF) chromatin remodeling complex. It is mutated in 3.5% of all human cancers with most events occurring in renal clear cell carcinomas (RCCC) (40%). However, depletion of PBRM1 is not sufficient for tumorigenesis and adult mice with a conditional PBRM1 knockout are phenotypically normal. Recent reports indicate that loss of both PBRM1 and VHL, the most commonly mutated gene in RCCC, in the kidney is sufficient to cause tumor formation. Through RNA-seq studies, we identified that PBRM1 regulates genes involved in cell adhesion, apoptosis, hypoxic response, and negative cell proliferation pathways and PBRM1 transcriptional targets are further upregulated when cells are grown in 3D culture, which causes metabolic stress and hypoxia. Additionally, we have found that histone 3 lysine 14 acetylation (H3K14Ac), a mark associated with stress and DNA damage, is necessary for the PBRM1 binding. Due to PBRM1’s association with stress pathways and epigenetic marks, we explored PBRM1’s role under both metabolic and oxidative stress. We used both normal epithelial and cancer cell lines. Consistently, we found loss of PBRM1 increased cell proliferation in epithelial cell lines as well as elevating reactive oxygen species (ROS) levels. However, knockdown of PBRM1 sensitized cells to high levels of ROS. Finally, using the MCF10a breast series, we found that knockdown of PBRM1 causes opposing growth phenotypes depending on the stage of cancer progression. We hypothesize that PBRM1 is regulating stress pathways and depending on cell type as well as the internal and external environment, PBRM1 can be growth promoting or growth inhibiting.
Modeling Aryl Hydrocarbon Receptor-mediated Gene Regulatory Networks

Wenjie Qi, David Filipovic and Sudin Bhattacharya

Department of Biomedical Engineering, Michigan State University, East Lansing 48824

The Aryl Hydrocarbon Receptor (AHR) is a ligand-inducible transcription factor (TF) that regulates genes involved in a variety of physiological functions. The AHR, together with the aryl hydrocarbon nuclear translocator (ARNT) protein, binds to specific DNA sequences containing the 5-nucleotide core motif 5’-GCGTG-3’ (“dioxin response elements”, or DREs) after being activated by the potent environmental pollutant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The 5-bp core DRE motif is not sufficient for AHR binding: 5’ and 3’ flanking sequences also play a role. Knowing the identity of valid AHR binding sites is critical for assembling AHR-mediated transcriptional regulatory circuits. We predicted valid AHR binding sites in hepatocytes cells via the application of supervised machine learning algorithms. K-nearest neighbor (k-NN) and Random Forest classification yielded test set accuracies of 80% and 84%, respectively. In extension of this preliminary work, we will apply more sophisticated classification models including boosted trees, support vector machines and artificial neural networks for more accurate prediction of AHR binding sites. We also found evidence that in addition to binding in proximal promoter regions, TCDD-activated AHR binds at distal enhancer regions to regulate the expression of target genes through DNA looping. In MCF-7 cells, of 332 differentially expressed genes, only 24 had AHR bound in their promoter regions. Reciprocally, only 38 of 2595 AHR ChIP-Seq peaks in MCF-7 cells were located in the promoter regions of differentially regulated genes, suggesting widespread long-range gene regulation mediated by AHR. Our combination of accurate prediction of AHR binding sites and mapping proximal and distal AHR binding to target genes will enable the construction of AHR regulatory networks for multiple tissues.
Exploring the role of histone modifications in epigenetic UV hypere resistance of *Saccharomyces cerevisiae*

Rachel M. Reardon, Amanda K. Walsh, and Jeffrey S. Thompson

Department of Biology, Denison University, Granville, Ohio

Epigenetic phenomena involve the ability of organisms to pass along cellular “memories” based on environmental conditions they experience. Progeny can inherit epigenetic profiles that make it easier to access genes useful in withstanding environmental stresses faced in prior generations. Our lab is investigating the potential for yeast to develop an epigenetically-inheritable cellular memory that improves their ability to survive UV damage. We have observed that cells given a pre-exposure to a low dosage of UV exhibit a significant increase in survival following subsequent exposures when compared to unexposed cells, up to a 200-fold difference, which persists for up to 8 generations. Further analysis indicates that these changes do not result from mutations, suggesting an epigenetic mechanism of inheritance. Since histone modifications are well-characterized as heritable epigenetic factors, these molecules were our first target of investigation for acquisition of the hyper-resistance phenotype. To test which modifications may be involved, we screened knockout strains of known histone-modifying enzymes for defects in the hyper-resistance phenotype. From this screen, we have identified a number of histone modifications that are important for this phenotype. For example, the Rtt109 knockout (H3K56 acetyltransferase) displays a significant increase in UV resistance, while knockouts of the corresponding deacetylases (Hst3 and Hst4) display a corresponding decrease in hyper-resistance. This suggests that deacetylation at H3K56 is important for the establishment and/or maintenance of the hyper-resistance phenotype. Modulation of H3K4 methylation also appears to be important, as loss of function in its demethylase reduces hyper-resistance. These results indicate that UV hyper-resistance is an epigenetically-inheritable phenotype in yeast cells, and further suggest that changes in histone modification states are important in its propagation.
Linker histones bind Nucleosome Core Particles (NCP) forming chromatosomes, which induces greater DNA compaction in chromatin fibers. However, precise molecular understanding of the impact of linker histones on the structure, mechanics, and dynamics of chromatin fibers is still lacking in the literature. In this contribution we will report results from 3 µs of fully atomistic Molecular Dynamics (MD) simulations of chromatin fibers formed by an octa-NCP structure, both with and without H1 linker histones bound in off-dyad positions. Our systems were built using as reference the 11 Å cryo-EM density map solved by Song et al. (*Science*, 2014, 344, 376) with 177 base pairs. Results show that inter-NCP stacking interactions are increased upon linker histone binding. H1 linker histones are tightly bound to the linker DNA due to the abundance of lysine amino acids, which prompts a local rearrangement of the DNA and reduces the flexibility of the linker DNA. These effects are translated into an increase in rigidity and compaction of the overall chromatin fiber when H1 linker histones are present in the system. Moreover, mutual correlation analyses of the systems with and without H1 show an enhancement of the correlated motions all along stacked NCPs. This suggests that H1 triggers a cascading stabilizing effect along the resulting chromatin fiber. We expect our findings to have direct implications in chromatin remodeling, epigenetics, and identification of histone posttranslational modification sites.
Bivalent histone reader, EBS, regulates floral phase transition in Arabidopsis

Zhenlin Yang¹,²⁺, Shuiming Qian³,⁴*, Ray N. Scheid³,⁴, Li Lu³,⁴, Xiangsong Chen³,⁴, Rui Liu¹, Xuan Du¹,², Xinchen Lv¹,², Melissa D. Boersma⁴, Mark Scalf⁵, Lloyd M. Smith⁵, John M. Denu⁴,⁶,⁷, Jiamu Du¹†, Xuehua Zhong³,⁴†

¹National Key Laboratory of Plant Molecular Genetics, CAS Center for Excellence in Molecular Plant Sciences, Shanghai Center for Plant Stress Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China
²University of Chinese Academy of Sciences, Beijing, China
³Laboratory of Genetics, University of Wisconsin-Madison, Madison, WI, USA
⁴Wisconsin Institute for Discovery, University of Wisconsin-Madison, Madison, WI, USA
⁵Department of Chemistry, University of Wisconsin-Madison, Madison, WI, USA
⁶Department of Biomolecular Chemistry, University of Wisconsin-Madison, Madison, WI, USA
⁷Morgridge Institute for Research, Madison, WI, USA
*These authors contributed equally to this work.
†Correspondence: Jiamu Du (jmdu@sibs.ac.cn) & Xuehua Zhong (xuehua.zhong@wisc.edu)

The ability of cells to perceive and translate versatile cues into differential chromatin and transcriptional states is critical for many biological processes. In plants, timely transition to a flowering state is crucial for successful reproduction. EARLY BOLTING IN SHORT DAY (EBS) is a negative transcriptional regulator that prevents premature flowering in Arabidopsis. Here, we revealed that bivalent bromo-adjacent homology (BAH)-plant homeodomain (PHD) reader modules of EBS bind H3K27me3 and H3K4me3, respectively. A subset of EBS-associated genes was co-enriched with H3K4me3, H3K27me3, and the Polycomb repressor complex 2 (PRC2). Interestingly, EBS adopts an auto-inhibition mode to mediate its binding preference switch between H3K27me3 and H3K4me3. This binding balance is critical because disruption of either EBS-H3K27me3 or EBS-H3K4me3 interaction induces EBS-mediated early floral transition. This study identifies a single bivalent chromatin reader capable of recognizing two antagonistic histone marks and reveals a distinct mechanism of interplay between active and repressive chromatin states.
Bromodomains are chromatin interaction domains that bind acetyl-lysine residues on histones and transcription factors, thereby controlling gene expression by directing assembly of transcriptional complexes on chromatin. Members of the bromodomain and extra-terminal domain (BET) family (Brd2, Brd3, Brd4, Brdt) each contain two bromodomains within a single polypeptide sequence. Pan-BET bromodomain inhibitors are currently in phase I/II clinical trials for multiple cancers and phase III trials for type 2 diabetes subjects with coronary artery disease. To aid mechanistic inquiries, we are removing a critical barrier in the study of BET bromodomain biology: the lack of inhibitors and chemical probes that selectively target individual BET proteins. Currently, all existing BET inhibitors target Brd2, Brd3, Brd4, and Brdt with equal nanomolar potency. This lack of selectivity may be responsible for the side effects of memory loss and lymphoid toxicity recently associated with existing pan-BET inhibitors. We are overcoming these barriers with a novel fragment-based ligand discovery strategy to discover selective Brd4 inhibitors by covalently targeting a unique cysteine within Brd4. These chemical tools will be necessary to distinguish the differential activities of BET proteins in cell and rodent models of disease and may lead to therapeutics that selectively target the Brd4 axis in cancer and diabetes.
BMI1 localization to sites of DNA damage as a potential mechanism for development of cisplatin resistance

Shruthi Sriramkumar¹, Heather M. O’Hagan¹,²

¹Medical Sciences, Indiana University School of Medicine, Bloomington IN, 47405, USA.
²Indiana University Melvin and Bren Simon Cancer Center, Indianapolis, IN, 46202, USA.

Background: Platinum based agents, cisplatin and carboplatin are most commonly used to treat ovarian cancer (OC) patients. These agents damage DNA by forming adducts with adjacent guanines. Resistance to platinum based agents is the major cause of mortality among OC patients. Aberrant DNA hypermethylation of genes and their subsequent transcriptional repression has been linked to cisplatin resistance in OC. However, the mechanism of initiation of these alterations is not known. Transient transcriptional repression occurs in the vicinity of DNA damage sites to promote repair. However, this transient transcriptional repression can persist at some loci causing stable silencing. We hypothesize that BMI1 ubiquitinates H2AX at K119 at sites of cisplatin-induced DNA damage and contributes to transcriptional repression. This recruitment occasionally causes stable gene silencing ultimately contributing to the development of cisplatin resistance.

Methods: Ovarian cancer cells were treated with IC50 dose of cisplatin and 8 hours later localization of BMI1 to sites of damage and H2AX ubiquitination was demonstrated by using immunofluorescence and western blot respectively. Gene expression changes were determined by qRT-PCR.

Results: Our preliminary data demonstrates BMI1 co-localization with the damage marker γH2AX and H2AX ubiquitination after cisplatin treatment. H2AX ubiquitination decreases when ATM is inhibited and on knockdown of NER proteins-XPA and CSB. We also demonstrate that candidate genes that are known to be silenced by DNA methylation in cisplatin resistant cells, are transcriptionally repressed following acute cisplatin treatment.

Conclusions: BMI1 localizes to sites of cisplatin-induced DNA damage where it ubiquitinates H2AX. A portion of this ubiquitination is dependent on ATM and NER proteins. We hypothesize that BMI1 localization is also dependent on ATM and NER proteins. Understanding the role of BMI1 in platinum resistance will enable us to design therapies to avert the development of drug resistant ovarian cancers.
Analysis of RNA Polymerase II protein-protein interactions as a consequence of FACT perturbation

Jason D. True, Guihong Qi, Amber L. Mosley

Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN

The histone chaperone Spt16 is a conserved and essential transcription regulator. Spt16 heterodimerizes with Pob3, and together they associate with Nhp6 forming the FACT complex. Spt16 regulates both transcription initiation and elongation, through either a repressive or activating role. In addition, Spt16 interacts with elongating RNAPII. Our lab wanted to determine how Spt16 affected the RNAPII interactome, so we performed affinity purification mass spectrometry (AP-MS) on Rpb3-FLAG (a subunit of RNAPII) in both SPT16 wildtype (WT) and temperature sensitive spt16-197 cells. In order to get a more quantitative comparison, we coupled this with tandem mass tagging (TMT), an isobaric labeling approach that enabled sample multiplexing, separated the samples through multidimensional protein identification technology (MudPIT), followed by analysis on a high-resolution Q-Exactive Plus Orbitrap mass spectrometer. Since immunoprecipitation can result in different yields of the bait protein, we normalized to Rpb3. Overall, we identified 2,055 proteins, of which ~50 were significantly decreased in spt16-197 compared to WT. Interestingly, three of the five subunits of the PAF complex (Paf1, Cdc73, and Ctr9) were decreased significantly (abundance ratio <0.65, p<0.05). In addition, the histone chaperone Spt6 and chromatin remodeler Snf2 decreased significantly (0.811 and 0.699 respectively). Most of the RNAPII subunits were unchanged (~1.0), suggesting that RNAPII assembly is not impaired. Spt16 association with RNAPII increased in the mutant although not significantly. However, Pob3 and Nhp6 association decreased suggesting that the increase in Spt16 association with RNAPII could not recapitulate the FACT interaction in spt16-197 cells. All four core histones and the variant H2A.Z were decreased in the mutant, although none were statistically significant. Altogether, it appears that while RNAPII assembles properly, it does not interact with proteins necessary for elongation in the absence of fully functional Spt16. Therefore, Spt16 helps recruit necessary elongation factors for productive elongation by RNAPII.
PLK1-dependent phosphorylation of EZH2 contributes to its oncogenic activity in castration-resistant prostate cancer

Ruixin Wang, Zhuangzhuang Zhang, and Xiaoqi Liu

Department of Biochemistry, Purdue University, West Lafayette IN

Enhancer of zeste homologue 2 (EZH2), the catalytic subunit of Polycomb-repressive complex 2 (PRC2), plays a critical role in repressing gene expression by tri-methylation of histone 3 at lysine 27 (H3K27me3). Emerging data have demonstrated that there is a link between EZH2 and oncogenesis as EZH2-mediated methylation acts as an important factor in epigenetic silencing of tumor suppressor genes in cancer. Expression of EZH2 is often upregulated in castration-resistant prostate cancer (CRPC), thus EZH2 has been proposed as a target for CRPC. Importantly, it has been demonstrated that EZH2 becomes hyper-phosphorylated in CPRC cells. Further, it has been shown that the oncogenic function of EZH2 is regulated by these post-translational modifications. Polo-like kinase 1 (PLK1), a regulator of various stages of mitosis, has been shown high activity in CRPC. However, whether PLK1 is involved in EZH2 phosphorylation is not known. Herein, we show that Plk1 physically interacts with EZH2 and negatively regulates H3K27 trimethylation (H3K27me3). Furthermore, Plk1 can phosphorylate EZH2 at T144, and Plk1-mediated phosphorylation of EZH2 is involved in inhibiting EZH2 activity toward H3K27me3. More importantly, EZH2 phosphorylation by Plk1 is inhibitory for PRC2-mediated gene repression but required for gene activation toward oncogenesis. Finally, by combination with Plk1 inhibitor BI2536, we show a robust sensitization of EZH2 inhibitors in CRPC cell lines, as well as in CRPC xenograft tumors. Our findings provide a new mechanism to define the oncogenic activity of EZH2 and suggest that inhibition of Plk1-mediated EZH2 activity may provide a promising therapeutic approach for CRPC.
Investigating X-Chromosome Inactivation in Human Embryonic Stem Cells

Aaron Williams¹, Marissa Cloutier¹, Surinder Kumar¹, Emily Buttigieg¹, Brandon Lee¹, Sandra Mojica-Perez², Andre Monteiro Da Rocha², Laura Keller², Gary Smith², and Sundeep Kalantry¹

¹Department of Human Genetics   ²Department of Molecular & Integrative Physiology
University of Michigan Medical School, Ann Arbor, MI 48109

X-inactivation equalizes X-linked gene expression between XX females and XY males via transcriptional gene silencing of one of the two X-chromosomes in early female embryos¹. During initiation of X-inactivation, XIST, a long-noncoding RNA, is expressed from and coats the inactive X-chromosome², which results in stable transcriptional silencing by recruitment of epigenetic factors in cis³. For this reason, XIST RNA coating, detected by RNA fluorescence in situ hybridization (FISH), is an excellent proxy for presence of X-inactivation⁴. Mouse embryonic stem cells (mESCs) have yielded a wealth of data on mouse X-inactivation dynamics. However, to what extent these findings apply to humans is unclear⁵. In collaboration with Gary Smith’s group, we have begun to profile X-inactivation in human embryonic stem cells (hESCs).

Unexpectedly, we found hESCs grown in mTeSR media showed loss of XIST RNA expression over successive passaging, while hESCs grown in xenofree (XF) media did not show loss of XIST RNA coating. We hypothesized that differences between the mTeSR and XF media led to the loss of XIST RNA coating in hESCs. mTeSR media contains Lithium (Li) while XF does not. To determine if Li was the causative agent in the loss of XIST RNA coating, we added Li to XF media. When grown in XF media with Li, hESCs displayed loss of X-inactivation, similar to that seen in mTeSR media. Li is a potent inhibitor of the GSK-3 protein, among other kinases⁶. This observation led us to hypothesize that GSK-3 inhibition by pharmacologic agents may also cause loss of X-inactivation. We are currently using three GSK-3 inhibitors to determine if inhibition of GSK-3 causes loss of XIST RNA coating: BIO⁷, LY2098, and Alsterpaullone⁹. Our results suggest that pharmacologic inhibition or activation of cell signaling pathways may have unknown effects on the epigenome broadly and on X-inactivation specifically. Our findings are surprising because X-inactivation is believed to be a cell autonomous phenomenon that is immune to extracellular influences¹⁰. Moreover, our findings suggest that certain media formulations should be avoided in the future if hESCs are to be used for therapeutic purposes.
Characterization of the mammalian DEAD-box protein DDX5 reveals functional conservation with \textit{S. cerevisiae} ortholog Dbp2 in transcriptional control and glucose metabolism

Zheng Xing\textsuperscript{1} & Elizabeth J. Tran\textsuperscript{1, 2}

AFFILIATION: \textsuperscript{1} Department of Biochemistry, Purdue University, West Lafayette, IN, USA
\textsuperscript{2} Purdue Center for Cancer Research, Purdue University, West Lafayette, IN, USA

DEAD-box proteins are a class of non-processive RNA helicases that dynamically modulate the structure of RNA and ribonucleoprotein complexes (RNPs). However, the precise roles of individual members are not well understood. Work from our lab revealed that the DEAD-box protein Dbp2 in \textit{Saccharomyces cerevisiae} is an active RNA helicase \textit{in vitro} that functions in transcription by promoting mRNP assembly, repressing cryptic transcription initiation, and regulating long non-coding RNA activity. Interestingly, Dbp2 is also linked to glucose sensing and hexose transporter gene expression. DDX5 is the mammalian ortholog of Dbp2 that has been implicated in cancer and metabolic syndrome, suggesting that the role of Dbp2 and DDX5 in glucose metabolic regulation are conserved. Herein, we present a refined biochemical and biological comparison of yeast Dbp2 and human DDX5 enzymes. We find that human DDX5 possesses a 10-fold higher unwinding activity than Dbp2, which is partially due to the presence of a mammalian/avian specific C-terminal extension. Interestingly, ectopic expression of \textit{DDX5} rescues the cold sensitivity, cryptic initiation defects, and impaired glucose import in \textit{dbp2}\textsuperscript{Δ} cells, suggesting functional conservation. Consistently, using mouse hepatocyte AML12 cells, we show that DDX5 promotes glucose uptake and glycolysis, a process that is upregulated in cancers. We will present evidence that DDX5 may be a novel entry point for therapeutic targeting of cancer-specific metabolism.
The chromatin remodeler PIE1 contributes to loss of the repressive epigenetic mark H3K27me3 in plants lacking the chromatin remodeler PKL

Jiaxin Long, Ben Carter, Brett Bishop, Hui-Chun Li, Emily Overway, Christopher K. Dugard, Ru Huang, Wei Jia, Heng Zhang, Nicholas C. Carpita, Pete E. Pascuzzi, Roger B. Deal, and Joe Ogas

H3K27me3 is a repressive epigenetic mark that plays a critical role in differentiation and development of plants and animals. We recently demonstrated that the SWR1-related chromatin remodeler PIE1 contributes to deposition of H3K27me3 in Arabidopsis, most likely by promoting incorporation of the histone variant H2A.Z. In addition, our data indicated that the CHD chromatin remodeler PKL promotes H3K27me3 by promoting nucleosome retention after the passage of a polymerase. In a genetic screen for mutants that alter the phenotype of pkl plants, we identified a novel allele of PIE1, pie1-6 that suppressed pkl-associated traits. In particular, the presence of pie1-6 suppresses the transcript phenotype of pkl plants as well as the reduction of H3K27me3. PKL is thought to promote retention of H3K27me3 in part by promoting maturation of prenucleosomes. Based on previous biochemical characterization of prenucleosomes, loss of PKL is therefore predicted to result in increased levels of H3K56Ac. We examined the ability of different histone deacetylase inhibitors to increase pickle root penetrance and observed that that pickle root penetrance is specifically responsive to an inhibitor of the histone deacetylases that act on H3K56Ac. Our combined data suggest an additional role for PIE1 in homeostasis of H3K27me3, in which PIE1 acts to promote loss of H3K27me3 in an H3K56Ac-dependent fashion in the absence of PKL.
POSTER SESSION ABSTRACTS
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GLTSCR1 and GLTSCR1L define a unique SWI/SNF subcomplex

Aktan Alpsoy

1Department of Medicinal Chemistry and Molecular Pharmacology | Center for Cancer Research, Purdue University, West Lafayette, IN, United States

BRG1/BRM-associated factors (BAF) complex is an ATP-dependent chromatin remodeler. The complex alters the DNA accessibility by ejecting or sliding nucleosomes; or exchanging the histone dimers. It consists of an ATPase subunit (BRM or BRG1), DNA/histone- binding and scaffolding proteins. Various types of BAF complexes have been identified in different tissues and developmental stages that differ in subunit composition and functions, most of which are yet-to-be characterized.

Glioblastoma tumor suppressor candidate region 1 (GLTSCR1) is a protein encoded in chromosome 19q arm, a frequently-deleted region in gliomas. The protein has not been characterized in terms of function or biochemical activity; however, it was reported to interact with BRG1 and some BRG1-associated factors. However, the context at which the interaction occurs has not been understood.

Our density sedimentation and coimmunoprecipitation experiments revealed that GLTSCR1 is a subunit of a novel SWI/SNF subcomplex, which lacks core subunits such as ARID1A/B, BAF170 or BAF47 while it uniquely possesses BRD9. We termed this complex as GBAF. GLTSCR1L, a paralog of GLTSCR1, can substitute GLTSCR1 in GBAF and its ectopic expression is enough to form GBAF and shift the complex stoichiometry from regular BAF to GBAF. Besides, we demonstrated that BRD4-BAF cooperation is dependent on GLTSCR1 but not GLTSCR1L, indicating that GLTSCR1 is a key candidate protein connecting chromatin remodeling and BRD4-mediated transcriptional regulation. Knocking out GLTSCR1 reduced the proliferation rate in androgen-independent prostate cancer cell line PC3. In addition, GLTSCR1 knockout androgen-sensitive prostate cancer line, LNCaP, displayed higher sensitivity to BET inhibitors. Transcriptomics and genome-wide binding studies will follow to unravel unique and redundant roles of GBAF on transcriptional regulation.
Human cytomegalovirus (HCMV) is the leading infectious cause of birth defects in the United States and presents a significant threat to the health of immunocompromised individuals. HCMV utilizes the host cell RNA polymerase II (Pol II) transcriptional apparatus for its gene expression. However, the extent to which HCMV follows the host transcriptional paradigm remains unclear. We performed PRO-Seq and PRO-Cap in uninfected and HCMV-infected primary human foreskin fibroblasts to identify sites of initiation, pausing and productive elongation by Pol II with extreme depth at single-nucleotide resolution. Using these methods, we determined that transcription of HCMV is similar to host transcription in that both utilize Pol II elongation control. 91,332 host and 14,006 HCMV transcription start regions (TSRs) were identified and some differences between host and viral core promoter elements were found. Late gene transcription in HCMV utilizes a set of viral factors. One of these factors recognizes TATT instead of TATA as an upstream element and we found that only TATT on the viral genome impacted initiation. We also observed that late in the lytic HCMV infection cycle, Pol II transcription of the HCMV genome is pervasive. A TSR was detected approximately every 17 bp in the HCMV genome, which far exceeds the density observed on the host genome. Chromatinization of the HCMV genome contributes to viral latency, but whether or how chromatin impacts HCMV during the lytic infection cycle is less well defined. We propose a model explaining our results in the context of what is already known, in which transcription of the HCMV genome takes place on a template that is only partially chromatinized.
Ex vivo screen identifies CDK12 as an epigenetic-driven metastatic vulnerability in osteosarcoma

Ian Bayles¹, Malgorzata Krajewska², Alina Saiakhova¹, James Morrow¹, Zachery Faber¹, Yuriy Fedorov¹, Drew Adams¹, Rani George², and Peter Scacheri¹

Case Western Reserve University¹ Dana Faber Cancer Center²

Osteosarcoma (OS) is an aggressive pediatric cancer with poor outcomes. Most patients present with lung metastases and current treatments were developed against primary tumors. We developed an approach that enables preclinical screening of compounds on metastatic OS cells in the context of the native lung microenvironment, allowing the identification of compounds targeting the main cause of mortality in these patients. Deploying this strategy to screen a library of epigenetically-related compounds, we found inhibitors of CDK12 to be most effective, reducing OS cell outgrowth in the lung by >90% at submicromolar doses. CDK12 inhibitors impeded elongating RNA Pol II-S2 in a gene length- and expression-dependent manner. These effects were accompanied by displacement of CDK12 from active enhancer elements and global changes in gene expression. We further identify OS cell models that differ in their response to CDK12 inhibition in the lung. The sensitivity discrepancies correlate with differences in the levels of known metastasis dependency genes and strong drivers of survival. Our studies provide a framework for rapid preclinical testing of compounds with anti-metastatic activity and highlight CDK12 as a potential therapeutic target in osteosarcoma.
Nucleosomal Inhibition of PHD-H3 Binding is Mediated by PTM Crosstalk: Support from Molecular Dynamics Simulations

Samuel Bowerman and Jeff Wereszcynsk

Department of Physics, Illinois Institute of Technology, Chicago, IL, USA

The H3 histone tail contains many post-translational modification (PTM) sites invoked by the cell to mediate chromatin remodeling. In particular, triple methylation of K4 serves as a signal for the recruitment of the BPTF PHD finger domain. Indeed, the mechanisms of histone-PHD binding have been extensively studied, but typically using truncated H3 peptides. Until recently, little work has been directed towards understanding the PHD-H3 interaction in the context of the full NCP complex. Here, we utilize MD simulations, in combination with the NMR measurements of our collaborators, to support a model in which the NCP core inhibits association with the PHD finger by competitively interacting with the H3 tail sequence, and we find that subsequent charge-altering PTMs to residues far from the PHD-binding sequence simultaneously reduces tail-NCP interactions and increases PHD recruitment. Taken together, these data support an elaborate network of PTM crosstalk in epigenetic regulation.
Mechanisms involved in repression of germline genes in somatic cells of *C. elegans*

Jerrin Cherian and Lisa Petrella

Department of Biological Sciences, Marquette University, Milwaukee, WI 53233

Organisms need to maintain proper gene expression at all times. In *C. elegans*, the conserved DRM complex regulates proper gene expression maintenance in somatic cells by repressing germline gene expression. DRM complex mutants maintain close to normal gene expression at 20°C; however, at 26°C, DRM mutants have increased ectopic germline gene misexpression accompanied by High Temperature larval Arrest (HTA) phenotype. How germline genes become active at 26°C is still not fully known. Although data from our lab shows changes in histone modifications between mutants and wild type, these marks do not change between 20°C and 26°C. I am investigating if changes in germline gene localization within the nucleus correlate to gene expression changes at 26°C. Initial results show that the promoter of a DRM regulated gene localizes to the nuclear periphery at 20°C and 26°C in both wild type and mutants. Since DRM targets do not move away from the periphery when expressed, I hypothesize that they could be moving near to expressive environment of nuclear pore protein (NPP) complexes. I have that found that knock-down of several NPP genes in a DRM mutant suppressed HTA. This suggests that disruption of the NPP structure dampens ectopic germline gene expression in DRM mutants. I speculate that DRM targets are expressed by localizing to the NPP when faced with moderate temperature stress. I am also working on identifying transcription factors (TFs) involved in misexpression of the DRM target genes at 26°C. I conducted an RNAi screen and found that knock-down of nine of 82 TFs tested lead to suppression of the HTA phenotype in DRM mutants. Identifying TFs facilitating germline gene misexpression will elucidate local changes in chromatin structure at DRM target loci. Overall, this study aims to identify mechanisms involved in proper cell fate maintenance in *C. elegans* under environmental stress.
Embryonic development requires the modulation of gene expression states via cellular epigenetic machinery. Polycomb repressive complex 2 (PRC2) is a key epigenetic regulator that methylates lysine at amino acid position 27 on histone H3 (H3K27me3). PRC2 and H3K27me3 are required to maintain imprinted X-chromosome inactivation. X-inactivation equalizes X-linked gene expression between XX female and XY male mammals via transcriptional silencing of one of the two X chromosomes in early female embryos. Imprinted X-inactivation results in the preferential inactivation of the paternal X-chromosome and initiates in all cells of the preimplantation mouse embryo. The parent-of-origin-specific pattern of inactivation of the paternal-X makes imprinted X-inactivation a paradigm of transgenerational epigenetic inheritance in mammals. PRC2 and H3K27me3 are enriched on the prospective inactive-X during the initiation of imprinted X-inactivation. We previously postulated that the inactive X-enriched PRC2 proteins are transmitted by the oocyte to the early embryo. Here, we test the hypothesis that oocyte-derived maternal PRC2 protein EED acts as a transgenerational epigenetic regulator that potentiates imprinted X-inactivation in the embryo. We generated mouse embryos devoid of maternal and zygotic EED (Eed_{mz-/-}). Eed_{mz-/-} embryos failed to initiate imprinted X-inactivation. Paternal X-linked genes that are normally silenced were expressed in Eed_{mz-/-} preimplantation embryos. Moreover, the maternal X-chromosome ectopically induced Xist lncRNA, a master regulator of X-inactivation in wild-type oocytes and embryos. Our results therefore suggest a dual role of maternal EED protein in imprinted X-inactivation initiation. The first is to deposit H3K27me3 at the maternal Xist locus, and thus keep maternal Xist silenced and the maternal X-chromosome active. The second is to silence genes on the paternal X-chromosome. Thus, EED functions transgenerationally to cause inactivation of the paternal X-chromosome while ensuring that the maternal X-chromosome remains transcriptionally active.
A functional proteomics screen for lysine methyltransferase substrate selectivity reveals a role for SMYD2 in circadian clock regulation

Evan M. Cornett¹, Bradley M. Dickson¹, Kevin M. Shaw¹, Robert M. Vaughan¹, Krzysztof Krajewski², Nicholas Spellmon³, Philip P. Versluis¹, Martis W. Cowles⁴, Joseph Brunzelle⁵, Zhe Yang³, Zu-Wen Sun⁴, and Scott B. Rothbart¹

¹ Center for Epigenetics, Van Andel Research Institute, Grand Rapids, Michigan 49503, USA.
² Department of Biochemistry and Biophysics, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, USA.
³ Department of Microbiology, Immunology, and Biochemistry, Wayne State University School of Medicine, Detroit, Michigan 48201, USA.
⁴ EpiCypher, Inc., Research Triangle Park, NC 27709, USA.
⁵ Advanced Photon Source, Argonne National Lab, Argonne, IL 60439, USA.

Lysine methylation plays an important role in the regulation of protein function, but it has proven challenging to connect lysine methyltransferases (KMTs) to non-histone protein substrates. Here, we report on the development of an unbiased and high-throughput functional proteomics platform to map KMT substrate selectivity. We use this platform to map the selectivity of three KMTs: G9a, SET7, and SMYD2. Notably, we identify five new SMYD2 substrates that could not have been predicted from previously available motif information, demonstrating the utility of this platform for making new KMT-substrate connections. Among the newly identified SMYD2 substrates is PER2, a core circadian clock component; consequently, we discovered SMYD2 functions as a negative regulator of this circadian clock gene. Collectively, this work confirms the utility of our platform for mapping the substrate selectivity of KMTs and highlights several ways the data generated with this platform can guide the study of lysine methylation signaling.
A central question in development is how chromatin is organized to ensure proper gene expression and cell fate. During early embryo development, before gene expression is globally upregulated, chromatin is found in an open state. As development proceeds and cells differentiate, chromatin undergoes both a general whole genome compaction and also becomes organized into open and closed domains based on if a gene will be expressed in a particular lineage. Although this process is highly regulated, many of the proteins involved in this progression are unknown. Here we show that loss of *C. elegans* synMuv B proteins causes changes in the developmental regulation of chromatin compaction both globally and at target loci. Previous work demonstrated that synMuv B mutants have ectopic expression of germline genes in somatic cells and demonstrate a high temperature larval arrest (HTA) phenotype. The HTA phenotype is rescued by knockdown of chromatin modifiers, suggesting that synMuv B proteins regulate gene expression programs at the chromatin level. We investigated if synMuv B proteins regulate developmental chromatin compaction utilizing extrachromosomal arrays and fluorescent *in-situ* hybridization. synMuv B mutants display a developmental delay in both general genome-wide chromatin compaction and compaction of tissue specific loci. The timing of compaction is sensitive to temperature in both wild type and mutant embryos but is delayed longer into mid-embryonic development in mutants. Open chromatin during this period may allow germline genes to be poised for ectopic expression in somatic tissues of synMuv B mutants. Interestingly, we found that the most anterior cells of the intestine are the last cells to adopt compact chromatin, suggesting an anterior to posterior pattern of chromatin compaction that has not been previously described. Understanding this pattern and synMuv B regulation of chromatin compaction will help elucidate pathways used to achieve proper gene expression and correct development.
Hypoxia-inducible factor 1 alpha (HIF1A) stimulates neuronal nitric oxide synthase (Nos1) transcription by modifying spatial chromatin organization


Mayo Clinic, Rochester, MN, USA

NOS1 is the source of nitric oxide, a neurotransmitter and regulator, in neurons, skeletal and cardiac muscle. NOS1-related diseases include impotence, gastrointestinal motor disorders, muscular dystrophies, neurodegenerative diseases and stroke. The mechanisms regulating Nos1 transcription are incompletely understood. Most tissues experience relative hypoxia (1-5% O$_2$ vs. 21% in air). Nos2 and Nos3 are transactivated by HIF1A and aryl hydrocarbon receptor nuclear translocator (ARNT). Here, investigated whether Nos1 is also subject to hypoxic control, and whether pharmacological manipulation of HIF1A could stimulate Nos1 expression. In NOS1$^+$ N1E115 mouse neuroblastoma cells and IM-FEN enteric neuron precursors, physiological hypoxia (4% O$_2$) for 3 days increased HIF1A protein, Nos1 mRNA and NOS1 protein levels. Pharmacological upregulation of HIF1A also increased NOS1. Nuclear HIF1A was detected in NOS1$^+$ human gastric neurons. In N1E115 cells, by chromatin immunoprecipitation-sequencing (ChIP-seq) we found hypoxia-induced Nos1 transcription to be associated with increased HIF1A and ARNT binding to 3 enhancer-promoter pairs located 86 kb (E1-P1), 76 kb (E2-P2) and 28 kb (E3-P3) upstream of the translation initiation site in Nos1 mRNA. Furthermore, hypoxia dramatically enhanced P1 and moderately increased P3 promoter activity, stimulated the activity of all enhancers, and upregulated transcriptional elongation throughout the Nos1 locus. Chromosome conformation capture by 3C and Hi-C revealed long-range interactions between E1-P1 and E3-P3. Hypoxia weakened these interactions while strengthening enhancer-promoter connectivity within both regions. Genomic deletion of E1 reduced the expression of Nos1 by 82%. ChIP-seq in freshly purified nitrergic neurons identified E1-P1 as the only active cis-regulatory region. We conclude that physiological hypoxia stimulates Nos1 expression by dramatically enhancing the activity of a distal enhancer-promoter pair and simultaneously modifying spatial chromatin organization. Our findings also suggest the activation of hypoxic pathways under physiological conditions. Pharmacologically increasing HIF1A protein stability may be useful for restoring impaired Nos1 expression in a variety of diseases.
PRMT5 is a substrate of HDAC6

Inosha D. Gomes and Mary Kay H. Pflum*

Wayne State University, Department of Chemistry, Detroit, MI, 48202

Gene expression is regulated by chromatin remodeling factors and histone modifications, such as phosphorylation, methylation and acetylation. Acetylation is catalyzed by histone acetyltransferase and histone deacetylase (HDAC) proteins. HDAC proteins remove acetyl groups from ε-N-acetyl lysine amino acids on nucleosome histones, which allow DNA to wrap around the histones more tightly and influences protein expression. The most studied HDAC substrates are histones, which led to the name histone deacetylase. The recent discovery of a wide variety of acetylated proteins in cells suggests that HDAC proteins likely deacetylate substrates in addition to histones. Unfortunately, identification of non-histone substrates is largely serendipitous. This poster describes identification of a novel substrate PRMT5, as a substrate of HDAC6 using substrate trapping mutants. Protein arginine methyltransferase 5 (PRMT5) is a protein arginine methyl transferase that catalyzes the symmetrical dimethylation of arginine residues within target proteins. PRMT5 is shown to associate with and methylate histone or non-histone proteins in cells, and plays key roles in cell development, survival and apoptosis. Ras-GAP SH3 binding protein 1(G3BP1) is a known substrate of PRMT5 and deacetylation of PRMT5 by HDAC6 decrease the dimethylation of G3BP1. Demethylation of G3BP1 known to promote stress granule formation which help to regulate gene expression and cell survival.
“Small talk” between the Non-Small-Cell Lung Cancer cells and normal bronchial epithelial cells via extracellular vesicles lead to NSCLC progression

Humna Hasan¹²³, Sean Humphrey¹², Sarunya Kitdumrongthum¹²⁴, Hana Kubo¹², Feng Tian³² and Andrea L. Kasinski¹²³

¹Department of Biological Sciences, ²Purdue Center for Cancer Research, and ³Graduate Program in Biological Sciences, Purdue University, West Lafayette, IN 47906, ⁴Department of Physiology, Faculty of Science, Mahidol University, Bangkok, Thailand.

Non-Small-Cell-Lung-Cancer (NSCLC) is essentially incurable, comprising over 85% of lung cancers. Its rapid growth and metastatic progression leads to increased mortality each year. For the cancer’s progression to occur, cell-to-cell communication is important. Cells communicate using a variety of mechanisms including the transfer of bioactive material from one cell to another by way of extracellular vesicles of different types. One class of extracellular vesicles formed in endosomal multivesicular bodies (MVB) that are released following fusion of the MVB with the plasma membrane are termed as ‘exosomes’. Classically, exosomes are circulating extracellular vesicles ranging in diameter from 40-140nm and serve as a vehicle of intercellular communication through delivery of bioactive molecules including proteins, lipids, and nucleic acids (DNA, mRNA, microRNA and other non-coding RNAs). This study aims to identify the ability of NSCLC cell-derived-exosomes to modulate the migratory, invasive, and proliferative activity of normal human bronchial epithelial cells (HBECs). Exosomes isolated from NSCLC cells and non-tumorigenic HBECs were characterized based upon size, quality, and expression of exosome-enriched protein markers (CD9 and CD63). Functional studies highlight that exosomes isolated from NSCLC cells promote HBECs to invade, migrate, and proliferate. Moreover, our results revealed that RNAs isolated from NSCLC cell-derived exosomes stimulated some of these behaviors indicating that exosomal RNA is critical to exosome function. Collectively, our findings suggest that intercellular communication between NSCLC and non-tumorigenic bronchial epithelial cells via exosomes can modulate the activity of the recipient cell. Also, Exosomes-associated RNAs are important bioactive molecules that might cause modulation of activity in the normal cells. However, the mechanisms by which exosomal RNAs bring these changes remain elusive. Future studies evaluating exosomal RNA using RNA seq analysis will provide more insight on the unique RNA content selectively sorted into the exosomes by the cancer cells.
POSTER #24

Epigenetically-regulated CAZymes enable efficient degradation of untreated lignocellulose by anaerobic gut fungi for bioenergy production

Casey Hooker\textsuperscript{1,2}, Ethan T. Hillman\textsuperscript{1,3}, Adrian Ortiz-Velez\textsuperscript{1}, Jonathan C. Overton\textsuperscript{1,2}, Scott Briggs\textsuperscript{4}, and Kevin Solomon\textsuperscript{1-3}

\textsuperscript{1}Department of Agricultural and Biological Engineering, Purdue University West Lafayette, IN
\textsuperscript{2}Lab for Renewable Resource Engineering (LORRE), Purdue University West Lafayette, IN
\textsuperscript{3}Purdue University Interdisciplinary Life Science Biotechnology Training Group (PULSe), Purdue University West Lafayette, IN
\textsuperscript{4}Department of Biochemistry, Purdue University West Lafayette, IN

Fungi are attractive platforms for elucidating eukaryotic gene regulation mechanisms given their relatively small genomes, ease of culturing, and short life-cycles. Despite this, many fungal lineages are either underrepresented, or entirely neglected in these analyses. For example, the early-diverging and only obligately anaerobic division of fungi, \textit{Neocallimastigomycota}, which are native to the digestive tracts of ruminants and hindgut fermenters, only has six published genomes to date. Hence, characterizing epigenetic mechanisms in these lineages may identify novel strategies that are overlooked or missed in widely studied yeast and other eukaryotic organisms. Here we have isolated 4 previously unclassified species of anaerobic fungi and characterize their growth and protein expression on diverse plant biomass to establish a baseline by which these organisms may be cultivated. Carbohydrate active enzyme expression is significantly remodeled to maximize substrate hydrolysis efficiency on diverse lignocellulosic feedstocks. The ability of these organisms to adjust protein expression and activity is mediated in part by histone modifications. We show that carbohydrate active enzyme expression is correlated with histone deacetylase, H3K56 acetyltransferase, and DNA methyltransferase transcript abundance. Similarly, by using multiple epigenetic inhibitors we are able to control H3K4 and H3K27 trimethylation and increase xylanase activity by almost 100%. Our studies demonstrate for the first time that early diverging anaerobic fungi use epigenetic mechanisms for gene expression and establish their role in lignocellulose hydrolysis. Our work provides insight into the mechanisms that control anaerobic fungal plant-degradation abilities and develops facile tools to enhance activity for efficient plant degradation, while identifying regulatory mechanisms that may be conserved in other eukaryotic systems.
Spt4, along with its DSIF complex partner Spt5, is thought to have roles in RNA Polymerase II (RNAPII) processivity, heterochromatin dynamics during transcription, and co-transcriptional mRNA processing. My current research goal is to investigate how Spt4 mechanistically regulates transcription elongation, and how the RNAPII interactome and global transcription dynamics are altered in the absence of Spt4. FLAG-tagged RNAPII complex and any interacting proteins in wildtype or spt4Δ yeast can be isolated and analyzed via affinity purification mass spectrometry (MS). My preliminary results suggest that in an spt4Δ strain, RNAPII has increased interactions with a possible premature transcription termination complex, and Cdc48, a protein involved in protein complex degradation by the proteasome. Cdc48 is a AAA ATPase thought to be responsible for pulling apart protein complexes in order for the subunits to be degraded by the 26S proteasome. Previous data from Verma et al. (2012) suggests Cdc48 may mediate Rpb1 degradation. To further investigate RNAPII as a substrate of Cdc48, 26S proteasomes are affinity purified (Pre1-Myc) from wildtype and cdc48-3 ts strains. The isolated proteasomes are then analyzed by MudPIT MS² to observe the accumulation of proteasome substrates that cannot be degraded without the help of Cdc48.
Sirtuins catalyze the NAD\(^+\)-dependent deacylation of acyl-lysine residues, producing O-acyl-ADP-ribose and nicotinamide. Humans encode seven sirtuins (Sirt1-7) that are considered pro-survival proteins, and decreased sirtuin activity promotes aging-related diseases, including type-II diabetes. However, how sirtuin activity is inhibited during aging is largely unknown. We are defining the physiological mechanisms that regulate sirtuin activity post-translationally, as elucidation of these mechanisms will illuminate unexploited means to prevent disease-associated decreases in sirtuin activity. As oxidative stress increases with age, we focus on the regulation of sirtuin activity by post-translational modification by cellular oxidants.

We show that Sirt1 can be nitrosated by S-nitrosoglutathione (GSNO). Colorimetric Zn\(^{2+}\) loss and circular dichroism assays revealed Sirt1 nitrosation correlated with Zn\(^{2+}\)-release and loss of \(\alpha\)-helical structure, suggesting the target of nitrosation is the Zn\(^{2+}\)-tetrathiolate conserved among sirtuins. Molecular dynamics simulations suggested Zn\(^{2+}\) loss due to Sirt1 nitrosation results in repositioning of the tetrathiolate subdomain away from the rest of the catalytic domain, disrupting NAD\(^+\) and acetyl-lysine substrate binding. Furthermore, Sirt1 nitrosation was reversed upon exposure to thiol-based reducing-agents, resulting in restoration of Sirt1 activity. This restoration was dependent on the presence of Zn\(^{2+}\), consistent with nitrosation of the Zn\(^{2+}\)-tetrathiolate as the source of Sirt1 inhibition.

More recently, we found that nuclear sirtuins (Sirt1, Sirt2, Sirt6) are inhibited \textit{in vitro} by nitric oxide (NO) and NO-derived oxidants, and are resistant to inhibition by oxidized glutathione, hydrogen peroxide, and hydrogen sulfide. Surprisingly, mitochondrial sirtuins (Sirt3 and Sirt5) displayed selective inhibition by peroxynitrite and were insensitive to all other assayed oxidants. These data suggest that, despite conservation of the Zn\(^{2+}\)-tetrathiolate across all sirtuins, S-nitrosation does not universally inhibit sirtuins. Additionally, we observed a concentration- and time-dependent increase in Sirt6 expression in pancreatic beta cells in response to NO, suggesting that NO modulates Sirt6 expression in beta cells under inflammatory conditions.
**CDC7 modulates silencing via H4 K16 acetylation and the histone chaperone CAF-1**

Tiffany J. Young\(^{1,2}\), Yi Cui\(^{2,3}\), Joseph Irudayaraj\(^{2,3,4}\), and Ann L. Kirchmaier\(^{1,2}\)

\(^{1}\)Department of Biochemistry, \(^{2}\)Purdue University Center for Cancer Research, \(^{3}\)Department of Agricultural and Biological Engineering, West Lafayette, IN 47907, \(^{4}\)Current Address: Department of Bioengineering, University of Illinois at Urbana Champaign, Urbana, 61820

CAF-1 is an evolutionarily conserved H3/H4 histone chaperone that plays a key role in replication-coupled chromatin assembly and is targeted to the replication fork via interactions with PCNA, which, if disrupted, lead to epigenetic defects. In *Saccharomyces cerevisiae*, when the silent mating-type locus *HMR* contains point mutations within the *E* silencer, Sir protein association and silencing is lost. Mutation of *CDC7*, encoding an essential S phase-specific kinase, or subunits of the H4 K16-specific acetyltransferase complex SAS-I, however, restore silencing to this crippled *HMR\(^{a}e^{*}\)*. Here, we observed that loss Cac1p, the largest subunit of CAF-1, also restores silencing at *HMR\(^{a}e^{*}\)*, and silencing in both cac1\(^{Δ}\) and cdc7 mutants is suppressed by overexpression of *SAS2*. We demonstrate Cdc7p and Cac1p interact *in vivo* in S phase, but not G1, consistent with observed cell cycle-dependent phosphorylation of Cac1p, and chromatin in both cdc7 and cac1\(^{Δ}\) mutants is hypoacetylated at H4 K16. Moreover, silencing at *HMRae\(^{a}e^{*}\)* is restored in cells expressing cac1p mutants lacking Cdc7p phosphorylation sites. Combined, our results support a model in which Cdc7p regulates replication–coupled histone H4 K16ac via a CAC1-dependent mechanism, and thereby silencing, but that other CAF-1-dependent chromatin assembly activities remain functional in the absence of phosphorylation of Cdc7p consensus sites on CAF-1.
Identifying critical genes and microRNAs that when lost, can drive neoplastic transformation of non-cancerous lung cells.

Chennan Li$^{1,2}$, Sagar M. Utturkar$^2$, Nadia Atallah$^2$, and Andrea L. Kasinski$^{1,2}$

Department of Biological Sciences$^1$, and Purdue Center for Cancer Research$^2$, Purdue University, West Lafayette, IN, 47907

One of the most important attributes that lead to the high incidence of lung cancer and poor survival of patients is the lack of accurate prevention strategy. Current therapies that target KRAS-activated genes are effective in clinics, but resistance often occurs. Thus, understanding how KRAS-driven cancer is initiated is important for designing better preventive therapeutics. Evidence suggests that this single genetic event usually results in cell senescence due to the presence of p53, indicating that epigenetic factors may contribute to the emergence of carcinoma in tumors with functional p53. In mouse models, intratracheal activation of Kras$^{G12D}$ induces locally-distributed lung hyperplasia. However, KRAS$^{G12V}$ does not induce a similar response in non-tumorigenic human bronchial epithelial cells (HBEC), even when p53 signaling is silenced, which suggests that full transformation of in vitro cultured lung cells requires additional genetic or epigenetic changes. MicroRNAs, a class of small non-coding RNA molecules that epigenetically modulate many genes are globally reduced in many cancers. Based on this, we hypothesize that loss of certain microRNAs may drive neoplastic transformation of normal lung cells. We propose to test this hypothesis using an unbiased genome-scale CRISPR loss-of-function screen.

Herein, a CRISPR small guide RNA (sgRNA) library and a Cas9-expressing vector are co-transduced in HBEC Kras$^{G12V}$ shRNA-p53 (KP) cells to identify genes and microRNAs that when lost, can confer in vitro two-dimensional growth advantage, anchorage independence, and in vivo tumorigenicity. To evaluate the contribution of Cas9-mediated gene knockout in vivo, an sgRNA library targeting murine microRNAs will be administered together with adeno-Cre viruses into the lungs of Kras$^{LSL-G12D}$; Rosa26$^{LSL-Cas9}$ mice. SgRNAs that can potentiate Kras$^{G12D}$-driven lung adenocarcinoma will be highly represented in the resulting tumors and identified through deep sequencing. Candidate microRNAs and relevant genes will be further validated. Studies on the mechanisms that initiate lung cancer will follow.
The molecular basis of multivalent DNA binding by the BRM AT-Hook and bromodomain

Brianna E. Lupo¹, Peirou Chu, Emma A. Morrison¹ and Catherine A. Musselman¹

¹Department of Biochemistry, University of Iowa Carver College of Medicine, Iowa City, IA

The remodeling of chromatin structure is critical in the control of gene expression. Chromatin is remodeled to repress or activate regions of DNA by altering the location of nucleosomes. The nucleosome core is comprised of a histone octamer wrapped by ~147 base pairs of DNA. Remodeling is largely facilitated through the action of ATPase complexes and involves repositioning of the DNA around the histone octamer, or eviction of the histones. The activity of the remodeling complexes is influenced by the local chromatin environment including nucleosomes architecture and post-translational modifications (PTMs), both of which can alter recruitment and activity of the complexes at chromatin.

The SWI/SNF family of chromatin remodelers includes the BAF complex, which consists of subunits of Brahma (BRM) or Brahma-related gene 1 (BRG1). These two ATPase subunits form distinct BAF complexes and are differentially expressed in various cancers. Both BRM and BRG1 contain a C-terminal bromodomain that binds acetylated histone tails. In addition, our lab has recently discovered that this bromodomain can also bind DNA. Adjacent to the bromodomain is an AT-hook, a small motif that binds DNA in the minor groove, and we have shown that the bromodomain and AT-hook bind DNA multivalently. However, the molecular details underlying this multivalent association are still largely uncharacterized.

We are using NMR spectroscopy and Isothermal Titration Calorimetry to further characterize the binding activity of the BRM AT-hook and bromodomain to nucleosomes and isolated DNA. The relative contribution of the AT-hook and the bromodomain, as well as the role of the linker between the AT-hook and bromodomain are being investigated.
Investigating the interaction between MED5 and CDK8 in Arabidopsis

Xiangying (Candy) Mao, Vikki Weake and Clint Chapple
Department of Biochemistry, Purdue University, IN, USA

Plant metabolic networks are precisely regulated by the spatial and temporal expression of suites of genes. Among the various transcription (co)factors, Mediator has been identified as a hub for transcription regulation. Using a forward genetic screen, our lab determined that MED5, an Arabidopsis Mediator tail subunit, is required for maintaining phenylpropanoid homeostasis. A semi-dominant mutant (ref4-3) characterized by a single amino acid substitution in MED5a (G383S) was isolated as a strong suppressor of phenylpropanoid pathway, indicated by decreased soluble phenylpropanoid metabolite accumulation, reduced lignin content and dwarfism. In contrast, knocking out MED5a and MED5b (med5a/5b) results in the accumulation of increased levels of phenylpropanoid pathway derivatives. Considering that the CDK8 kinase module is a repressive module in Mediator, we tested the hypothesis that Arabidopsis MED5 represses phenylpropanoid pathway by interacting with CDK8.

To test this hypothesis, CDK8 knockout lines (cdk8-1) were crossed with ref4-3, and the phenylpropanoid content of the resulting double mutants was evaluated. In ref4-3 cdk8-1 plants, the concentration of sinapate esters and total lignin content are as low as they are in ref4-3, yet the growth defect in ref4-3 is largely rescued. To further determine the genes targeted by MED5 and CDK8 in maintaining proper plant growth, we performed an RNA-seq analysis which showed that a majority of the genes involved in salicylic acid (SA) biosynthesis and signaling are up-regulated in ref4-3 compared to wild type and ref4-3 cdk8-1. Consistent with this observation, SA, which has been previously implicated in dwarfing in lignin-modified plants, is accumulated to elevated levels in ref4-3 but not in wild type and ref4-3 cdk8-1. Nevertheless, blocking SA biosynthesis is not sufficient to restore the growth deficiency of ref4-3, suggesting that the hyperaccumulation of SA is more likely to be an effect rather than a cause for its dwarf phenotype.

At the molecular level, to elucidate how ref4-3 regulates downstream gene targets in a CDK8-dependent manner, we performed RNA polymerase II (Pol II) ChIP-seq analysis in wild type, ref4-3, cdk8-1 and ref4-3 cdk8-1. The Pol II ChIP-seq data provides additional information for us to identify the genes that are causative for the dwarfism of ref4-3.

Taken together, this study identifies the genetic interaction between MED5 and CDK8 in Arabidopsis, which enhances our understanding in the function of Mediator in plant metabolism and its role in lignin-modification-induced dwarfism.
Histone H3 Tail Conformation Regulates Nucleosome Association by the BPTF PHD Finger

Emma A. Morrison¹, Samuel Bowerman², Kelli Sylvers¹, Jeff Wereszczynski², and Catherine A. Musselman¹

¹University of Iowa, Iowa City, IA 52242
²Illinois Institute of Technology College of Science, Chicago, IL 60616

Post-translational modification (PTM) of histone proteins is one of the principal mechanisms of chromatin regulation. These chemical modifications are thought to act either directly by impacting chromatin structure or indirectly by recruiting cofactors to modified nucleosomes or regulating their activity once there. The specific recognition of histone PTMs by cognate effector domains in chromatin-regulatory complexes is key to these processes. The mechanism of binding of histone effector domains to PTMs has largely been studied with modified histone tail peptides, which does not take into account the context of the nucleosome. We have used the BPTF PHD finger, a well-characterized effector for tri-methylated Lys4 on histone H3 (H3K4me3), as a model system to probe the effect of the nucleosomal environment on effector domain binding.

In this study, we demonstrate that the conformation of the H3 tail within the nucleosome core particle (NCP) abrogates binding by the BPTF PHD finger by approximately two orders of magnitude and probe the basis for this inhibition. A combination of solution NMR-based investigations and molecular dynamics simulations demonstrates that the H3 tail robustly interacts with the DNA component of the nucleosome core. Furthermore, this H3 tail-DNA interaction is competitive with PHD finger binding. Our results support a model where the H3 tails predominantly exist in a dynamic ensemble of conformations collapsed onto the nucleosomal DNA and in equilibrium with an ensemble of extended states. Charge-altering chemical modification or mutation of the H3 tail that perturbs this linked equilibrium in turn affects effector domain binding. Thus, histone tail accessibility, which is modulated by histone PTMs, is involved in dictating specificity, providing key mechanistic insight into the active role of histone tails in chromatin signaling and supporting the existence of higher order PTM cross talk.
Role of miRNAs in opioid addiction and neuroplasticity

Kaushik Muralidharan¹ and Andrea Kasinski¹,²

Department of Biological Science¹, Purdue Centre for Cancer Research², Purdue University, West Lafayette, IN 47907

Opioid addiction is a brain disorder, where a person engages in compulsive use of an opioid substance that produces a rewarding stimulus despite being aware of its detrimental effects. In the United States, drug overdose due to opioid addiction is the leading cause of accidental deaths. Behavioral studies indicate that chronic opioid administration results in the disruption of neuroplasticity in brain reward and cognition. However, there is a knowledge gap about genes that opioids target and the mechanisms by which these genes are modulated following exposure. While majority of the research uses behavioral approaches to determine how opioids affect the brain, there is little known about the key players involved in mediating addiction. One of the most important player implicated in opioid addiction is ΔFosB, a truncated product of FosB. Acute or chronic exposure to opioids induces the production of ΔFosB in the reward center of the brain and it is unusually stable; leading to desensitization to drug and thereby promoting self-administration via unknown mechanisms. Among other candidates, low levels of G9a, a DNA methyltransferase also directly correlates with addiction. Further, recent studies have demonstrated the crucial role of epigenetic modifications and non-coding RNA (ncRNA) in regulating genes involved in addiction, more specifically, microRNAs (miRNAs). Studies have shown altered expression of a subset of miRNAs in addiction-related neuroplasticity. However, there is very little known in the field about miRNAs in addiction-related neuroplasticity. This study aims to identify the role of miRNAs in regulation of gene expression in addiction-related neuroplasticity and the role of epigenetics and possible synergism with miRNAs in regulating genes related to addiction. This study will not only elucidate the biological processes that drive addiction but also lay the foundation for identifying potential therapeutic targets that can be exploited to combat the national problem.
In addition to somatic mutations, aberrant enhancer activation is now recognized as a hallmark feature of cancer. Our lab recently identified signature changes in gene enhancer activity in colorectal cancer (CRC) through epigenomic comparisons of CRC samples and normal colonic crypts. These signature enhancers activate a specific transcriptional program that plays an important role in CRC, but the key transcription factors (TFs) that drive their acquisition remains unknown. Using three integrative approaches, we curated a prioritized list of 48 TFs predicted to regulate signature CRC enhancer activity. Briefly, we first screened for TF binding sites enriched at signature enhancers using the HOMER motif discovery tool. Second, we mined available ChIP-seq data for >200 TFs to identify TFs that physically bind to signature enhancers. Third, transcriptome data was mined to identify factors specifically expressed in the colon and differentially expressed in CRC tumors. To pinpoint which of the 48 factors regulate the signature enhancers, we performed a medium-throughput screen in which each TF was knocked down via siRNA, followed by qPCR of three genes regulated by signature enhancers: MYC, PHLDA1, & FOXQ1. Twelve TFs scored as hits and are therefore potential regulators of the CRC enhancer signature. Among the hits was ARID1A, a component of the BAF chromatin remodeling complex known to be required for maintaining H3K27ac at active enhancers. The 12 candidates will be evaluated further to determine their potential effects on chromatin accessibility and H3K27ac levels at signature enhancers in CRC.
PRMT5 is a novel epigenetic regulator of DNA repair genes and a therapeutic target to improve radiation therapy

Jake L. Owens¹, Elena Beketova¹, Xuehong Deng¹, and Chang-Deng Hu¹²

1: Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN, 47907
2: Purdue University Center for Cancer Research, Purdue University, West Lafayette, IN, 47907

Cancer is one of the leading causes of death globally. Over half of all cancer patients receive radiation therapy because it is potentially curative. Radiation therapy utilizes ionizing radiation to generate DNA damage to induce cancer cell death. Resistance to radiation therapy thus contributes to much of cancer related death. Therefore, improving radiation therapy will save many lives.

The most promising approach to improve radiation therapy is targeting proteins critical to DNA repair. Upon recognition of extensive DNA damage, repair proteins are upregulated and recruited to the sites of damage to facilitate repair. However, the mechanism for how these proteins are selectively induced upon DNA damage is unknown.

Here, we present evidence that protein arginine methyltransferase 5 (PRMT5) acts as a master epigenetic activator of genes involved in DNA repair. Analysis of RNA-seq data revealed several putative PRMT5 target genes involved in DNA repair including RAD51, BRCA1, BRCA2, PRKDC (DNAPKcs), and NHEJ1. Mechanistically, using several assays (e.g. qPCR, Western blotting, ChIP-qPCR) we determined that PRMT5 is quickly upregulated and recruited to the promoters of genes involved in DNA repair to activate gene expression through symmetric dimethylation of Histone 4 arginine 3 (H4R3me2s).

To extend our findings, we also analyzed data from tens-of-thousands of cancer patients encompassing 43 cancer types. In agreement with our hypothesis that PRMT5 activate expression of genes critical to DNA repair, PRMT5 expression positively correlates with the expression of these genes across all 43 cancer types. Using immunocytochemistry and clonogenic assays, we confirmed that PRMT5 is required to repair radiation-induced DNA damage and ensure cancer cell survival. Lastly, targeting PRMT5 significantly enhances the tumor killing effect of radiation in xenograft tumors in mice. Given that PRMT5 is overexpressed in several cancers, PRMT5 may be explored as a therapeutic target to improve RT outcomes across various cancer types.
Stability of whole-blood DNA methylation profiles under different storage durations and conditions

Xiaoqing Pan1, Yingchuan Li2, Michelle L. Roberts1, Pengyuan Liu3,1, Theodore A. Kotchen4, Allen W. Cowley, Jr.1, David L. Mattson1, Yong Liu1, Mingyu Liang1#, Srividya Kidambi4#

1Center of Systems Molecular Medicine, Department of Physiology, Medical College of Wisconsin, Milwaukee, WI 53226; 2Department of Critical Care Medicine, Shanghai JiaoTong University affiliated Shanghai The Sixth People’s Hospital, Shanghai, China; 3Sir Run Run Shaw Hospital and Institute of Translational Medicine, Zhejiang University, Zhejiang, China; 4Department of Medicine, Medical College of Wisconsin, Milwaukee, WI 53226;

*co-first authors #co-corresponding authors

AIM: To test whether DNA samples stored for a prolonged period (20 years) under various storage conditions could be used for comparative methylation studies using reduced representation bisulfite sequencing.

PATIENTS & METHODS: Five groups of human blood DNA samples (n=5-6/group) were compared. The groupings were based on the anticoagulant used and storage conditions.

RESULTS: Methylation profiles of defined genomic regions in the DNA or blood samples archived for 20 years were similar across storage temperatures, including 4°C. The level of inter-sample similarity in archived samples was not significantly different than that in recently collected samples.

CONCLUSION: Archived samples, including DNA stored at 4°C for 20 years, are suitable for comparative studies of DNA methylation.
Control of cellular metabolism is critical for cell homeostasis and ultimately, for cell and organismal viability. There is a direct link between metabolism and control of gene expression. The mechanism for this cross-talk, however, remains incompletely understood. The SIN3 complex is one of the major multi-subunit histone modifying complexes present in cells. The very large majority contain the deacetylase HDAC1, while a subset additionally contain a histone H3K4me3 demethylase. We recently found that SIN3 links epigenetic regulation and metabolism, specifically as related to methionine catabolism and global histone methylation. SIN3 regulates expression of SAM-S, the key enzyme necessary to generate the major cellular methyl donor. Additionally, SAM-S and SIN3 act in opposition to affect global histone methylation. To further investigate the connections between SAM-S and SIN3, we performed a genome-wide transcriptome analysis and generated a metabolomic profile in cells with altered SIN3 and SAM-S levels. Comparing the gene regulatory and metabolic pathways controlled by SIN3 and SAM-S, we find a complex interaction between these factors. For some pathways, SIN3 and SAM-S act in a redundant fashion. For others, however, reduction of SAM-S counteracts effects of SIN3 knockdown, similar to the effect observed for global histone H3K4me3. Through correlation analysis, we determined that glycolysis is a key metabolic pathway controlled by SIN3 and SAM-S. These data indicate that SIN3 is key factor to link epigenetic and metabolic control.
High-Throughput Locus-Specific DNA Methylation Validation Platforms in Aging Quantification Research

Emily Putnam, Yap Ching Chew, Wei Guo, Xiaojing Yang, Mingda Jin, Keith Booher and Xi Yu Jia

Zymo Research Corp., 17062 Murphy Ave, Irvine, CA, 92614

DNA methylation plays an important role in normal organismal development and in cellular differentiation in higher organisms. Changes in DNA methylation have been shown to correlate with disease risk, response to therapy and survival in a wide range of clinical conditions such as cancer and autoinflammatory diseases. Recent advances in next-generation sequencing and microarray technology have made it possible to map DNA methylation genome-wide, at a high resolution. However, these genomic assays tend to be costly, labor-intensive and impractical in the clinic. Thus, high-throughput DNA methylation assays that measure a small number of genomic regions in large cohorts are needed for validating biomarker candidates discovered from genomic studies. For this reason, Zymo Research has developed two targeted bisulfite sequencing platforms for measuring DNA methylation at multiple loci with multiple samples: Methyl-Check™ and SWARM™ (Simplified Whole-panel Amplification Reaction Method). The presentation will introduce these methods and demonstrate their utility in the development of a DNA methylation panel for aging and other research applications.
Adaptation of a CRISPR Interference Method for Probing Chromatin Properties of Repressor Domains

Ana-Maria Raicu¹, David N. Arnosti ²

¹Cell and Molecular Biology Program, and ²Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, Michigan 48824-1319

raicuana@msu.edu

Transcriptional repressors are involved in chromatin modification and regulate gene expression through binding to cofactors and modifiers across the genome. Taking a genome-wide approach to studying repressor activity sheds light on the large body of binding sites of corepressors such as the retinoblastoma tumor suppressor (Rb). However, binding events seen in Chromatin Immunoprecipitation and other genome-wide studies do not always correlate with direct transcriptional repression by these corepressors. Repressors and corepressors often bind thousands of sites but only induce transcriptional effects at a small fraction of associated genes. The specific developmental stage, chromatin environment, or associated basal transcription machinery can influence the effectiveness of repressors and corepressors in ways that still need to be further explored.

To uncover the mechanisms of transcriptional control and the physiological importance of repressor binding to DNA in the context of development, we are engineering a vector fusing a nuclease dead Cas9 enzyme to a repressor protein to direct it to various genomic targets via the recruitment of gene specific single guide RNAs. We will couple dCas9 repression with RNA-seq data to identify relevant and specific targets - a method that can help us interpret the many binding events seen in ChIP-seq experiments.

This adapted CRISPR interference method will allow for comparative analysis of repression pathways in eukaryotes and show how genomic, temporal, and tissue-specific contexts impact corepressors. Furthermore, it will give us insight into the progression of cancers arising from the misregulation of these fundamental pathways.

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Characterization of the novel DNA binding activity of the BRG1 AT-hook-bromodomain and effect of cancer mutations.

Julio C. Sanchez, Liyang Zhang, Amber Liu, Miles Pufall, Catherine A. Musselman,

Department of Biochemistry, The University of Iowa

Iowa City, IA 52242

The packaging of eukaryotic DNA with histones into chromatin acts to both compact it into the nucleus as well as provide a mechanism for regulation of the genome. Spatial and temporal remodeling of chromatin structure is critical for all DNA-templated processes and dysregulation of these pathways is associated with a number of diseases. The BRG/BRM Associated Factors (BAF) complex, is an ATP-dependent remodeling complex that plays an important role in gene regulation, several subunits of which are mutated in human cancers. The Brahma related gene 1 (BRG1) subunit of BAF, which provides the ATPase activity, contains a bromodomain (BD) at its C-terminus. BDs are well-characterized readers of acetylated lysines on histones, and the BRG1-BD has been shown to bind H3K14ac. However, we have recently discovered that in addition to binding acetyl-lysine, the BRG1-BD associates with DNA, a novel function for BDs. In addition, we have demonstrated that an adjacent AT-hook contributes to a multivalent mechanism of association with DNA, increasing affinity and specificity (Morrison et al., Nature Communications, 2017). Notably, the newly identified DNA binding interface harbors several cancer-associated mutations. Here we present our continued studies on this newly recognized composite DNA binding domain. We use systematic evolution of ligands by exponential enrichment (SELEX-seq) to generate a biophysical model of sequence specificity for the domain that we are exploring structurally using NMR spectroscopy and X-ray crystallography. To determine the kinetic and thermodynamic basis of association we use biolayer interferometry (BLI). In addition, we are investigating the structural basis of the interaction with DNA by using binding inhibitors in the minor groove and major groove of DNA. Together, our results are revealing the molecular details of how this novel DNA binding domain functions to navigate chromatin, and how dysregulation of the ATBD may impact BAF function.
Converting DNA information into double-stranded RNA in a coupled reaction

Jasleen Singh¹, Vibhor Mishra¹,², Feng Wang¹, Hsiao-Yun Huang¹,², Craig Pikaard¹,²

¹Department of Biology and Department of Molecular and Cellular Biochemistry, Indiana University, Bloomington, IN, USA
²Howard Hughes Medical Institute, Indiana University, Bloomington, IN, USA

In plants, 24nt siRNA-directed DNA methylation silences transposons, repeats and specific genes. Here, we demonstrate DNA-templated synthesis of 24nt siRNAs in vitro by multisubunit NUCLEAR RNA POLYMERASE IV (Pol IV), RNA-DEPENDENT RNA POLYMERASE 2 (RDR2) and DICER-LIKE3 (DCL3). Pol IV acts first, transcribing the template DNA and terminating soon after encountering the non-template DNA strand. The termination complex triggers RDR2 copying of Pol IV transcripts into duplex RNA, with second strands often including 3’ non-templated nucleotides due to RDR2’s terminal transferase activity. DCL3 cuts resulting duplexes from either end, tolerating 5’ monophosphates or triphosphates and blunt or overhanging 3’ ends, primarily yielding 24nt products. The results suggest explanations for why Pol IV-RDR2 transcripts are short, why siRNAs vary in size and often have DNA-mismatched 3’ ends, and reveal a unique reaction mechanism in which DNA information is converted into dsRNA via the coupled reactions of DNA- and RNA-dependent RNA polymerases.
Analysis of the contribution of the chromatin remodeler and tumor suppressor chd5 to neural differentiation and tumor suppression in Danio rerio

Erin Sorlien¹, Mary Witucki¹, Ellen Denning¹, Taylor Sabato¹, Kwok-Ki Ho¹, Joe Ogas¹

¹Department of Biochemistry, Purdue University, West Lafayette IN 47906

Loss of the ATP-dependent chromatin remodeler CHD5 is associated with formation of a range of tumors including melanoma, glioma, and is strongly linked to development of neuroblastoma. CHD5 is a vertebrate-specific CHD protein that is expressed preferentially in neural tissue. Knockdown of CHD5 in mouse cortices has been shown to result in defective neural differentiation and neural migration, and tissue-specific knockout of Chd5 in mice results in altered neural phenotypes accompanied by autism-like phenotypes. However, no tumor models currently exist to study the role of CHD5 in tumor suppression. We have used CRISPR/Cas9 to generate chd5 knockout (chd5⁻/⁻) zebrafish that are protein nulls as determined by western analysis. As previously observed in mice, chd5⁻/⁻ fish do not exhibit a gross morphological phenotype compared to wild-type. We have used a variety of the powerful tools available in zebrafish to further explore the contribution of chd5 to vertebrate processes. In particular, we are using a targeted chemical-genetic screen in zebrafish embryos, double-mutant analysis for tumor phenotypes, and genome-wide analyses of embryonal neural tissue to characterize differentiation defects in chd5⁻/⁻ embryos, consistent with described roles for CHD5 in human diseases. We anticipate that the chd5-dependent phenotypes identified by our analyses will generate robust testable hypotheses regarding how loss of the Chd5 remodeler contributes to tumor formation and neural development in vertebrates.
High-resolution epigenome mapping reveals unique genomic signatures of UHRF1-dependent DNA methylation inheritance

Rochelle L. Tiedemann¹, Qian Du², Bradley M. Dickson¹, Alison A. Chomiak¹, Wanding Zhou¹, Kevin M. Shaw¹, Benjamin K. Johnson¹, Susan J. Clark², Peter A. Jones¹, Scott B. Rothbart¹

¹Van Andel Research Institute, Center for Epigenetics, Grand Rapids, Michigan
²Garvan Institute, Genomics and Epigenetics Division, Sydney, Australia

The E3 ubiquitin ligase UHRF1 is an established cofactor for replication-coupled DNA methylation inheritance. Our group and others have made recent progress dissecting biochemical mechanisms linking UHRF1 to DNA methylation control. Our current model posits a step-wise pathway in which UHRF1 engages nucleosomes through histone and DNA binding. This multivalent nucleosome readout directs UHRF1 ubiquitin ligase activity toward the N-terminal tail of histone H3, a binding site for DNMT1. While this elegant chromatin regulatory process is now taking shape, it remains unclear whether the reliance on UHRF1 for DNA methylation maintenance is localized or genome-wide. Here we present comparative, high-resolution, genome-scale analysis of DNA methylation maintenance following acute depletion of UHRF1 and DNMT1 in a human colorectal cancer cell line. As expected, DNMT1 depletion resulted in global reduction of DNA methylation genome-wide. However, depletion of UHRF1 revealed distinct attributes of the genome that rely on UHRF1 for DNA methylation maintenance. Notably, these newly identified UHRF1-dependent DNA methylation signatures are consistent with the observed loss of DNA methylation that occurs during aging and cancer progression. We propose that UHRF1 misregulation is a key contributor to DNA methylation erosion that occurs through human lifespan and cancer.
Regulation of transcription termination of RNA Polymerase II (RNAPII) is critical for the proper maintenance of gene expression. NNS (Nrd1-Nab3-Sen1) dependent termination is an alternative RNAPII termination pathway to the polyadenylation-dependent termination pathway. NNS-dependent termination plays a role in regulation of noncoding RNA (ncRNA) processing or degradation and disruption of the pathway can impact adjacent events such as expression of nearby genes. Recruitment of NNS pathway components is achieved through a domain unique to the largest subunit of RNAPII referred to as the C-terminal domain (CTD), which contains a repeating heptad sequence, Y1S2P3T4S5P6S7, and acts as a docking site for transcription regulatory proteins. Ssu72 is a phosphatase that regulates phosphorylation levels at serine 5 and serine 7 of the CTD repeats, however, its precise role in the regulation of NNS termination is unknown. We propose that Ssu72 regulates recruitment of the NNS complex through its control of the phosphorylation status of the RNAPII CTD.

Our studies are focused on an Ssu72-L84F mutant yeast strain in which shows evidence of termination abnormalities. In order to study RNAPII localization, we performed chromatin immunoprecipitation followed by exonuclease treatment (ChIP-exo), a single nucleotide resolution ChIP assay, with a Ssu72-L84F yeast strain. We normalized and analyzed each genotype to calculate differential RNAPII occupancy. Our results show many genes including mRNA encoding genes had differential RNAPII occupancy as a result of the Ssu72-L84F mutation. Global studies highlight the read-through effects of ncRNA as well as mRNA genes. Read-through with the Ssu72-L84F mutant was confirmed with northern blot analysis and RNA sequencing analysis. We show that the levels RNAPII CTD phosphorylation are decreased in Ssu72-L84F mutants by western blot, implying that Ssu72-L84F confers a gain of function to the strain. Our results suggest that increasing Ssu72 activity might promote read-through of genes downstream of non-coding genes.
Histone post-translational modifications (PTMs) are key determinants of local chromatin structure and critical for regulation of gene expression. The PTMs can be specifically recognized by histone effector (or reader) domains, which interactions are thought to recruit or modulate the activity of histone regulatory complexes. The chromodomain family of histone reader domains are commonly found in histone modifying complexes and preferentially bind H3K9me3 or H3K27me3 to recruit these complexes to chromatin. Chromobox protein homolog 8 (CBX8) is a chromodomain containing protein found in the Polycomb repressive complex 1 (PRC1), a histone ubiquitin ligase complex. ChIP-seq analysis shows CBX8 localizes to genomic regions containing H3K27me3 in vivo, consistent with a classical targeting mechanism. However, previous in vitro studies found weak affinity of CD8 for H3K9me3 and H3K27me3, and were not able to distinguish any specificity. Here, we find that CD8 binds nucleosomes through multivalent engagement of DNA and H3K27me3. NMR experiments reveal that the CD8 binds H3K9me3 and H3K27me3 weakly, but with a distinct preference for H3K27me3, and allowed for determination of the molecular basis for this specificity. Notably, we find that CD8 robustly interacts with nucleosomes independent of methyl-lysine binding, driven instead by contacts with nucleosomal DNA. We determine the structural basis for CD8 interaction with linear DNA and unmodified nucleosomes and find that the DNA binding interface is unique from the H3K27me3 binding pocket. Finally, we show that CD8 binds linear DNA and methylated histone peptides simultaneously and in a cooperative manner, with DNA binding enhancing the CD8 affinity for methylated histone tails. Taken together, our data is consistent with a model in which CBX8-PRC1 initial recruitment occurs through the robust association of CD8 with nucleosomal DNA and that retention of the complex at condensed H3K27me3 facultative heterochromatin is achieved through the multivalent engagement of DNA and H3K27me3.
The effects of linker histone isoforms on the structure and dynamics of the chromatosome

Dustin C. Woods¹ and Jeff Wereszczynski²

Department of Chemistry¹ and Department of Physics², Illinois Institute of Technology

Experiments have shown that linker histones can bind to DNA in either an on- or off-dyad mode on the nucleosome (NCP) and its presence can have cascading effects that may affect greater chromatin dynamics. However, the molecular mechanisms that drive the binding mode, how these modifications are influenced by specific linker histone residues, and the major biological implications of these variations in chemical composition and conformation, remain poorly understood. Here, we report on the chemical-physical properties of the chromatosome from a series of molecular dynamics simulations of three different linker histone isoforms bound in on- and off-dyad binding modes. Results show that the flexibility of the linker DNA is reduced upon linker histone binding, producing more compact chromatosome conformations. Additionally, an analysis of relative binding energies suggests that Globular Histone H5 (GH5) exhibits a stronger binding affinity to the NCP than the other linker histones, Globular Histone H1 (GH1) and the Globular Histone H5 penta-mutant (GH5-pMut), and prefers the on-dyad binding mode. Moreover, full-correlation analysis of the individual histones shows that GH1 samples a distinct conformational space apart from histones GH5 and GH5-pMut, independent of binding mode. Overall, these atomistic simulations provide insight into the physical and chemical effects of linker histone isoforms on the structure and dynamics of the chromatosome.
Enhancer RNAs (eRNAs) are non-coding RNAs transcribed from active enhancer regions with an increasing role in regulating gene transcription. However, their manifestation in adaptive immune cells are incompletely understood. Here we characterize eRNAs in B, CD4, and CD8 cells by integrating data from ATAC-seq and ribo-depleted stranded RNA-seq. We show that nearly 60% of all intronic/intergenic ATAC-seq peaks have eRNA transcripts. Majority of these eRNAs (>70%) occur simultaneously on both positive and negative strand of DNA (bi/semi directional), while the remaining are observed only on one strand (Uni-directional). Bi/semi directional eRNA regions are highly enriched in cell-specific transcription factors, active enhancer marks, and super enhancers. Interestingly, a subset of cell-specific eRNAs are originated from chromatin remodeler CTCF containing sites, implicating potential role in chromatin structure organization. Furthermore, we find that disease associated SNPs are highly enriched in regions giving rise to bi/semi directional eRNAs. Thus eRNAs are pervasive and play a key role in identity of immune cells.
Identification of Novel HDAC1 Substrates Using a Mutant Trapping Strategy

Ethan Zhang, Nalawansha Dhanusha, Mary Kay H. Pflum

Wayne State University

Identification of Non-histone substrates of HDACs is proven to be difficult and there is no systematic way to profile substrate for each HDACs. Here, we are introducing the first mutant trapping technology to identify HDAC isoform specific substrates. We introduced a mutant C151A within the catalytic active site of HDAC1, which is involved in relying the release of acetate upon deacetylation. With this key cysteine mutant, the substrate protein with acetylated lysine will bind to the HDAC1 mutant in a retained manner since the product is not readily formed. In this case, by doing co-immunoprecipitation, we will have a better chance identify the substrate of individual HDAC isoforms. Successfully, we identified and validated two new non-histone substrates of HDAC1 using this mutant trapping strategy.
Within a cell, DNA is constantly being damaged by endogenous and exogenous factors, including UV radiation. In order to maintain genomic stability, cells have developed DNA damage response pathways to repair this damage, some of which depend on various epigenetic elements. One of these pathways, nucleotide excision repair (NER), has been shown to be inducible in *Saccharomyces cerevisiae*. In our lab we have found that cells previously exposed to UV radiation show increased survival when irradiated again compared to control cells. We wanted to know whether this acquired hyper-resistance to UV can be stably inherited, whether it is genetic or epigenetic, and whether NER is involved in this phenotype. We found that hyper-resistance is heritable over at least 7 generations, but disappears by 14 generations, which points to an epigenetic mode of inheritance. Experiments with strains lacking genes for certain histone modifications or variants show diminished hyper-resistance, indicating that this hyper-resistant phenotype is likely driven by non-genetic changes such as histone modifications and use of the histone variant H2A.Z. We also observed damage levels in UV-exposed cells by measuring cyclopyrimidine dimer (CPD) abundance before and after each exposure. Our results revealed that pre-exposed cells survive better not because of more efficient NER, but because they receive less CPD damage during the second irradiation. Preliminary experiments suggest that UV-exposed cells avoid damage by upregulating genes involved in spore wall formation in response to UV exposure. We propose that this transcriptional change is accompanied by epigenetic modifications that are passed onto the offspring, instructing them to build modified cell walls and thereby protecting their DNA from future irradiation. Overall, this research offers valuable insights into the ways that eukaryotes can use epigenetic memory to adapt to stressful environments and maintain genomic integrity.