Welcome

Studies on Transcription, Chromatin, and Epigenetics have begun to provide a revolutionary conceptual framework for explaining how molecules are synthesized, organisms are made, phenotypes are modified by the environment, and traits are inherited independently of the coding capacity of DNA. The application of new and exciting methodologies to better understand epigenetic changes genome-wide (Epigenomics) is an important pillar of systems biology, which is being introduced into the practice of individualized medicine. Moreover, the fact that epigenetic mechanisms are amenable to reversal by small drugs has given rise to the new area of epigenetic therapeutics, with many agents being tested in clinical trials. Thus, epigenetics is promising to provide deeper mechanistic insight into fundamental biological processes and new diagnostic and therapeutic tools for their management.

Noteworthy, scientists from the American Midwest have significantly contributed to the birth and popularization of the field of transcription, chromatin, and epigenetics. The realization of this fact led to the organization of the first Midwest Chromatin and Epigenetics Meeting that was held at the University of Iowa (Iowa City, IA) in 2006. This meeting sought to provide a forum for the free exchange of ideas, establish collaborative networks, and educate scientists of all levels. In subsequent years similar themed Midwest Meetings were held at Michigan State University (E. Lansing, MI) and at the University of Chicago (Chicago, IL), which expanded this mission to larger audiences. To continue building on this tradition of excellence, we are now honored and delighted to welcome you to the 2014 Midwest Chromatin and Epigenetics Meeting being held at the University of Wisconsin (Madison, WI). We have chosen to meet at the Wisconsin Institute for Discovery (WID) since its modern physical plan and outstanding scientific environment ensure a successful conference.

We have designed a program, which offers an opportunity to present your latest findings to an intellectually engaged and eclectic scientific community dedicated to the study of epigenetics. The program has been designed to be rich and varied with keynote speeches, invited talks, and poster presentations given by researchers representing laboratories not only from the Midwest but also from other parts of the country and abroad. The presentations will highlight fundamental discoveries in epigenetics, as well as the applications of this science to biology and medicine. Attendees will benefit from being immersed in the rapidly emerging conceptual advances and the development of state-of-the-art technology spanning the wide range of epigenomic research, including its application to system biology. We also expect to provide demonstrations by industry on new reagents and methodologies, as well as numerous opportunities for networking. As a special highlight, a promising young researcher will be selected from the meeting to receive the Journal of Biological Chemistry (JBC) Herb Tabor Young Investigator Award. This prestigious award will be presented at the meeting by an Associate Editor (John Denu) of the JBC.

Thus, we are highly optimistic that the Midwest Chromatin and Epigenetics Meeting will continue to be an excellent opportunity to renew old acquaintances and to meet new colleagues from industry, government and academia. Thus, it is in the spirit of scientific celebration that we welcome you to what promises to be one of the most exciting meetings to date on this rapidly advancing field of research.

Co-organizers

Raul Urrutia    John Denu    Lori Wallrath
# Meeting Schedule

*All events will take place in the H.F. Deluca Forum at the Discovery Building unless otherwise noted.*

## Sunday, May 18, 2014

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<tr>
<th>Time</th>
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<tr>
<td>3:00PM – 5:00PM</td>
<td>Registration</td>
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<tr>
<td>5:00PM – 6:00PM</td>
<td><strong>Keynote Address</strong>&lt;br&gt;<em>Chromatin Regulation: New Concepts and Methods</em>&lt;br&gt;Gerald Crabtree, Stanford University</td>
</tr>
<tr>
<td>6:00PM – 7:30PM</td>
<td>Opening Reception</td>
</tr>
<tr>
<td>7:30PM – 8:00PM</td>
<td>Predicting Cell-Type Specific Regulatory Networks</td>
</tr>
<tr>
<td>8:00PM – 8:30PM</td>
<td><em>Genome Architecture in Action: Gene Regulation via 3D Chromatin Organization in a Malarial Parasite</em>&lt;br&gt;Ferhat Ay, University of Washington</td>
</tr>
<tr>
<td>8:30PM – 9:00PM</td>
<td><em>Learning Integrative Regulation Programs Across Diverse Human Cell Types</em>&lt;br&gt;Anshul Kundaje, Stanford University</td>
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## Monday, May 19, 2014

<table>
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<tr>
<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>8:00AM - 8:30AM</td>
<td>Breakfast</td>
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<tr>
<td>8:30AM – 8:45AM</td>
<td>Welcome and Opening Remarks</td>
</tr>
<tr>
<td>8:45AM – 9:05AM</td>
<td>H2Bub1 Promotes DNA Replication Fork Progression by Facilitating Nucleosome Assembly&lt;br&gt;Kelly Trujillo, University of Nebraska Medical Center</td>
</tr>
<tr>
<td>9:05AM – 9:25AM</td>
<td>Histone Modifications Regulate Nucleosome Assembly&lt;br&gt;Zhiguo Zhang, Mayo Clinic</td>
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<tr>
<td>9:25AM – 9:45AM</td>
<td>Structural Insights into the Mode of Action of Histone Chaperones&lt;br&gt;Georges Mer, Mayo Clinic</td>
</tr>
<tr>
<td>9:45AM – 10:05AM</td>
<td>Mitotic Regulatory Function of Histone H3 and Chromatin Modifying Enzymes Gcn5p and Rpd3p&lt;br&gt;Min Hao Kuo, Michigan State University</td>
</tr>
<tr>
<td>10:15AM – 10:45AM</td>
<td>Morning Break &amp; Exhibitor Showcase&lt;br&gt;Forum wedge, Atrium</td>
</tr>
<tr>
<td>10:45AM – 11:05AM</td>
<td>Pathological Changes in Muscle Gene Expression Caused by Mutant lamin&lt;br&gt;Lori Wallrath, University of Iowa</td>
</tr>
<tr>
<td>11:05AM – 11:25AM</td>
<td>Mechanisms Driving Genome Reprogramming and Transcriptional Activation in the Early Embryo&lt;br&gt;Melissa Harrison, University of Wisconsin-Madison</td>
</tr>
<tr>
<td>11:25AM – 11:45AM</td>
<td>Nucleosome Binding and Cell-Type Specific Gene Expression&lt;br&gt;Monique Floer, Michigan State University</td>
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</table>
12:00PM – 1:15PM
Lunch
Open Court Central & East

- Cell Fate and Maintenance of Cell Identity #2 -
  Session Chair: Lori Wallrath, University of Iowa

1:15PM – 1:35PM
Deciphering the Regulatory Code of the Drosophila Suppressor of Hairy-Wing Protein
Pamela Geyer, University of Iowa

1:35PM – 1:50PM
Establishing the Erythroid Cell Transcriptome:
Coregulator Matrix Model of GATA Factor Function
Andrew DeVilbiss, University of Wisconsin-Madison

1:50PM – 2:10PM
Intersection of Chromatin and signaling Pathways in the Acquisition of Pluripotency
Rupa Sridharan, University of Wisconsin-Madison

2:30PM – 2:45PM
Brief Break
Forum wedge

- The Language of Histone Modifications -
  Session Chair: Raul Urrutia, Mayo Clinic

2:45PM – 3:05PM
How Metabolism Informs the Epigenome
John Denu, University of Wisconsin-Madison

3:05PM – 3:25PM
Mechanisms of PRC2 Regulation
Catherine Musselman, University of Iowa

3:25PM – 3:40PM
Yeast Histone Demethylase Jhd2 Mediates a Conserved Cross-Talk Between H3K4 methylation and H3K14 Acetylation
Kayla Harmeyer, Purdue University

3:40PM – 4:00PM
H3S10 Phosphorylation by the JIL-1 Kinase Regulates Heterochromatin, H3K9me2 Distribution and Gene Expression in Drosophila
Kristen Johansen, Iowa State University

4:15PM – 4:30PM
Brief Afternoon Break
Forum wedge

- DNA Methylation -
  Session Chair: John Svaren, University of Wisconsin

4:30PM – 4:50PM
DNA Methylation Patterning in Development and Cancer
Keith Robertson, Mayo Clinic

4:50PM – 5:10PM
Molecular Mechanism of Action of Plant de Novo DNA Methyltransferases
Xuehua Zhong, University of Wisconsin-Madison

5:10PM – 5:30PM
DNA Methylation Changes Underlie Hyperglycemia and the Metabolic Memory State of an Adult Zebrafish Model of Type 1 Diabetes
Michael Sarras, Jr., Rosalind Franklin University of Medicine and Science

5:45PM – 8:30PM
Reception & Exhibitor Showcase
Atrium

6:30PM – 7:30PM
Odd number poster presentations

7:30PM – 8:30PM
Even number poster presentations
<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Speaker</th>
<th>Institution</th>
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</thead>
<tbody>
<tr>
<td>8:00AM – 8:20AM</td>
<td>The Development of Chromatin Remodeling Inhibitors to Investigate and Treat a Wide Spectrum of Human Cancers</td>
<td>Emily Dykhuizen</td>
<td>Purdue University</td>
</tr>
<tr>
<td>8:20AM – 8:40AM</td>
<td>The Long and Short of Drug Targeting Bromodomain Proteins</td>
<td>Danette Daniels</td>
<td>Promega Corporation</td>
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<tr>
<td>8:40AM – 9:00AM</td>
<td>Targeting MLL1 in Treatment of Leukemia</td>
<td>Yali Dou</td>
<td>University of Michigan</td>
</tr>
<tr>
<td>9:00AM – 9:20AM</td>
<td>Structural Studies on Sirtuin Inhibitor</td>
<td>Manfred Jung</td>
<td>University of Freiburg</td>
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<tr>
<td>9:30AM – 9:45AM</td>
<td>Brief Morning Break</td>
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<tr>
<td>9:45AM – 10:05AM</td>
<td>GLI1 and MLL/COMPASS Complex to Control Tumor Microenvironment-Associated Gene Expression</td>
<td>Martin Fernandez-Zapico</td>
<td>Mayo Clinic</td>
</tr>
<tr>
<td>10:05AM – 10:25AM</td>
<td>The Role of Chromatin in RB Repression of RNA polymerase III</td>
<td>Bill Henry</td>
<td>Michigan State University</td>
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<tr>
<td>10:25AM – 10:40AM</td>
<td>Functional characterization of the Mcs5c Locus Reveals an Age-Dependent Chromatin Interaction that may Influence Gene Expression</td>
<td>Amanda Henning</td>
<td>University of Wisconsin-Madison</td>
</tr>
<tr>
<td>10:40AM – 10:55AM</td>
<td>Remodeling of Chromatin States during Hairy Mediated Repression in the Drosophila Embryo</td>
<td>Kurtulus Kok</td>
<td>Michigan State University</td>
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<tr>
<td>11:05AM – 11:15AM</td>
<td>Brief Morning Break</td>
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<tr>
<td>11:15AM – 11:35AM</td>
<td>Writers, Readers and Erasers of the Histone Code in Cancer</td>
<td>Raul Urrutia</td>
<td>Mayo Clinic</td>
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<tr>
<td>11:35AM – 11:55AM</td>
<td>Varying the Chromatin Landscape in Human Cancers</td>
<td>Peter Lewis</td>
<td>University of Wisconsin-Madison</td>
</tr>
<tr>
<td>11:55AM – 12:15PM</td>
<td>Epigenetic Plasticity During Human Mammary Cell Differentiation and Gene Silencing of SOD3 in Breast Cancer</td>
<td>Frederick Domann</td>
<td>University of Iowa</td>
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<tr>
<td>12:30PM – 1:30PM</td>
<td>Business Meeting</td>
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**Tuesday, May 20, 2014**

- Therapeutic Targets and Small Molecule Development -
  Session Chair: Wei Xu, University of Wisconsin

- Epigenetics in Development and Disease #1 -
  Session Chair: Kristen Johansen, Iowa State University

- Epigenetics in Development and Disease #2 -
  Session Chair: Gwen Lomberk, Mayo Clinic

Business Meeting
Exhibitor Showcase - Atrium
Lunch - Open Court Central & East
### - Tools -

**Session Chair:** Rupa Sridharan, University of Wisconsin

<table>
<thead>
<tr>
<th>Time</th>
<th>Title</th>
<th>Speaker</th>
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<tbody>
<tr>
<td>1:30PM – 1:50PM</td>
<td><em>ChIP-seq is Broken, How Do We Fix It?</em></td>
<td>Alex Ruthenburg, University of Chicago</td>
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<tr>
<td>1:50PM – 2:10PM</td>
<td><em>What is the Role of Chromatin Structure in Maintaining Cell Fate? Cell-Type-specific Chromatin Tools Allow us to Profile Terminally Differentiated Cells</em></td>
<td>Carla Margulies, Ludwig-Maximilians-University Munich</td>
</tr>
<tr>
<td>2:10PM – 2:30PM</td>
<td><em>Universal Homogenous Bioluminescent Assay to Monitor the Effect of Modulators on Various Classes of Methyltransferases in Vitro</em></td>
<td>Said Goueli, Promega Corporation</td>
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<tr>
<td>2:45PM – 3:00PM</td>
<td>Brief Afternoon Break</td>
<td>Forum wedge</td>
</tr>
<tr>
<td>3:00PM – 3:20PM</td>
<td><em>Structural and Functional Studies of JMJD2 Demethylase Substrate Specificity</em></td>
<td>Raymond Trievel, University of Michigan</td>
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<tr>
<td>3:20PM – 3:40PM</td>
<td><em>Mis16 Recognizes Histone H4 and Scm3sp Simultaneously to Recruit CENP-A into Centromeres</em></td>
<td>Uhn-Soo Cho, University of Michigan</td>
</tr>
<tr>
<td>3:40PM – 4:00PM</td>
<td><em>Evolution of Multi-Subunit RNA Polymerases and Co-evolution of Eukaryote Complexity with the RNAP II CTD</em></td>
<td>Zachary Burton, Michigan State University</td>
</tr>
<tr>
<td>4:00PM</td>
<td><em>Announcement of the Herb Tabor Young Investigator Award Winner</em></td>
<td>Closing Remarks &amp; Acknowledgements</td>
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Dr. Jerry Crabtree is a Professor of Pathology and Developmental Biology at Stanford University School of Medicine. Dr. Crabtree has a B.S. from West Liberty State College in West Virginia and an M.D. from Temple University Medical School in Philadelphia. The Crabtree Lab is studying the interaction between the signaling pathways and genetic circuits regulating embryonic development. To modulate and explore these circuits, his laboratory members are also designing and synthesizing small molecules that rapidly and reversibly activate or inhibit the products of specific genes critical to these circuits, thereby allowing precise temporal analysis of their functions.

Keynote Speaker

Chromatin Regulation: New Concepts and Methods

One of the remarkable outcomes of exom sequencing of human diseases has been the emerging concept that misfunction of chromatin is a major contributor to human disease, particularly human cancer. Perhaps the most commonly mutated chromatin regulator in human disease are the subunits of BAF or mWI/SNF complexes\(^1,2\). These complexes use the energy from ATP to control chromatin states and in many respects act as master regulators of the epigenetic state by controlling the transcription of polycomb genes and the placement of PRC1 and PRC2 complexes over the genome as well as other histone marks\(^3,4\). These complexes (BAF or mSWI/SNF) are polymorphic assemblies of 15 subunits encoded by 29 genes\(^5\). In the transition from pluripotency to multipotent neural stem cells to committed neurons the complexes switch subunits from an esBAF to npBAF to nBAF complex\(^5\). Recapitulating this subunit switch in human fibroblasts converts them to neurons\(^6,7\) indicating an unexpected instructive role for these complexes. Recent exome sequencing studies have indicated that the subunits are frequently mutated in a number of neurologic syndromes characterized by delayed language acquisition\(^2,8\). Recently we have been able to define the full subunit composition of these complexes and find that they are mutated in more than 20% of all human malignancies\(^1\). The complexes can act as oncogenes (by evicting polycomb from oncogenic drivers), or more commonly as tumor suppressors\(^9\). We find that BAF complexes are essential for the binding and function of Topolla over the genome. Indeed, 11,000 of 16000 Topolla sites over the genome are dependent upon BAF complexes\(^10\). When subunits are mutated by conditional deletion the cells develop anaphase bridges and a temporary arrest. Studies will be presented indicating that the misfunction of Topolla is a major contributor to the ability of BAF complexes to act as tumor suppressors.

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Predicting cell-type specific regulatory networks

Sushmita Roy1,3 and Rupa Sridharan2,3
1 Assistant Prof., Biostatistics and Medical Informatics, UW Madison, Wisconsin
2 Assistant Prof., Cell and Regenerative Biology, UW Madison, Wisconsin
3 Wisconsin Institute for Discovery, UW Madison, Wisconsin

Regulation of gene expression in mammalian systems is complex and is controlled by many factors including transcription factor proteins, and chromatin components such as DNA methylation and post-translational modifications of histones; and nucleosome occupancies and three-dimensional organization of the genome. Efforts from human ENCODE, mouse ENCODE and NIH epigenome roadmap projects have resulted in rich datasets that measure the epigenomic states of cells from different tissues. A key challenge is to systematically combine these datasets to gain insight into the regulatory networks that govern cell fate in mammals. To tackle this challenge we are developing computational methods to combine different regulatory genomic datasets to address problems specifically in mammalian gene regulation: (a) prediction of long range interactions among enhancer and promoter elements, (b) identifying modular regulatory programs determining cell fate in cellular reprogramming, (c) inferring dependencies among histone modifications. We are developing complementary approaches to define the landscape of long-range interactions at different genomic resolutions. To understand long-range regulation within an 1MB locus, we are using supervised machine learning approaches that trains a classifier on a small, high confidence set of promoter-enhancer interactions obtained from 5C (Sanyal et al. 2012), a variant of chromatin capture conformation methods. We used our classifier to predict whether an enhancer-promoter pair interact or not in three cell-lines K562 (leukemia), GM12878 (lymphoblastoid) and HeLa S3 (cervical cancer). Compared to a correlation approach, our approach has improved sensitivity at the same false discovery rate, infers cell-line specific interactions and provides a systematic framework to identify important features of such interactions. A classifier trained using data from one cell line can correctly predict up to 83% of the interactions in other cell-lines demonstrating the ability of our approach to generalize to new cell types. In parallel, we are developing more unsupervised, clustering based methods to understand large-scale chromatin organization. We recently developed methods to reconstruct regulatory modules across hierarchically related biological contexts such as species phylogenies (Roy et al, Genome Research 2013), and to reconstruct regulatory networks to identify both regulators for individual genes as well as sets of genes in a module (Roy et al, Plos comp bio, 2013). We are extending these approaches to identify regulatory network changes during transitions from a differentiated cell type to a pluripotent cell. We applied our module reconstruction approach to histone modification data measuring eight chromatin marks in gene promoters in mouse embryonic fibroblast cells (MEF), iPSCs (induced pluripotent) and pre-iPSCs (partially induced pluripotent stem cells) and identified dozens of modules exhibiting distinct mark combinations in promoters and on open reading frames, separating genes based on the combinatorial patterns of histone modifications. We are examining these patterns to understand their impact on cell-type specific expression changes. Our computational methods can be used to systematically dissect regulatory networks in cell fate determination and provide insight into the interplay of chromatin organization and transcription factor proteins on downstream gene expression.
Genome architecture in action—Gene regulation via 3D chromatin organization in a malarial parasite

The development of the human malaria parasite Plasmodium falciparum is controlled by coordinated changes in gene expression throughout its complex life cycle, but the corresponding regulatory mechanisms are incompletely understood. To study the relationship between genome architecture and gene regulation in Plasmodium, we assayed the genome architecture of P. falciparum at three time points during its erythrocytic (asexual) cycle. Using chromosome conformation capture coupled with next-generation sequencing technology (Hi-C), we obtained high-resolution chromosomal contact maps, which we then used to construct a consensus three-dimensional genome structure for each time point. We observed strong clustering of centromeres, telomeres, ribosomal DNA and virulence genes, resulting in a complex architecture that cannot be explained by a simple volume exclusion model. Internal virulence gene clusters exhibit domain-like structures in contact maps, suggesting that they play an important role in the genome architecture. Midway during the erythrocytic cycle, at the highly transcriptionally active trophozoite stage, the genome adopts a more open chromatin structure with increased chromosomal intermingling. In addition, we observed reduced expression of genes located in spatial proximity to the repressive subtelomeric center, and colocalization of distinct groups of parasite-specific genes with coordinated expression profiles. Overall, our results are indicative of a strong association between the P. falciparum spatial genome organization and gene expression. Understanding the molecular processes involved in genome conformation dynamics could contribute to the discovery of novel antimalarial strategies.
Learning integrative regulation programs across diverse human cell types

Sofia KyriazopoulouPanagiotopoulou¹, Serafim Batzoglou¹, Anshul Kundaje¹,²
¹Stanford University, Dept. of Computer Science, Stanford, CA,
²Stanford University, Dept. of Genetics, Stanford, CA

Large scale efforts such as ENCODE and The Roadmap Epigenomics Project are generating massive compendia of diverse functional genomic data to interrogate the human transcriptome, regulome and epigenome in diverse cellular contexts. Using integrative computational approaches we have learned one of the most comprehensive maps of dynamic regulatory elements (~2.5 million enhancers) across 127 diverse primary cells, tissues and cell lines. We have identified modules of tissue and lineage specific regulatory elements and their potential downstream target genes using novel denovo enhancer gene linking methods. This comprehensive reference regulatory landscape presents a tremendous opportunity to dissect the complexity of regulation programs driving common and lineage specific transcriptional programs. We introduce a powerful machine learning framework based on Boosting algorithms and multitask regression to learn context specific rules of transcriptional regulation based on the sequence composition and chromatin state of regulatory elements as well as the expression patterns of potential regulatory proteins, such as transcription factors, chromatin modifiers, and signaling molecules. Our method simultaneously (1) models chromatin state dynamics of proximal and distal regulatory elements (e.g. enhancers), (2) learns predictive cisregulatory sequence grammars (i.e. combinations of sequence motifs representing transcription factor binding preferences), (3) discovers combinatorial pathways that regulate overlapping modules of target genes in different subsets of cell types and lineages, and (4) utilizes biological prior knowledge from protein protein interaction databases and functional interaction maps to improve robustness and biological interpretability of the models. We learn high accuracy models jointly on the ENCODE and Roadmap data that not only recover an extensive collection of key lineage specific regulators and sequence grammars but also a large number of novel higher order regulatory pathways affecting modules of coexpressed and coregulated gene modules. We demonstrate the generalizable power of our approach by predicting context specific regulation of 100s of annotated disease associated gene sets from the MSigDB database. Our models provide a multiscale, context specific view of the regulatory architecture of the human genome and the dynamic regulation programs that result in the phenotypic diversity of cells and tissues in the human body.
H2Bub1 Promotes DNA Replication Fork Progression by Facilitating Nucleosome Assembly

Kelly M. Trujillo¹,², Matthew Northam¹, Shikha Sharma¹, and Mary Ann Osley²

¹University of Nebraska Medical Center; Department of Biochemistry and Molecular Biology; Omaha, NE and ²University of New Mexico Health Science Center; Department of Molecular Genetics and Microbiology; Albuquerque, NM

The monoubiquitylation of histone H2B (H2Bub1) is canonically established during transcription elongation, where it promotes nucleosome assembly and processivity of the RNA Pol II complex. Here we explore a novel and transcription-independent function of H2Bub1 in DNA replication. We show that H2Bub1 is enriched on nucleosomes near origins in yeast, and that levels of H2Bub1 are maintained throughout the cell cycle at these sites by the de novo recruitment of Bre1 to newly assembled chromatin behind active replication forks. In the absence of H2Bub1, the pre-replication complex is formed and activated, but upon replication stress, fork progression is impeded and the replisome becomes unstable. We attribute these phenotypes to a defect in nucleosome assembly, which is sensed by the replisome. We postulate that this signal functions to impede MCM mediated unwinding, leading to reduced fork progression, which contributes to replisome instability.

Histone modifications regulate nucleosome assembly

DNA replication coupled (RC) nucleosome assembly plays an important role in the maintenance of genome stability and epigenetic information. Histone chaperone Asf1, a key regulator of RC nucleosome assembly, presents newly synthesized histone H3-H4 for Rtt109-catalyzed H3K56 acetylation (H3K56ac) in budding yeast. Following H3K56ac, H3-H4 of the Asf1-H3-H4 complex is transferred to other histone chaperones including Rtt106 for deposition onto replicating DNA. More recently, we show that the Rtt101Mms1 E3 ubiquitin ligase ubiquitylates H3K56ac and regulates RC nucleosome assembly. Cells lacking Rtt101 or expressing H3 ubiquitylation defective mutants exhibit defects in nucleosome assembly and are sensitive to genotoxic agents. H3 ubiquitylation regulates the association of H3-H4 with histone chaperone Asf1 and Rtt106. These results reveal a novel crosstalk between histone acetylation and ubiquitylation and a mechanism whereby histone ubiquitylation regulates RC nucleosome assembly.
Structural Insights into the Mode of Action of Histone Chaperones

Histone chaperones contribute to nucleosome assembly and histone exchange during DNA replication, transcription and repair. We will present recent structural biology results that help us better understand the mechanism of action of histone chaperones.

Mitotic regulatory function of histone H3 and chromatin modifying enzymes Gcn5p and Rpd3p

Faithful segregation of the duplicated chromosomes during mitosis is essential to maintain cellular function and species perpetuation. Bipolar attachment of mitotic spindles to the kinetochores aligns chromosomes in the metaphase midplate before cells enter the anaphase when sister chromatids are irreversibly segregated. Prior to segregation, the poleward pulling force by spindles generates tension between sister chromatids. Tension thus is a key criterion for the spindle assembly checkpoint (SAC) to ensure proper alignment of metaphase chromosomes. We showed previously that the budding yeast histone H3 at pericentromeres plays an essential role in tension sensing. This novel mitotic function of H3 is carried out by the Tension Sensing Motif, TSM, of H3 that recruits the Shugoshin protein, Sgo1p, to the pericentromeres. Unlike other eukaryotes in which heterochromatic marks are enriched at pericentromeres and are responsible for Shugoshin recruitment, the budding yeast pericentromeres are euchromatic and lack appreciable epigenetic marks that distinguish them from chromosomal arms. Thus, how Sgo1 is selectively recruited to pericentromeres in budding yeast remains an enigma. Our recent data strongly suggest that the Gcn5p histone acetyltransferase and Rpd3p histone deacetylase play antagonistic roles in the H3 TSM-Sgo1p pericentric interaction. Furthermore, although TSM is not part of the flexible N’ tail domain of H3, our genetic and ChIP experiments suggest that Sgo1p may use the tail domain as a secondary docking site when the integrity of TSM becomes impaired. Overall, our discoveries reveal a network involving different domains of H3, multiple histone modifying enzyme, and the Shugoshin protein in maintaining the function of the SAC to ensure faithful segregation during mitosis.
Lamins are ubiquitously expressed intermediate filament proteins that line the inner membrane of the nuclear envelope. They provide structural support for the nucleus and regulate gene expression through contacts made with chromatin. Mutations in the human LMNA gene encoding A-type lamins cause laminopathies, a collection of diseases that include muscular dystrophy (MD). Our laboratory has generated a Drosophila model of laminopathies in which human disease mutations are modeled in Drosophila lamin for functional analyses. Muscle-specific expression of mutant lamins causes cytoplasmic aggregation of nuclear envelope proteins, induction of genes regulated by the redox-sensing transcription factor Nrf2, and lethality. Under normal conditions, Nrf2 resides in the cytoplasm, where it is sequestered by Keap-1. Under conditions of oxidative stress, Nrf2 dissociates from Keap-1 and translocates to the nucleus where it activates specific target genes. Paradoxically, we found that the muscles expressing mutant lamins were not under oxidative stress, rather a condition known as reductive stress. This raised the question as to how the Nrf2 target genes were activated. We posit that Nrf2 target gene activation occurs due to cytoplasmic lamin aggregation. Cytoplasmic protein aggregates typically bind to the adaptor protein p62/SQSTM1, which also binds Keap-1. Thus, we propose that p62/SQSTM1 sequesters Keap-1, allowing Nrf2 to translocate into the nucleus and activate gene expression. Our findings provide a novel mechanism by which mutant lamins regulate gene expression and suggest classification of laminopathies as protein aggregation disorders.
The transition from a specified germ cell to a population of totipotent cells occurs immediately following fertilization during the initial stages of embryonic development. At this developmental stage the zygotic genome is transcriptionally quiescent, and development is controlled by maternally deposited mRNAs and proteins. Widespread zygotic transcription initiates later, after the cells have become totipotent and are poised to differentiate. Despite the fact that this delayed transcriptional activation of the zygotic genome is nearly universal among metazoans, the mechanisms governing this process remain unknown. We and others have demonstrated that the Drosophila protein Zelda (ZLD) acts globally to facilitate the transcriptional activation of the zygotic genome. Our data suggested that ZLD may establish a permissive chromatin environment, allowing additional transcription factors access to their underlying binding sites. We directly tested this hypothesis by measuring chromatin accessibility in the presence and absence of ZLD in early embryos. We find that ZLD is specifically required to establish regions of open chromatin at the maternal-to-zygotic transition. To further elucidate the genomic reorganization that occurs during these initial stages of development, we assessed histone marks on a genome-wide level at discrete stages through early embryogenesis. We will discuss how these data compare to similar analyses in other organisms and how they relate to transcriptional activation and the establishment of a totipotent state.
**Nucleosome binding and cell-type specific gene expression**

*Alison Gjidoda, Mohita Tagore, Michael McAndrew and Monique Floer*

How nucleosome occupancy at transcriptional regulatory regions is altered to allow expression of inducible genes in mammals is not clear. We have used a quantitative assay to analyze changes in nucleosome occupancy upon induction of three pro-inflammatory cytokines in primary mouse macrophages and find that distal and proximal enhancers are rapidly cleared of nucleosomes. We also show that the cleared enhancers become associated with cis-regulatory transcription factors required for induction of the associated genes. Surprisingly, we find that the promoters of two of these genes are not cleared of nucleosomes under conditions that lead to high levels of gene expression, and we speculate that competing nucleosomes at the promoters may contribute to the high degree of stochasticity found at these and other inducible genes. Our studies further revealed that enhancers of these inducible genes are only lowly occupied in macrophages prior to induction, suggesting that a permissive chromatin architecture may keep enhancers accessible to subsequent binding of cis-regulatory transcription factors. Using siRNA we have begun to dissect the involvement of lineage-specific transcription factors (e.g. PU.1, C/EBPβ, Runx) and nucleosome remodelers (e.g. Brg1/Brm) in determining cell-type specific chromatin architecture at enhancers of inducible genes in macrophages.

**Deciphering the regulatory code of the Drosophila Suppressor of Hairy-wing protein**

Differential gene transcription generates distinct cell types during development. Cell-specific transcription requires factors that bind cis-regulatory DNA elements, such as enhancers, silencers and insulators. Alterations in the function of these cis-regulatory elements may be a common cause of human disease, as over 85% of disease-associated sequence variations map outside of coding regions of genes. Our ability to predict how these disease associated sequence variants affect gene transcription is challenged because most transcription factors are multifunctional, demonstrating context-specific regulation of target genes. Advances in our ability to infer disease mechanisms will depend on the definition of rules that govern how a single transcription factor establishes multiple regulatory outputs. To this end, we are investigating mechanisms of transcriptional regulation of the Drosophila Suppressor of Hairy-wing [Su(Hw)] protein, a multi-functional regulator that possesses activator, repressor and insulator functions. The twelve zinc-finger (ZnF) Su(Hw) protein binds ~3,000 sites within the Drosophila genome. To define mechanisms of Su(Hw) transcriptional regulation, we conducted a genetic screen for new su(Hw) alleles. We identified two separation of function alleles, encoding proteins that possess only the insulator or repressor functions of Su(Hw). In each case, the mutant allele encodes a full-length protein with a single ZnF disrupted. Both ZnF mutants bind chromosomes in vivo, showing reduced occupancy relative to the wild type protein. Interestingly, genome-wide mapping demonstrates that the separation of function mutants bind different sequence subclasses of the ~30-bp Su(Hw) consensus site. We postulate that Su(Hw) binding at these sequence subclasses involves differential ZnF usage, which may alter the conformation of DNA-bound Su(Hw) and recruitment of factors needed for transcriptional regulation. These data suggest that the insulator and repressor functions of Su(Hw) are dictated by a DNA code.
Establishing the Erythroid Cell Transcriptome: Coregulator Matrix Model of GATA Factor Function differentiation.

The establishment and maintenance of cell type-specific transcriptional programs require an ensemble of broadly expressed chromatin remodeling and modifying enzymes. Many questions remain unanswered regarding the contributions of these enzymes to specialized genetic networks that control critical processes such as lineage commitment and cellular differentiation. The transcription factor GATA-1 is a critical driver of erythrocyte development. GATA-1 functions with its coregulator, Friend-of-GATA-1 (FOG-1), to activate and repress genes required for the erythroid developmental program. As some GATA-1 targets have little or no FOG-1 requirement, presumably other trans-acting factors contribute to GATA-1 function at specific loci. Through a screen of prioritized chromatin modifying and remodeling enzymes, we identified a histone lysine methyltransferase, Pr-Set7/SetD8, as a critical GATA-1 corepressor. Setd8 is the sole enzyme known to monomethylate histone H4 at lysine 20 (H4K20me1). To interrogate SetD8 function in the context of erythroid cell biology, we utilized a genetic complementation system in GATA-1-null G1E cells, in which GATA-1 fused to an estrogen receptor ligand-binding domain (GATA-1) can be conditionally replaced at physiological levels. GATA-1 selectively induced H4K20me1 at repressed, but not activated, loci, and endogenous SetD8 mediated GATA-1-dependent repression of a cohort of its target genes. GATA-1 utilized different combinations of SetD8, FOG-1, and the FOG-1-interacting Nucleosome Remodeling and Deacetylase (NuRD) complex component Mi2ß to repress distinct target genes. Additionally, studies are underway that demonstrate a balance between SetD8 and interacting chromatin modifying enzymes is necessary for primary murine erythroid maturation. Implicating SetD8 as a context-dependent GATA-1 corepressor expands the repertoire of coregulators mediating establishment/maintenance of the erythroid cell genetic network and provides a biological framework for dissecting the cell type-specific functions of this important coregulator. We propose a coregulator matrix model in which distinct combinations of epigenetic regulators are required at different GATA-1 target genes, and the unique attributes of the target loci mandate these combinations.
Intersection of chromatin and signaling pathways in the acquisition of pluripotency

Transcription factor-mediated reprogramming of somatic cells into induced pluripotent stem cells (iPSCs) involves a dramatic change in chromatin state. We performed a comprehensive quantitative mass spectrometry-based proteomics screen to identify post-translational modifications (PTMs) of histones that change in global abundance during reprogramming and found that pluripotent cells are enriched for histone PTMs associated with active regulatory regions, but surprisingly depleted for PTMs associated with transcriptional elongation. By contrast, in somatic cells and pre-iPSCs, that are a late intermediate of the reprogramming process, repressive modifications such as H3K9me2/me3 act as a barrier to achieving pluripotency. Using pre-iPSCs we found that the addition of both ascorbic acid (AA) and 2i(MAP kinase and GSK inhibitors) synergistically allowed the acquisition of a fully reprogrammed state at a very high efficiency. However, minimal conversion occurred with either component alone. When uncoupled, AA treatment had to strictly precede 2i and mediated perturbation of the epigenome. Among the AA-dependent activities, there was critical early requirement for histone demethylase effects while Tet-mediated 5-hydroxymethylation was required throughout the conversion to iPSCs. Under hypoxic conditions the concentration of AA required for enhanced 5-hydroxymethylation and iPSC conversion was considerably reduced. Transcriptional response to the individual stimuli was almost completely divergent and both components were required for the rewiring of the pluripotency network. Taken together our results provide important insights into changes in the epigenome and transcriptome and the pathways that lead to the acquisition of pluripotency.
Enzymes that modify chromatin require co-substrates that are major metabolic intermediates, like acetyl-CoA, NAD^+, SAM, O_2, alpha-KG. We are testing the hypothesis that certain chromatin modifying enzymes/complexes have evolved to exquisitely ‘sense’ metabolite levels and respond accordingly, modifying specific chromatin loci for altered gene expression. A class of chromatin modifying enzymes that provide an excellent example of this connectedness are the sirtuins (SIRT1-7), which are NAD^+ dependent protein deacylases. In particular, SIRT6 is plays crucial roles in genomic and metabolic stability. Overexpression of SIRT6 in mice lowers LDL and triglyceride levels, improves glucose tolerance, and increases mitochondrial respiration. Despite in vivo evidence indicating that SIRT6 is a potent histone deacetylase, SIRT6 is among the mammalian sirtuins that display extremely inefficient deacetylase activity in vitro, suggesting that an activating mechanism might exit in cells. Recently, we demonstrated that the catalytic activity of SIRT6 is directly activated by biologically relevant free long-chain fatty acids (LCFA), several of which are linked to the health benefits of dietary PUFAs. These results suggest the SIRT6 deacetylase activity (H3K9ac) is stimulated under metabolic states (i.e., fasting and dietary supplementation) that increase particular free fatty acids such as Ω3 and Ω6 forms. Non-esterified LC-FAs are known to control gene expression by regulating the activity and/or abundance of several transcription factors involved in glycolytic and lipogenic pathways. This work provides evidence that LCFA stimulate SIRT6 to down-regulate carbohydrate and lipid metabolism in response to dietary input and metabolic status.
Mechanisms of PRC2 regulation

Chromatin has evolved as an elegant mechanism for the regulation of the genome. The structure of chromatin is quite dynamic, exhibiting dramatic fluctuations throughout the lifecycle of the cell that lead to functional regulation of the underlying genomic material. The pattern of post-translational modifications (PTMs) on histone proteins is known to be critical in chromatin structure and dynamics and is linked to essentially every DNA-template process. However, the mechanisms by which these patterns of modifications are established and modulated are still largely unclear. Polycomb Repressive Complex 2 (PRC2) methylate lysine 27 on the N-terminal tail of histone H3 (H3K27me3), and is critical for establishing and maintaining gene repression patterns important in development, stem cell maintenance and cancer. The PRC2 complex is made up of four core subunits, including SUZ12, RbAp46/48,EED and the catalytic subunit EZH2. However the exact composition of PRC2 is modulated by different isoforms of the core subunits as well as by a number of sub-stoichiometric associated co-factors. The differential composition of the complex appears to dynamically regulate its activity in a functionally relevant manner, fine-tuning the proper H3K27me3 patterning. However, the molecular mechanisms underlying how these co-factors alter the activity of the complex and thus the pattern of H3K27me3 are largely unknown. I will discuss recent work that elucidates how the co-factor PHF1 regulates PRC2 in a manner that is dependent on the local epigenetic landscape.

Yeast histone demethylase Jhd2 mediates a conserved cross-talk between H3K4 methylation and H3K14 acetylation

Gene expression has been shown to be regulated through epigenetic modifications to the N-terminal tail of histones. Among these modifications is methylation of lysine residues. In budding yeast the primary sites of methylation are lysines 4, 36, and 79 on histone H3. The enzyme Jhd2 is a histone demethylase that functions to remove H3K4 methylation in S.cerevisiae. Jhd2 is a homologue of the human JARID1 family of histone demethylases, which has four members: JARID1A, B, C and D. JARID1B is of particular interest because it has been shown to be up regulated in 90 percent of primary breast cancers. Furthermore, JARID1A has been shown to be up regulated in gastric cancer. Studies have shown a cross-talk between H3K4 methylation and H3K14 acetylation. A recent report suggests that Jhd2 mediates the cross-talk between these H3K4 trimethylation and H3K14 acetylation; however, the mechanism in which Jhd2 acts to facilitate this cross-talk is unknown. Our study shows that Jhd2 mediates a cross-talk between H3K4 trimethylation and H3K14 acetylation and that this is mediated through the interaction between Jhd2 and the H3 N-terminal tail in that acetylation disrupts the interaction. By mutating H3K14, H3K4 trimethylation can be modulated in a Jhd2 dependent manner. Additionally, deletion of a histone acetyl transferase(HAT) GCN5, the HAT associated with the SAGA complex, results in a decrease for not onlyH3 acetylation but also in global and gene specific H3K4 trimethylation. This decrease inH3K4 trimethylation is dependent on Jhd2. We further demonstrate that deletion of GCN5causes an increase in Jhd2 binding to chromatin. Finally, we were able to show that knock-down of Gcn5 in Drosophila melanogaster also causes a decrease in H3K4 trimethylation levels. Through our study we show that the histone demethylase Jhd2 mediates a cross-talk between H3K14 acetylation and H3K4 trimethylation through modulating its interaction with chromatin, and that this mechanism for maintaining chromatin modifications is conserved through other eukaryotes.
H3S10 phosphorylation by the JIL-1 kinase regulates heterochromatin, H3K9me2 distribution and gene expression in Drosophila

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A large number of histone modifications have been correlated with epigenetic regulation. The modifications have been classified as either repressing or activating; however many of these marks have complex and seemingly conflicting roles. JIL-1 kinase mediated H3S10 phosphorylation associates with active chromatin and contributes to an open chromatin structure. In order to explore the effect of the epigenetic H3S10 mark on gene expression we compared the global changes in gene expression in wild-type and JIL-1 null salivary glands. Salivary glands are post-mitotic allowing us to also compare the binding sites of JIL-1 with the locations of the interphase H3S10ph mark and the H3K9me2 silencing mark. Surprisingly, we found that almost as many genes were activated as repressed in the absence of the H3S10ph mark but that downregulation of genes in the mutant was correlated with higher levels of the H3K9me2 mark whereas upregulation was correlated with diminished H3K9 dimethylation. These findings suggest a model where the H3S10ph mark is not required for gene expression per se but indirectly regulates transcription by controlling the distribution of the repressing H3K9me2 mark. However, it is now recognized that some H3S10ph antibodies’ labeling can be occluded by the concomitant presence of the H3K9me2 mark. Using a non-occluded antibody we find that a double H3S10phK9me2 mark is present in pericentric heterochromatin as well as on the 4th chromosome of wild-type polytene chromosomes but not in preparations from null JIL-1 or Su(var)3-9 (H3K9 methyltransferase) larvae. When a Lac-I-Su(var)3-9 transgene is overexpressed, H3K9me2 dimethylation is upregulated on the chromosome arms creating extensive ectopic H3S10phK9me2 marks, suggesting that H3K9 dimethylation occurred at euchromatic H3S10ph sites. This is further supported by the finding that under these conditions euchromatic H3S10ph labeling by the occluded antibodies is abolished. These findings are consistent with the model that JIL-1 kinase activity under normal conditions antagonizes Su(var)3-9 activity by keeping H3K9me2 levels low relative to H3S10ph levels at certain transcriptionally active regions. However, it also implies the existence of a different mechanism for regulating the interactions between kinase and methyltransferase activity in the context of pericentric heterochromatin and the 4th chromosome that instead of competition promotes creation of the double mark. Supported by NIH grant GM62916.
DNA methylation patterning in development and cancer

Global patterns of DNA methylation, mediated by the combined action of DNA methyltransferases (DNMTs) and ten-eleven translocation (TET) proteins, are critical for normal differentiation. DNA hydroxymethylation, the product of TET-mediated oxidation of 5-methylcytosine (5mC), remains poorly understood but also appears essential for developmental processes. Disruption of the DNMTs and TETs by mutation or aberrant expression, and the concomitant abnormalities to 5mC and 5hmC patterns and gene transcription, are common defects that contribute to tumor initiation and promotion. To study the dynamics of 5mC and 5hmC regulation we acutely depleted all DNMTs and TETs and examined the impact on localization of each epigenetic mark, the impact on transcription, and how these changes interface with key histone modifications. Key findings from the DNMT depletions include DNMT3B occupancy regulating methylation during differentiation and an unexpected interplay between DNMT1 and DNMT3B in regulating gene body methylation. TET depletion yielded widespread reduction of 5hmC that differentially impacted high versus low CpG density promoters and different chromatin environments, and caused marked CpG island hypermethylation. Taken together, these results provide novel insight into the division of labor among DNMTs and TETs in regulating DNA epigenetic marks.

Molecular mechanism of action of plant de novo DNA methyltransferases

Epigenetic regulation of gene expression plays critical roles in maintaining genome integrity, imprinting, cellular differentiation, normal growth and development, disease formation as well as in responding versatile environmental signals. Two longstanding and fundamental questions in the field of epigenetics are how certain DNAs are targeted by methylation and how DNA methylation is stably and faithfully maintained. DOMAINS REARRANGED METHYLTRANSFERASE (DRM) is a key de novo methyltransferase in plants, but how DRM acts mechanistically is poorly understood. Here, we report the crystal structure of the methyltransferase domain of tobacco DRM (NtDRM) and reveal a molecular basis for its rearranged structure. Our crystal structure further reveals that DRM forms a functional homo-dimer critical for catalytic activity. We also show that Arabidopsis DRM2 exists in complex with the siRNA effector ARGONAUTE4 (AGO4) and preferentially methylates one DNA strand, likely the strand acting as the template for RNA polymerase V mediated non-coding RNA transcripts. This strand-biased DNA methylation is also positively correlated with strand-biased siRNA accumulation. These data suggest a model in which DRM2 is guided to target loci by AGO4-siRNA and involves base-pairing of associated siRNAs with nascent RNA transcripts.
DNA Methylation Changes underlie Hyperglycemia and the Metabolic Memory State of an Adult Zebrafish Model of Type I Diabetes

Studies from human cells, rats, and zebrafish have documented that hyperglycemia induces the demethylation of specific cytosines throughout the genome. Using a zebrafish diabetic model, we previously documented that a subset of these changes become permanent and may provide, in part, a mechanism for the persistence of complications referred to as the metabolic memory phenomenon. Clinically, MM is defined as the persistence of diabetic complications even after glycemic control has been pharmacologically achieved. In our zebrafish model, MM occurs following pancreatic regeneration which returns fish to a euglycemic state. We reported that specific changes in DNA methylation are associated with altered gene expression patterns underlying the secondary complications seen in DM/MM fish. In this report we present two sets of findings: 1) elucidating the molecular machinery that is responsible for the hyperglycemia induced DNA demethylation observed and 2) a detailed bioinformatics analysis of DNA methylation patterns that result from the hyperglycemic state and are maintained into the MM state. In regard to the molecular machinery of de-methylation, RNA expression analyses and enzymatic activity assays indicate that the ten-eleven translocation family of enzymes (Tet) were activated by hyperglycemia resulting in the conversion of 5mC back to the unmethylated form via the Tet-de-pendent iterative oxidation pathway. In addition, evidence is provided that the Poly-ADP ribose polymerase enzyme's (Parp) activity is required for activation of Tet activity as the use of a Parp inhibitor prevented the accumulation of the Tet induced intermediates. Remarkably, this inhibition was accompanied by a complete restoration of the tissue regeneration deficit that is also induced by hyperglycemia. In regard to the second set of findings, we focused on the diabetic limb (caudal fin) and analyzed 1) what functional gene groups are prominent during DM, 2) which genes of these groups persist into MM, and 3) what is the positional relationship of altered DNA methylation to these genes. We found that the DM/MM states are associated with changes in the expression of genes of the DNA replication and DNA metabolism groups. Aberantly methylated DNA regions (MRs) were 6-13 kb upstream of the transcription start site of a subset of functionally important genes within these groups. Lastly, MRs were associated with potential transcription factor (TF) binding sites whose methylation is known to perturb TF binding. Translatability of this data to the human genome was also investigated.
The development of chromatin remodeling inhibitors to investigate and treat a wide spectrum of human cancers

Recent exome sequencing of human tumors has uncovered a surprising new role for chromatin-associated processes in the development of cancer. Of particular note is the frequent identification of mutations in one or more subunit of the SWI/SNF (or BAF) chromatin remodeling complex. For some of these cancers, it has been found that the residual complex is actually oncogenic, suggesting that the BAF complex itself is a good therapeutic target. Several additional lines of data suggest that inhibition of chromatin remodeling complexes could be therapeutic; however no inhibitor of an ATP-dependent chromatin remodeler has yet been identified. To this end, we have performed a large, high throughput screen monitoring BAF mediated transcription. After further validation, we have identified a handful of compounds that regulate transcription in a manner similar to the BAF knockout. In order to confirm that the compounds act directly on the BAF complex, we are synthesizing focused small molecule libraries for the optimization of target identification probes. While some of the compounds may not inhibit the BAF complex directly, deciphering their targets will give us great insight into the mechanism of BAF-mediated transcription, and possibly BAF-mediated tumor suppression. With a BAF inhibitor in hand, we will have the tools for understanding the tumor suppressive mechanism of the BAF complex, as well as confirming the therapeutic potential of targeting this class of epigenetic regulators in cancer.

The long and short of drug targeting bromodomain proteins

Bromodomain proteins comprise a functionally diverse family, and as numerous members have been implicated in disease, they represent an exciting new class of drug targets. The demonstrated success with specific inhibitors of the BET family of bromodomains has inspired continued screening of the larger non-BET family. Many of these however have added complexity by containing additional histone interacting or modifying regions such as PHD, HAT, and/or PWWP domains. Bromodomain inhibitor compounds have traditionally been identified in an in vitro system consisting of a purified bromodomain alone and modified histone peptides. In efforts to create a more physiological assay, we have utilized and optimized bioluminescence resonance energy transfer (BRET) to directly measure proteins binding to chromatin in living cells. With this approach, we demonstrate in vivo IC50s for bromodomain inhibitors can be calculated and potency differences between histones determined. More importantly, in live cell assays using non-BET family inhibitors against some of the more complex bromodomain proteins, we observe the ability to disrupt binding of the individual bromodomain alone, but not the full-length protein from chromatin. Further deletional studies reveal the domains contributing to the abrogation of inhibition when the compound is presented to the full-length protein. These data suggest that in certain cases, targeting of more than the bromodomain alone may be necessary for effective disruption of binding to chromatin, and in hope will help guide compound screening and development not only for bromodomain proteins, but also other chromatin readers which may be therapeutic targets.
**Targeting MLL1 in treatment of leukemia**

Mixed lineage leukemia protein 1 (MLL1) is one of the histone H3 lysine 4 (K4) methyltransferases in mammals and plays important roles in Hox gene regulation. Rearrangements of MLL1 gene including translocation, amplification and tandem duplication are common in acute leukemia that have extremely poor prognosis. Although MLL mutations are drivers of leukemogenesis, wild type MLL1 is critical for viability of leukemia cells. Genetic deletion of MLL1 in cells carrying oncogenic MLL1 fusion proteins prevent onset of leukemia, suggesting its potential as a therapeutic target. Based on biochemical characterization of the MLL1 complex, we have developed a potent and specific small-molecule inhibitor (MM-401) that targets MLL1 complex assembly and thus, its methyltransferase activity. We demonstrate that MM-401 selectively inhibits MLL1 dependent H3 K4 methylation in vitro and in cells. Furthermore, MM-401 shows high efficacy against MLL leukemia by inducing apoptosis, cell cycle arrest and myeloid differentiation while having little effects on normal bone marrow cells. Genome-wide transcriptome analyses show that MM-401 alters gene expression in the same manner as MLL1 deletion, underscoring the importance of MLL1 methyltransferase activity in disease progression as well as ‘on-target’ specificity of MM-401. We envision broad applications of MM-401 in basic and translational research.

**Structural studies on sirtuin inhibitor**

Sirtuins are NAD⁺ dependent protein deacylases that involved in the posttranslational modification of histones but also other proteins. The regulation of protein activity and stability affected by sirtuins shows a strong involvement in the pathogenesis of cancer, neurodegeneration and metabolic diseases. Here, we present structural studies on inhibitors of human Sirt2 in complex with the enzyme. We present different X-ray structures of inhibitor-hSirt2 cocomplexes that constitute the first examples with druglike inhibitors. These can now serve as a basis for rational optimization for existing and new Sirtuin inhibitors which was lacking so far. We also present the initial results of such optimization strategies as well as cellular data concerning tubulin hyperacetylation and functional effects.
GLI1 and MLL/COMPASS complex to control tumor microenvironment-associated gene expression


Cancer is characterized by aberrant patterns of gene expression, not only caused by genetic alterations, but also epigenetic ones. Although with the use of high-throughput genomic technologies, the genetic pathways regulating tumor growth have been extensively studied, the knowledge regarding the molecular mechanism by which the epigenetic alterations contribute to cancer development is limited. In the current study we have identified a new transcriptional mechanism involving the transcription factor GLI1 and histone methyltransferase MLL/COMPASS complex to differentially activates genes involved in the regulation of the tumor microenvironment. GLI1, a C2-H2 type of zinc finger transcription factor, is a transcriptional activator known as downstream effector of Hedgehog pathway, being crucial for many aspects of mammalian development, but it can also be an effector of signaling cascades involved in tumorigenesis, e.g. TGFβ pathway. Combined expression, luciferase assays and chromatin immunoprecipitation indicate that GLI1 interacts directly with TGFβ1 promoter. We have demonstrated that GLI1 acts as a downstream mediator of TGFβ signaling, one of the most potent inducers of the proper TGFβ1 ligand, creating a positive feedback loop. TGFβ requires GLI1 to promote TGFβ1 expression, RNAi knockdown of GLI1 completely abrogated the induction of its mRNA expression by this cytokine. Mechanistically, both, GLI1 and TGFβ treatment increase the methylation of the Histone 3 at lysine 4 (H3K4me3) in TGFβ1 promoter. This chromatin modification is a prevalent mark associated with transcriptional activation and is deposited by a family of histone methyltransferases (HMT) that share a conserved SET domain, the SET1/MLL family. We have found that several components of the MLL/COMPASS complex such as MLL1, LEDGF and ASH2L bind in the same region of the promoter. We confirmed that GLI1 physically associates with LEDGF and that this interaction is the responsible of the HMT complex recruitment to the TGFβ1 promoter region. Knockdown experiments of GLI1 or LEDGF impairs the binding of the complex and the increase of H3K4me3 levels on the promoter after TGFβ treatment. Like GLI1 LEDGF is also required for TGFβ1 mRNA induction by TGFβ signaling. This studies have been extended to other two important genes involved in the regulation of the tumor microenvironment, IL6 and IL7, sharing the same transcriptional mechanism mediated by GLI1 and the MLL/COMPASS complex. Thus these results provide evidence of a novel epigenetic mechanism mediated by the transcription factor GLI1 through the recruitment of the MLL complex that defines a specific histone modification signatures regulating the activation of important mediators of tumor development.
The role of chromatin in RB repression of RNA polymerase III

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RNA polymerase (Pol) III transcribes numerous small non-coding RNAs whose functions are integral to cellular proliferation. We discovered that Pol III activity is dynamically governed at multiple levels, both during normal proliferation and in cancer. In particular, the proto-oncoprotein c-Myc activates Pol III transcription while the p53 and RB tumor suppressor proteins repress Pol III activity. Consistently, elevated levels of Pol III-derived transcripts are frequently observed in human tumors, and may represent a hallmark of cancer. In the current study, we focused on the role of chromatin in the RB repression mechanism. Previous studies have demonstrated that some Pol III genes maintain a nucleosome within an upstream promoter proximal region that contributes to activated Pol III transcription. We show that RB repression is more effective on chromatin templates than on naked DNA, suggesting that histone modulation per se is important for Pol III repression. RB simultaneously associates with HDAC2 at some Pol III target genes in vivo and can stimulate HDAC2 recruitment to Pol III reporter genes in vitro. Significantly, HDAC activity contributes to RB-mediated repression only in the context of chromatin. We further found that RB orchestrates a network of additional chromatin modifying factors during Pol III repression, including SWI/SNF ATP-dependent chromatin remodeling complexes, DNA methyltransferases (DNMT), and DNA topoisomerase. Interestingly, RB could mediate DNA methylation and recruitment of DNMT1 and DNMT3a to naked DNA templates, suggesting that pre-existing chromatin marks are not essential for DNMT activity at these promoters. A model emerges that RB repression can be separated into a reaction pathway composed of nucleosomal-dependent and independent steps.
Identifying genetic factors that contribute to breast cancer risk is critical for both risk assessment and the development of new therapeutics. It is thought that many such risk factors are high-frequency, low-penetrant alleles, which are largely found to reside in non-coding regions, making functional studies difficult. Quantitative trait loci analysis in the mammary cancer resistant Wistar-Kyoto (WKy) rat model has led to the discovery of the Mammary Carcinoma Susceptibility 5c, (Mcs5c), risk locus which was found to decrease tumor multiplicity by 55% in congenic rats possessing the resistant allele. The Mcs5c locus spans 170kb on rat chromosome 5 and lies in a 1Mb gene desert. This region shares homology with mouse chromosome 4 and human chromosome 9. Mammary gland transplant experiments have determined that Mcs5c acts in a mammary gland autonomous manner. Expression analysis on surrounding genes has identified Pappa as the likely target of Mcs5c action, as its expression was found to be upregulated by 30% in mammary epithelial cells of susceptible rats at 7 and 9 weeks of age, with no expression differences at 12 weeks. The ages at which differential expression are observed represent a critical time during mammary gland development when animals are most susceptible to cancer initiation.

We hypothesized that Mcs5c mediates the observed changes in gene expression through an enhancer element that physically interacts with Pappa via chromatin folding. Chromosome conformation capture (3C) was used to detect such interactions, and found that a conserved region within Mcs5c strongly interacts with Pappa's first intron in 6 and 7 week old mammary epithelial cells over a distance of approximately 590kb. There was no observed genotype-dependent difference, with both resistant and susceptible epithelial cells showing a positive interaction at these time points. The interaction between these regions in 4 and 12 week epithelial cell samples; however, was much weaker, and is barely above the background noise. In this way, we have observed an age-dependent differential chromatin interaction occurring between a region within Mcs5c and the first intron of Pappa. Studies are currently underway to determine if this interaction is tissue-dependent in addition to age-dependent.

The site of Mcs5c interaction at the Pappa gene suggests a role for DNA methylation in the observed expression changes, as the region lies in a “CpG island shore” which are often found to be sites of differential methylation. Studies are currently underway to investigate the methylation state of Pappa, and we are also interested in characterizing histone-associated marks at both Mcs5c and Pappa, hypothesizing that Mcs5c epigenetically regulates Pappa expression, as facilitated by the observed chromatin interaction.

This research highlights the functionality and complexity of a non-coding risk locus. In addition to the novel discovery of an age-dependent enhancer element within Mcs5c, we believe Pappa to be a potential therapeutic target, as it is involved in a signaling pathway commonly mis-regulated in breast cancer.
Precise regulation of gene expression is essential for animal development. Context-specific switching between activation and repression is a central process in cell fate determination, and is orchestrated by transcriptional repressors through changes in chromatin structure. The nonpermissive chromatin environment in gene silencing is created by multi-subunit protein complexes, involving histone modifying enzymes and chromatin remodelers that are recruited by DNA binding repressors through co-repressors. In order to elucidate distinct repression mechanisms, we assessed chromatin changes on a genome-wide scale using Drosophila Hairy repressor as a paradigm in the blastoderm embryo. Hairy is a member of Hairy/Enhancer of Split (HES) family proteins and retains multiple repression domains through which it interacts with co-repressors Gro, dCtBP and dSir2. Misexpressing Hairy or mutant versions of Hairy that lack specific motifs required for co-repressor interaction allowed us to correlate dynamic changes in various histone marks with the modes of repression. We found that Hairy induces wide-spread histone deacetylation, especially in the distal regulatory regions of target genes, consistent with its long-range repression mechanism. Strikingly, local loss of acetylation marks is also associated with repression, more commonly at nearby promoters, indicating Hairy may employ short-range repression mechanism in some cases. The mutation in the CtBP interaction domain partially impairs repression ability of Hairy on some genes but completely abolishes its activity on others. These results support the model that Hairy acts in a context-dependent manner utilizing different repression mechanisms, and CtBP contributes to repression in a quantitative and gene-specific fashion. Comprehensive characterization of chromatin states and cofactor interactions mediated by Hairy help us understand the repression systems in Drosophila, and more generally, pathways of transcriptional repression in metazoans. In addition to providing mechanistic insights, lessons from this study will be constructive to interpret genome-wide transcription factor binding, chromatin modifications and understand the activity of cis-regulatory elements.
Writers, Readers, and Erasers of the Histone Code in Cancer

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Our laboratory is focused on better understanding the regulation and function of epigenetic writer, reader, and eraser complexes during cancer development. Regarding eraser complexes, our work led to the discovery of the first low affinity SID (Sin3 Interacting Domain), which mediates the sequence-specific recruitment of several tumor suppressor proteins to gene networks that inhibit cell proliferations in pancreatic cancer. Our studies on histone code readers unraveled a role for HP1γ-Histone Methyl Transferases in the regulation of mRNA elongation of oncogenic gene pathways. This is important since members of this family of small chromatin proteins had only been considered as transcriptional repressors. As it relates to writers of the histone code, we have identified and characterized several EZH2-type Histone Methyl Transferases, which also promote cancer development and growth. In this lecture, we will discuss how these findings help to build a theoretical framework that can aid to explain fundamental epigenetic mechanisms at the molecular level as well as how these mechanisms operate to either positively or negatively affect neoplastic transformation and cancer pathobiology.

Varying the Chromatin Landscape in Human Cancers

An emerging literature documents a growing list of mutations in chromatin-modifying genes found in human cancers, highlighting the role that aberrant epigenetic regulation plays in oncogenesis. Our research is directed at understanding how chromatin assembly and modifying machineries contribute to epigenetic memory, and how misregulation of these pathways contribute to tumorigenesis. Recent studies have focused on histone H3.3, a histone variant enriched at specific genomic loci including actively transcribed genes and telomeres. Multiple proteins associate with H3.3 for its chromatin deposition. Two of them, ATRX and Daxx are mutated in a variety of cancers, including pancreatic neuroendocrine tumors. Additionally, specific mutations in genes encoding H3.3 are frequently found in pediatric chondroblastoma and glioblastoma. These gain-of-function mutations inhibit the enzymatic activity of specific SET-domain-containing lysine methyltransferases. I will present evidence demonstrating how these H3.3 mutations or loss of ATRX-Daxx can lead to altered gene expression and loss of cellular differentiation.
Expression of the antioxidant enzyme EcSOD in normal human mammary epithelial cells was not recognized until recently. Although expression of EcSOD was not detectable in non-malignant human mammary epithelial cells (HMEC) cultured in conventional two-dimensional (2D) culture conditions, EcSOD protein expression was observed in normal human breast tissues, suggesting that the 2D-cultured condition induces a repressive status of EcSOD gene expression in HMEC. With the use of laminin-enriched extracellular matrix (lreECM), we were able to detect expression of EcSOD when HMEC formed polarized acinar structures in a 3D-culture condition. Repression of the EcSOD-gene expression was again seen when the HMEC acini were sub-cultured as a monolayer, implying that lreECM-induced acinar morphogenesis is essential in EcSOD-gene activation. We have further shown the involvement of DNA methylation in regulating EcSOD expression in HMEC under these cell culture conditions. EcSOD mRNA expression was strongly induced in the 2D-cultured HMEC after treatment with a DNA methyltransferase inhibitor. In addition, epigenetic analyses showed a decrease in the degree of CpG methylation in the EcSOD promoter in the 3D versus 2D-cultured HMEC. More importantly, >80% of clinical mammary adenocarcinoma samples showed significantly decreased EcSOD mRNA and protein expression levels compared with normal mammary tissues and there is an inverse correlation between the expression levels of EcSOD and the clinical stages of breast cancer. Combined bisulfite restriction analysis of some of the tumors also revealed an association of DNA methylation with the loss of EcSOD expression in vivo. Furthermore, overexpression of EcSOD inhibited breast cancer metastasis in both the experimental lung metastasis model and the syngeneic mouse model. This study suggests that epigenetic silencing of EcSOD may contribute to mammary tumorigenesis and that restoring the extracellular superoxide scavenging activity could be an effective strategy for breast cancer treatment. (Supported by NIH CA115438)
ChIP-seq is broken, how do we fix it?

Despite serving as a central experimental technique in epigenetics research, chromatin immunoprecipitation coupled to deep sequencing (ChIP-seq) suffers from several serious drawbacks: 1.) it is a relative measurement unthered to any external scale in a way that obviates comparison amongst experiments; and 2.) it employs antibody reagents that have differing specificity, affinity and off-target binding for different epitopes, which are in turn variable in abundance, yet none of these factors are taken into account in present analysis. To address these substantial problems, we have developed an approach to calibrate ChIP-seq data using a panel of nucleosomes derived from recombinant and semisynthetic sources as internal standard epitopes (ICE-ChIP-seq). We reconstitute nucleosomes bearing a given mark on a library of DNAs composed of a constant strong nucleosome positioning sequence that is flanked by a variable “barcode” that encodes each member’s concentration, then spike this calibration ladder into the input of a native ChIP-seq experiment. After immunoprecipitation with modification-specific antibodies followed by sequencing, the tag counts resulting from the exogenous semisynthetic nucleosome DNA series serve as an internal-standard calibration curve for absolute quantification of mark density with the positional accuracy of ChIP-seq in a genome-wide data set. We present the proof of concept for this methodology, demonstrating that this method is both highly precise and accurate by comparison of replicates and other readouts (digital droplet PCR, qPCR, radioactivity) and reveal starkly bimodal histone modification densities of H3K4me3 that approach 100% at some highly active gene promoters. We also critically assess untested assumptions inherent in conventional ChIP-seq: remarkably, the signal is linear over a > 6 order of magnitude range of modification density and the measured histone modification density is independent of input.

What is the role of chromatin structure in maintaining cell fate?

Cell-type-specific chromatin tools allow us to profile terminally differentiated cells

Differential gene activity defines the function of distinct cell types during development and in the adult. Gene activity is thought to be largely determined by the action of transcription factors and chromatin features present at enhancers and promoters. This gene expression state must be maintained for the lifetime of the cell, and yet be able to change when required. We study chromatin and gene expression in functionally distinct terminally differentiated cells of the adult Drosophila melanogaster. We aim to determine how chromatin regulates and maintains gene activity in these cell types, and to define the principles that underlie post-mitotic chromatin plasticity. To readily track chromatin features at the resolution of specific cell types within complex tissues, we have developed and validated CAST-ChIP, a broadly applicable biochemical procedure that profiles chromatin-associated proteins in distinct cell types. RNA polymerase II (Pol II) CAST-ChIP identifies ~1500 neuronal and glia-specific genes in differentiated cells within the adult brain. Using this and complementary techniques we have mapped nucleosome positions and several chromatin features, including H2A.Z, histone H3 K36 methylation and K27 methylation and acetylation, as well as the binding sites of insulator proteins. Surprisingly, our data indicated that in contrast to developmental systems, RNA polymerase II binding is much more indicative of gene activity than the presence of histone marks.
Universal Homogenous Bioluminescent Assay to monitor the Effect of Modulators on Various Classes of Methyltransferases in vitro

Methylation/demethylation of DNA and Proteins play major roles in modulation of the epigenome and has been implicated in a wide variety of human diseases. Recent biochemical and biological data suggest that the enzymatic activities of several of these enzymes have pathogenic roles in cancer, inflammation, and neurodegenerative diseases. Thus, pharmacological modulation of these enzymes by small molecules will be beneficial in developing novel therapeutics for multiple unmet medical needs. Towards this goal of searching for activators/inhibitors of these enzymes for the development of next generation of drugs, screening assays for these modulators are urgently needed. To address these unmet needs, we have developed a novel assay that monitors the activities of these enzymes and their modulation by small molecules. The assay is bioluminescent based, HTS formatted and highly sensitive. The assay is universal since it is based on monitoring the formation of the universal product S-adenosylhomocysteine (SAH), i.e., capable of detecting changes inactivity of a broad range of methyltransferases such as DNA, protein, and small molecules methyltransferases. In addition, the assay has been validated for all classes of protein methyltransferases (Lysine and Arginine), and with different types of substrates (small peptides, large proteins, or even nucleosomes). This enables determining the specificity of these enzymes and their substrate requirements. The assay has high signal to background and low C.V. The assay is robust (Z' value > 0.7) and has been validated using various plate densities such as 96-, 384, and 1536-well plates. A strong feature of this assay is its utility with broad range of substrates with no limitations on the use of high concentrations of substrates or the composition of the substrates (short vs. long peptides), thus enabling the generation of kinetic data and determining the mechanism of action of various modulators of methyltransferases of interest.

Structural and Functional Studies of JMJD2 Demethylase Substrate Specificity

The JMJD2 family of lysine demethylases has been linked to diverse biological processes, including transcriptional regulation, embryonic stem cell self-renewal, development, cell cycle control, and androgen and estrogen receptor target gene expression. Humans possess four JMJD2 homologs, termed JMJD2A, JMJD2B, JMJD2C, and JMJD2D, which exhibit different methylation site and state specificities. JMJD2A, JMJD2B, and JMJD2C possess dual site selectivity for trimethylated Lys9 and Lys36 in histone H3 (H3K9me3 and H3K36me3) while displaying weaker activity toward H3K9me2 and H3K36me2. In contrast, JMJD2D efficiently demethylates di-and trimethylated H3K9 (H3K9me2/3) but is inactive toward H3K36me2/3. Crystallographic studies of human JMJD2A and JMJD2D in complex with different methylated histone H3 peptides have yielded insights into the structural variations in their histone binding clefts that define their respective substrate specificities. Correlatively, kinetic studies using methylated H3K9 and H3K36 peptides coupled with mutational analyses of JMJD2A and JMJD2D have demonstrated that individual residues in their histone binding clefts play pivotal roles in governing substrate recognition. Together, these findings illustrate that subtle variations in the histone binding clefts of the JMJD2 demethylases impart substantial differences in their respective substrate specificities.
Mis16 recognizes histone H4 and Scm3sp simultaneously to recruit CENP-A into centromeres

The Mis16-Mis18 complex, which contains Mis16 and Mis18 in fission yeast and Mis18α, Mis18β, M18BP1, and RbAp46/48 in human, has been identified as a trans-acting component for newly synthesized CENP-A recruitment into centromeres. How the Mis16-Mis18 complex accomplishes these tasks at the molecular level, however, is still elusive. Recently, we have determined the crystal structure of S. japonicas Mis16, one of the components in the Mis18 complex, at 2.3Å resolution. Structural similarity of Mis16 with its orthologs, p55 in drosophila and RbAp46 in human, implies that Mis16 associates with the helix 1 of histone H4(H4α1), which is later confirmed by pull-down and the co-crystal structure of Mis16 with the H4α1 peptide. Interestingly, the temperature-sensitive mutation (Y41H) of Mis16, which disrupts CENP-A recruitment at non-permissive temperature, locates near the histone H4 binding site, indicating that histone H4 recognition is critical for Mis16 function as a CENP-A recruiting factor. We also identified that Mis16 associates with the C-terminal region of Scm3sp. Evidences we provided here strongly support that Mis16 performs its function as a CENP-A recruiting factor by recognizing histone H4 and Scm3sp concurrently.

Evolution of multi-subunit RNA polymerases and co-evolution of Eukaryote complexity with the RNAP II CTD

One class of Eukaryotic interfering RNA polymerase and multi-subunit RNA polymerases (RNAPs) each have two double psi beta barrel (DPBB) motifs (a distinct pattern for compact 6-beta sheet barrels) at their active site. Between beta sheets 2 and 3 of the beta subunit type DPBB of multi-subunit RNAPs is a sandwich barrel hybrid motif (SBHM) that interacts with conserved initiation and elongation factors in the three domains of cellular life that diverged from the last universal common ancestor (LUCA). Analysis of RNAP core protein motifs, therefore, indicates that RNAP evolution can be traced from the RNA-protein world to LUCA to the present day, spanning about 4 billion years. In the Eukaryotic lineage, I posit that splitting RNAP functions into RNAPs I, II and III and innovations developed around the carboxy terminal domain (CTD) of RNAP II and the extensive CTD interactome may largely describe how greater structural, cell cycle, epigenetic and signaling complexity co-evolved in Eukaryotes relative to Eubacteria and Archaea. Essentially, this is a story of genesis told from the point of view of multi-subunit RNAPs including an Old and New Testament of gene transcription and regulation.
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1  |  Histone Carbonylation and Epigenomics

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Protein carbonylation is a process by which elevated levels of reactive oxygen species leads to the production of reactive lipid aldehydes, which can covalently modify lysine, cysteine, and histidine residues of proteins. Often considered a biomarker of oxidative stress, this class of post-translational modification is implicated in many inflammatory disease states including obesity and neurodegeneration. Broadly, we aim to understand the mechanism by which oxidative stress initiate an altered metabolic program in the context of obesity and its connectivity to protein carbonylation. Previous studies show that 4-HNE (4-hydroxynonenal), a reactive lipid aldehyde that carbonylates proteins, is found at significantly increased levels in the adipose tissue of obese compared to lean controls in both mouse and human tissues. Furthermore, in the obese state, protein modification by 4-HNE is increased up to 3-fold. Interestingly, using an antibody directed against 4-HNE modified proteins, we have identified the core histones as a preferential target of 4-HNE in both adipocyte cell culture models and in murine adipose depots. These studies show significant increases in histone H4 carbonylation in adipose tissue from high fat vs. chow fed mice as well as increased histone modification in response to cytokine induced oxidative stress in cultured adipocytes. Due to the central role histone modifications play in regulating chromatin dynamics, this preliminary work has opened new avenues of research to investigate a novel mechanistic connection between oxidative stress and transcriptional regulation of metabolic pathways.

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2  |  Targeting epigenetic regulatory proteins using genetically encoded inhibitors

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The goal of our project is to develop inhibitors against epigenetic proteins to understand their function in gene regulation and elucidate their potential as new drug targets. Currently, the lack of selective inhibitors is a major bottleneck to achieve this goal. Therefore, we are generating highly potent designer proteins called ‘monobodies’, based on fibronectin type III domain, against epigenetic targets. Monobodies have strong propensity to bind to functional “hot spots” including both clefts and planer protein-interaction surfaces. Furthermore, unlike antibodies, monobodies lack cysteines making their folding independent of disulphide bond formation. Hence, monobodies can be expressed as genetically encoded intracellular inhibitors in reducing cellular environments.

By performing combinatorial library selection using phage display and yeast surface display we have generated monobodies with high affinity and specificity against several epigenetic proteins, primarily histone readers and writers, many of which are considered challenging targets for small molecule inhibitor discovery. Currently, we have monobodies against the PR domains of PRDM proteins (lysine methyltransferase family), WDR5, EED, MBT domains and chromodomains of CHD proteins. Biochemical and structural characterization suggested that a majority of these monobodies act as selective inhibitors of their target proteins. We are investigating the effects of expressing these monobody inhibitors in cells. Our results show that monobodies have remarkable potential in controlling the function of epigenetic regulatory proteins.
Establishing the Erythroid Cell Transcriptome: Coregulator Matrix Model of GATA Factor Function differentiation.

Andrew DeVilbiss, University of Wisconsin–Madison

The establishment and maintenance of cell type-specific transcriptional programs require an ensemble of broadly expressed chromatin remodeling and modifying enzymes. Many questions remain unanswered regarding the contributions of these enzymes to specialized genetic networks that control critical processes such as lineage commitment and cellular differentiation. The transcription factor GATA-1 is a critical driver of erythroid development. GATA-1 functions with its coregulator, Friend-of-GATA-1 (FOG-1), to activate and repress genes required for the erythroid developmental program. As some GATA-1 targets have little or no FOG-1 requirement, presumably other trans-acting factors contribute to GATA-1 function at specific loci. Through a screen of prioritized chromatin modifying and remodeling enzymes, we identified a histone lysine methyltransferase, Pr-Set7/SetD8, as a critical GATA-1 corepressor. SetD8 is the sole enzyme known to monomethylate histone H4 at lysine 20 (H4K20me1). To interrogate SetD8 function in the context of erythroid cell biology, we utilized a genetic complementation system in GATA-1-null G1E cells, in which GATA-1 fused to an estrogen receptor ligand-binding domain (GATA-1) can be conditionally replaced at physiological levels. GATA-1 selectively induced H4K20me1 at repressed, but not activated, loci, and endogenous SetD8 mediated GATA-1-dependent repression of a cohort of its target genes. GATA-1 utilized different combinations of SetD8, FOG-1, and the FOG-1-interacting Nucleosome Remodeling and Deacetylase (NuRD) complex component Mi2β to repress distinct target genes. Additionally, studies are underway that demonstrate a balance between SetD8 and interacting chromatin modifying enzymes is necessary for primary murine erythroid maturation. Implicating SetD8 as a context-dependent GATA-1 corepressor expands the repertoire of coregulators mediating establishment/maintenance of the erythroid cell genetic network and provides a biological framework for dissecting the cell type-specific functions of this important coregulator. We propose a coregulator matrix model in which distinct combinations of epigenetic regulators are required at different GATA-1 target genes, and the unique attributes of the target loci mandate these combinations.

Differentiation-dependent changes in the epigenomic landscape of human myeloid leukemia cells

Ann Kirchmaier, Purdue University

The recently identified epigenetic mark 5-hydroxymethylcytosine (5hmC) is derived from 5-methylcytosine (5mC) by the action of members of the TET family of oxygenases. Enrichment of unmethylated cytosine (C) or 5mC at promoters is often associated with active or silent transcriptional states, respectively. However, 5hmC may also help define regulator signals controlling expression of genes critical for stem cell maintenance and cellular differentiation. To assess how DNA modification patterns are influenced by myelopoiesis, we have mapped regions enriched for 5mC or 5hmC throughout the genome during promyelocyte to granulocyte differentiation in the HL-60 cell model. Genome-wide differentiation state-dependent 5mC and 5hmC levels at promoters, gene bodies and intergenic regions have been compared to previous reports on gene expression and histone modifications and correlations between these data sets highlight co-regulated genes within multiple cellular pathways during differentiation. These findings are anticipated to provide new insights into myelopoiesis and to serve as an informative backdrop for epigenetic therapies based on differentiation strategies for treatment of myeloid malignancies.
Establishment and maintenance of euchromatic and heterochromatic domains contributes to modulation of gene expression. Euchromatin is believed to be a transcriptionally accessible state, while heterochromatin formation blocks gene expression. Euchromatic genes that are juxtaposed with heterochromatin by rearrangement or transposition are silenced in some of the cells in which they would normally be active, resulting in a variegating phenotype (PEV). However, genes that are normally resident in constitutive heterochromatin are still appropriately expressed. The fourth chromosome of Drosophila melanogaster, a heterochromatic domain (based on criteria such as chromatin modification marks and a lack of recombination), provides an ideal model in which to study this dichotomy of gene expression in the context of a repressive environment. To understand what features of fourth chromosome genes permit expression in the context of heterochromatin, we are utilizing transgenic MIMIC fly lines with insertion sites on the fourth to incorporate reporter constructs. We hypothesize that there are unique DNA signatures on the fourth chromosome that allow for gene expression despite the presence of inhibitory chromatin marks. Genes on the fourth often exhibit a characteristic chromatin pattern: the transcription start sites show an enrichment of H3K4me2/me3 and H3K9ac, whereas the body of the gene is enriched for the pericentric heterochromatin marks H3K9me2 and H3K9me3, as well as HP1a. By contrasting the expression pattern of hsp70-white (a euchromatic reporter gene) with that of a fourth chromosome gene at specific fourth chromosome MiMIC sites, we can determine which features allow for or inhibit expression of the transgene. We have currently investigated two MiMIC insertion sites for their ability to induce PEV, and plan to test other MiMIC sites on the fourth. This approach will begin to tease out what aspects of the fourth chromosome and its associated genes allows for expression in a nonpermissive chromatin state.

CHD proteins are ATP-dependent chromatin remodelers that can repress or activate gene expression. PICKLE (PKL) is a CHD remodeler in Arabidopsis thaliana that plays a critical role in repression of developmental identity genes. In particular, PKL is necessary for repression of seed genes after germination. However, a role for PKL in the developing seed has not been established. Here we show that plants lacking PKL exhibit a 60% reduction in seed set as well as delayed asynchronous embryo development. Interestingly, these seed phenotypes exhibit a parent-of-origin effect: loss of PKL in the maternal plant is sufficient to result in these phenotypes, but the paternal allele of PKL appears to be dispensable for seed development. We hypothesized that the delayed asynchronous embryo development observed in plants lacking maternal pkl is due to a delay in fertilization. Consistent with this hypothesis, aniline blue staining of pollen tubes reveals a defect in pollen tube development in pkl siliques. It was previously observed that PKL promotes both activation and repression of genes enriched for the epigenetic modification trimethylation of histone H3 lysine 27 (H3K27me3). Several transmittal tract genes are enriched for H3K27me3, and we are currently testing the hypothesis that PKL acts maternally to promote pollen tube development by promoting activation of these genes.

In the seed, H3K27me3 plays an important role in developmental processes including endosperm cellularization and gene imprinting. Interestingly, we have observed phenotypes characteristic of a defect in H3K27me3 in pkl seeds including abnormal embryo patterning, enlarged size, and susceptibility to paternal genomic excess. These findings suggest that PKL may maternally promote expression or repression of H3K27me3-enriched genes during seed development. Unfortunately, transcript analysis of H3K27me3-dependent genes in pkl seeds is confounded by the asynchronous embryo development phenotype. PICKLE RELATED2 (PKR2), a close homolog of PKL, is not required for synchronous embryo development and is preferentially expressed in seed endosperm. Preliminary data suggest that loss of PKR2 results in a failure to activate some H3K27me3-dependent genes in the seed. In addition, pkl pkr2 seeds are reduced in size relative to pkl seeds, suggesting that PKL and PKR2 may act antagonistically to determine expression states of some H3K27me3-enriched genes in seeds. We are currently investigating this hypothesis.
The chromatin remodeler chd5 promotes head development during embryogenesis of Danio rerio.

Brett Bishop, Kim Tyler, Amanda Smith, Sylvia Bonilla, Yuk Fai Leung, and Joe Ogas, Purdue University

CHD proteins are ATP-dependent chromatin remodelers that play critical roles in regulation of gene expression and developmental identity in eukaryotes. The CHD protein CHD5 has been shown to act as a tumor suppressor in humans and mice. Loss of CHD5 leads to the development of a variety of tumors, including tumors of breast and gastric tissue along with neuroblastoma, a common childhood cancer. Neuroblastoma arises during embryogenesis, suggesting that CHD5 plays a role during this critical phase of development. We are utilizing the zebrafish model system to examine the role of the vertebrate-specific chd5 chromatin remodeler during embryogenesis. In particular, we are examining the possibility that chd5 promotes neurogenesis and inhibits tumorigenesis in zebrafish based on prior characterization of CHD5 function in mammalian systems. Expression analysis reveals that chd5 is preferentially expressed in the brains of adult fish as previously observed in mammals. During early embryogenesis, however, chd5 is widely expressed throughout the developing fish prior to being restricted to the brain during subsequent stages of development. Loss of function studies reveal that chd5 plays a variety of roles during zebrafish embryogenesis. Transient knockdown of chd5 leads to an increase in apoptosis in the brain, eye and trunk of developing embryos. In addition, reduced expression of chd5 leads to defects in craniofacial development, including a decrease in head and eye size as well as defects in cartilage formation. We also observe that patterns of gene expression are altered in the developing brains of fish with reduced expression of chd5, which is consistent with prior identification of CHD5 as a factor that promotes neurogenesis in developing mouse embryos. Our analyses demonstrate that the chd5 remodeler promotes vertebrate embryogenesis and lays the foundation for using zebrafish to characterize the mechanism by which chd5 chromatin remodelers contribute to determination of developmental identity in vertebrate systems.

Possible Roles and Interactions Between the Nematode DRM Complex and Histone Modifications Under High Temperature Stress

Brian Mikeworth, Marquette University

All organisms face multiple environmental challenges in regards to maintaining homeostasis and fertility. It has recently been observed that members of the nematode DRM complex, such as lin-35 and lin-54, play a role in germline gene regulation in both somatic and germ cell lines. Our lab has been determined that loss of lin-35, the nematode homologue of mammalian Rb, leads to high-temperature L1 larval arrest phenotype (HTA) at 26°C but not at 20°C, the standard nematode culture temperature. HTA is due to the ectopic expression of germline genes in somatic tissues, which leads to a failure in intestinal tissue function and starvation (Petrella et al., 2010). While investigating changes in somatic cells of lin-35 mutants we discovered defects in the primordial germ cells (PGCs) at 26°C, including changes in histone modification enrichment patterns. By rescuing lin-35 in the intestine, lin-35 mutants are able to fully develop into adults. Although we were able to rescue the HTA phenotype, adult lin-35 mutants are sterile when grown at 26°C where wild type worms remain fertile. Therefore, it is possible that the adult germ line is dysfunctional due to changes in chromatin domains, which are known to be important for germ line function. To assess this hypothesis we are analyzing histone modification patterns in both lin-35 L1 PGCs and fully developed adult germ lines at low and high temperature using immunohistochemistry. By determining the role lin-35 plays in maintaining histone enrichment patterns in the germ line under temperature stress, we will gain a better understanding of how the DRM complex functions to maintain chromatin states and proper gene regulation.
Transcriptional regulation by Sox10 in myelinating glial cells

Camila Lopez-Anido, University of Wisconsin–Madison

In the nervous system, many axons are wrapped by a myelin sheath to maintain axonal integrity and speed nerve impulses. Myelin is formed by specialized myelinating glia: Schwann cells and oligodendrocytes in the peripheral and central nervous system, respectively. While there are distinct morphological aspects and regulatory pathways in these two cell types, myelination in both systems requires the transcriptional activator SRY (Sex-Determining Region Y)-box10 (SOX10), which is implicated in demyelinating and dysmyelinating diseases. SOX10 has been shown to interact with cell-type-specific transcription factors at some loci to induce myelin gene expression, but it is largely unknown how SOX10 transcriptional networks compare between Schwann cells and oligodendrocytes. Here, we used in vivo ChIP-Seq analysis of peripheral nerve (sciatic nerve) and spinal cord to identify shared and unique SOX10 binding sites and their correlation with transcriptional profiles between Schwann cells and oligodendrocytes. Through careful analysis of unique SOX10 regulatory binding sites, we elucidated how this critical regulator exhibits different functions in two similar, yet distinct, cell types. We found that cell-type-specific transcription factors overlap with unique SOX10 binding sites, and that these unique sites are linked to cell-type-specific gene expression. Interestingly, we found that shared myelin genes expressed in both Schwann cells and oligodendrocytes have distinct patterns of Sox10 binding in the two cell types. These data are consistent with previous analyses of regulatory elements that activate gene expression in transgenic experiments. Ultimately, these experiments help elucidate mechanisms that establish the cell type-specific genomic occupancy of SOX10, which define the unique characteristics of Schwann cell and oligodendrocyte differentiation.

Nucleosome acetylation sequencing to study the establishment of chromatin acetylation.

Chitvan Mittal, Iowa State University

The establishment of post-translational chromatin modifications is a major mechanism for regulating how genomic DNA is utilized. However, current in vitro chromatin assays do not monitor histone modifications at individual nucleosomes. Here we describe a strategy, nucleosome acetylation sequencing, that allows us to read the amount of modification at each nucleosome. In this approach, a bead-bound trinucleosome substrate is enzymatically acetylated with radiolabeled acetyl CoA by the SAGA complex from S. cerevisiae. The product is digested by restriction enzymes that cut at unique sites between the nucleosomes and then counted to quantify the extent of acetylation at each nucleosomal site. We find that our trinucleosomes synthesis strategy is highly modular and efficient and can be used to generate nucleosomal systems in which nucleosome composition differs across the array. We also show that we can sensitively, specifically, and reproducibly follow enzyme-mediated nucleosome acetylation. Applying this strategy, when acetylation proceeds extensively, its distribution across nucleosomes is relatively uniform. However, when substrates are used that contain nucleosomes mutated at the major sites of SAGA-mediated acetylation, or that are studied under initial rate conditions, changes in the acetylation distribution can be observed. Current studies are using this technique to determine how free linker DNA and transcriptional activators affect SAGA activity and acetylation distribution.
Bromodomain proteins comprise a functionally diverse family, and as numerous members have been implicated in disease, they represent an exciting new class of drug targets. The demonstrated success with specific inhibitors of the BET family of bromodomains has inspired continued screening of the larger non-BET family. Many of these however have added complexity by containing additional histone interacting or modifying regions such as PHD, HAT, and/or PWWP domains. Bromodomain inhibitor compounds have traditionally been identified in an in vitro system consisting of a purified bromodomain alone and modified histone peptides. In efforts to create a more physiological assay, we have utilized and optimized bioluminescence resonance energy transfer (BRET) to directly measure proteins binding to chromatin in living cells. With this approach, we demonstrate in vivo IC50s for bromodomain inhibitors can be calculated and potency differences between histones determined. More importantly, in live cell assays using non-BET family inhibitors against some of the more complex bromodomain proteins, we observe the ability to disrupt binding of the individual bromodomain alone, but not the full-length protein from chromatin. Further deletional studies reveal the domains contributing to the abrogation of inhibition when the compound is presented to the full-length protein. These data suggest that in certain cases, targeting of more than the bromodomain alone may be necessary for effective disruption of binding to chromatin, and in hope will help guide compound screening and development not only for bromodomain proteins, but also other chromatin readers which may be therapeutic targets.

Initially delineated as purely for packaging of the genome into the nucleus, it has been realized that histones play an active and integral role in regulating the underlying DNA. The post-translational modification of histones allows for dynamic regulation of chromatin structure and is essential in the regulation of all DNA templated processes. The acetylation of histone lysines has been shown to be one of the most prominent post-translational modifications on histones. Histone acetylation can directly modify chromatin structure or recruit co-factors through recognition by effector domains such as bromodomains. The activity of bromodomain proteins is thought to have an integral role in gene regulation. BRG1 is an ATPase that is the catalytic subunit of the ATP dependent switching/sucrose non-fermenting (SWI/SNF) chromatin remodeling complex. Its activity is essential for recruitment of the transcription machinery and it has been shown to be mutated in multiple cancer cell lines. BRG1 contains a C-terminal bromodomain, which is believed to be essential in specific recruitment of BRG1 to histone H4 acetylated at lysine 8 (H4K8ac). Interestingly, in vitro studies of BRG1 with acetylated peptides showed poor specificity and affinity, demonstrating little to no affinity for H4K8ac and weak affinity for the doubly modified H3K9acK14ac peptide. This suggests that there are additional factors that guide the BRG1 bromodomain to H4K8ac nucleosomes outside the acetylated histone tail. To elucidate the mechanism underlying the bromodomain facilitated recruitment of BRG1 to chromatin, we will utilize 2D-NMR to investigate the association of the BRG1 bromodomain with acetylated lysines in the context of the full nucleosome.
Cytosine DNA methylation is a mechanism of epigenetic gene regulation and genome defense conserved from bacteria to humans. In plants, de novo DNA methylation is established by DOMAINS REARRANGED METHYLTRANSFERASE2 (DRM2) via a pathway termed RNA-directed DNA methylation. The Arabidopsis genome also encodes an evolutionarily conserved but catalytically inactive methyltransferase DRM3 reported to be involved in DNA methylation. However, the molecular mechanism of DRM3 action and its relationship with other components in RNA-directed DNA methylation pathway remain to be elusive. Here, we analyzed the whole genome bisulfite sequencing data on drm3 and found that DRM3 acts as a general factor and has moderate effects on global DNA methylation and small RNA abundances. Unlike its mammalian homology Dnmt3L that interacts with the de novo DNA methyltransferase Dnmt3a, our biochemical purification revealed that DRM3 and a plant specific RNA Polymerase V (Pol V) co-purify with each other. We further showed that DRM3 is partially required for accumulation of two known Pol V-dependent noncoding RNA transcripts. Together, our results indicate that DRM3 controls DNA methylation through its functional interaction with Pol V and by regulating Pol V transcription activity. These findings suggest a mechanistic basis for selective Pol V targeting for gene silencing.

**Drosophila NIAM: A chromatin protein that negatively regulates cell proliferation**

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Regulation of cell proliferation is a key step in the initiation and management of cancer. In humans, the nuclear factor NIAM (Nuclear Interactor of ARF and Mdm2) associates with chromatin, maintains chromosome stability, and inhibits cellular proliferation independent of the tumor suppressor ARF. We identified CG31111 as a potential orthologue of NIAM and designed it dNIAM. Both human and fly proteins possess an N-terminal lysine-rich region and C-terminal phenylalanine/tyrosine-rich (FYRN/FYRC) domains, which are exclusively found in proteins that modify chromatin structure. Consistent with a role in chromatin structure, dNIAM localizes to sites of active transcription on polytene chromosomes. Knock-down of dNIAM in the developing eye causes over-proliferation of eye tissue, demonstrating conservation of the anti-proliferation function. We are in the process of testing for genetic interactions between dNIAM and chromatin remodeling machines.

**MicroRNA Changes associated with Epithelial to Mesenchymal Transition (EMT) in breast cancer**

Elizabeth Peterson, University of North Dakota

Two highly related transcription factors, Snail and Slug, regulate the expression of genes that are essential for a process called Epithelial to Mesenchymal Transitions, or EMT. Snail and Slug repress genes by associating with gene promoters and inhibiting their transcription. Snail and Slug therefore cause cells to change their shape and migrate by repressing genes such as E-cadherin, which are cell adhesion proteins. Our previously published microarray data identified several genes that are upregulated following Snail and Slug expression, suggesting that they might also activate genes. One possible way that Snail and Slug function as activators of genes (i.e. indirect activation) is that they could repress the expression of microRNAs that in turn repress these genes. Recent work by many authors has implicated small RNAs, mainly microRNAs, in breast cancer, and specifically in metastasis of breast cancer. To test whether Snail and Slug can cause changes in microRNA expression, we used Qiagen microRNA PCR arrays to test a small subset of microRNAs for changes in expression. Our preliminary data suggests that Snail and Slug expression result in changes in levels of a few microRNAs that are important in breast cancer. We now propose to use small RNA-sequencing to determine global changes in microRNAs induced by Snail and Slug that directly lead to EMT, which will help identify new targets in breast cancer.
Epigenetic silencing of repetitive DNA sequences is critical for the maintenance of genome integrity. Silencing of proviral sequences in somatic and germ-line lineages requires cytosine methylation, whereas silencing in embryonic stem cells requires post-translational modifications of histones H3 and H4. The loss of silencing and aberrant overexpression of some repetitive elements is implicated in tumorigenesis. Human genes ATRX and DAXX, which encode for proteins involved in the specific deposition of histone variant H3.3 into nucleosomes, are implicated as tumor suppressors in several human cancers. In preliminary experiments, we found that Daxx co-immunoprecipitates with histone methyltransferase activity from human cell nuclear extract. We used affinity and conventional chromatography to purify a complex that contains several proteins including Daxx and SETDB1, a histone lysine methyltransferase. Previous work demonstrated that SETDB1 is involved in endogenous retroviruses silencing in mouse embryonic stem cells through catalysis of H3K9me3. Furthermore, analysis of RNA sequencing data from pancreatic neuroendocrine tumors with mutations in DAXX revealed an upregulation of endogenous retroviruses. These data suggest that a Daxx- and SETDB1-containing complex is required for proviral silencing in some cell lineages, possibly through a combination of H3.3-deposition and H3K9 methylation.

The development of chromatin remodeling inhibitors to investigate and treat a wide spectrum of human cancers

Emily Dykhuizen, Purdue University

Recent exome sequencing of human tumors has uncovered a surprising new role for chromatin-associated processes in the development of cancer. Of particular note is the frequent identification of mutations in one or more subunit of the SWI/SNF (or BAF) chromatin remodeling complex. For some of these cancers, it has been found that the residual complex is actually oncogenic, suggesting that the BAF complex itself is a good therapeutic target. Several additional lines of data suggest that inhibition of chromatin remodeling complexes could be therapeutic; however no inhibitor of an ATP-dependent chromatin remodeler has yet been identified. To this end, we have performed a large, high throughput screen monitoring BAF mediated transcription. After further validation, we have identified a handful of compounds that regulate transcription in a manner similar to the BAF knockout. In order to confirm that the compounds act directly on the BAF complex, we are synthesizing focused small molecule libraries for the optimization of target identification probes. While some of the compounds may not inhibit the BAF complex directly, deciphering their targets will give us great insight into the mechanism of BAF-mediated transcription, and possibly BAF-mediated tumor suppression. With a BAF inhibitor in hand, we will have the tools for understanding the tumor suppressive mechanism of the BAF complex, as well as confirming the therapeutic potential of targeting this class of epigenetic regulators in cancer.
The chromatin-based regulatory mechanisms that underwrite gene expression play a critical role in determination and maintenance of developmental identity. CHD5 is a vertebrate-specific ATP dependent chromatin remodeler that has been demonstrated to play a major role in tumor suppression in mammals. Loss of CHD5 has been implicated in the formation or progression of numerous human cancers including, neuroblastoma, leukemia, lymphoma, colorectal cancer, and lung cancer. Neuroblastoma arises during embryogenesis, raising the prospect that CHD5 may play one more roles during this critical stage of development. We used morpholinos to transiently knock down chd5 in zebrafish and observed that the resulting embryos exhibited abnormal head development, including small eyes and heads as well as increased apoptosis in the brain. In order to further characterize the role of chd5 in zebrafish development, we have generated transgenic zebrafish that overexpress a dominant negative version of chd5. Specifically, we have used the transposon based Tol2 kit to generate transgenic lines of zebrafish carrying a dominant negative version of chd5 under the control of the inducible heat shock promoter. In addition, we are using these lines to verify the morpholino-induced phenotype as well as to examine when chd5 is necessary to promote head development. These lines will allow us to examine whether chd5 knockdown results in tumor phenotypes in zebrafish and thereby establish whether zebrafish is a suitable model system for elucidating the mechanism by which CHD5 remodelers suppress tumor formation.

The human RNA polymerase II-associated factor complex (hPAFc) has been shown to regulate transcription elongation and transcription-coupled histone modifications. The entire hPAFc and its individual subunits have been implicated in human diseases and cancers. In line with its role in transcriptional regulation, our lab had previously shown that hPAFc recognizes the activated histone mark ‘H3R17me2’ and regulates transcription of estrogen receptor (ER)-target genes in breast cancer cells. These findings prompt us to further investigate the functional roles of hPAFc, especially the key scaffold subunit Ctr9, in breast cancer. By analyzing the published microarray datasets, we found that Ctr9 levels positively correlate with ER in human breast tumors. This is consistent with our finding that Ctr9 expression is higher in ER+ breast cancer cell lines as compared with ER- cells. The expression of none of the subunits in hPAFc is directly responsive to estrogen treatment. However, Ctr9 regulates ER protein stability through post-transcriptional mechanism, implying that Ctr9 could have significant function in estrogen signaling. Using Ctr9 inducible knock-down cell line, we observed that knockdown of Ctr9 led to apparent morphology change, decrease of proliferation, and reduced colony formation in ER+ MCF7 cells. In contrast, neither knockdown nor overexpression of Ctr9 affected ERα-MDA-MB-231 cells. Moreover, we demonstrated that knockdown of Ctr9 significantly decreased the expression of oncogenic MUC1 which is known to stabilize ER protein and overexpressed in ERα+ breast cancer cell lines and tumors. Collectively, our study reveals a novel role for Ctr9, and probably the entire hPAFc, in regulating MUC1 oncogene transcription and driving ER+ breast cancer cell growth. Future work will elucidate the mechanism by which Ctr9 regulates MUC1 transcriptionally in ER+ breast cancer cells. This study is supported by Department of Defense ERA of Hope Scholar Award W81XWHY-11-1-0237 to W.X.
Epigenetic regulation induces alterations of gene expression by modifying chromatin structure. Perturbations of the epigenetic machinery often lead to the deregulation of cardiac gene expression, resulting in defective cardiac development and cardiac hypertrophy. Histone tails can be methylated, which are recognized as a marker for transcriptional activation or repression. However, the regulatory roles of histone methylation status on gene expression remain to be elucidated. Due to a groundbreaking discovery of histone demethylases such as Jumonji (Jmj) family factors, histone methylation is now considered as a reversible epigenetic mark. Jarid2/Jumonji is the founding member of the JMJ factor family that functions as histone demethylases. Interestingly, Jarid2 is enzymatically inactive but functions as a transcriptional regulator. Jarid2 critically regulates developmental processes including cardiovascular development as well as ES cell differentiation. Jarid2 knockout (Jarid2 KO) mice exhibit cardiac defects including hyper-trabeculation with noncompaction of the ventricular wall. Therefore, we set out to determine molecular mechanisms of Jarid2 critical for cardiac development and ES cell pluripotency/differentiation. To identify the cardiac-specific role of Jarid2, we generated deletion of Jarid2 in early cardiac progenitors using Nkx2.5-Cre Knock-in mice. Mice with a deletion of Jarid2 in cardiac progenitors (Jarid2Nkx-KI) recapitulate partial phenotypic defects observed in whole body mutants (Jarid2 KO) including hyper-trabeculation, thin myocardium and ventricular septal defects. We have identified a group of genes that are occupied by Jarid2, SETDB1, H3K9me3 or H3K27me3, and upregulated in Jarid2 mutant hearts by overlapping ChIP-chip and microarray analyses, which are potential transcriptional targets of Jarid2. 172 genes including Bmp10 are identified as dysregulated genes and are occupied by Jarid2, SETDB1 and H3K9me3. 174 genes including Isl1 are identified as dysregulated genes that show accumulation of Jarid2 and H3K27me3. Among those, we have confirmed that Isl1, Bmp10 and Ifgbp2 are upregulated in Jarid2Nkx-KI hearts by qRT-PCR. Also, increased levels of Isl1 and p-Smad1/5/8, which is a downstream target of Bmp10, were detected in Jarid2Nkx-KI hearts.

Islet1 (Isl1) is a LIM-homeodomain transcription factor, which plays crucial roles in early cardiac development and a marker for cardiac progenitors. Jarid2 occupancy was shown at the Isl1 promoter region by ChIP asays, and the occupancy was reduced in Jarid2Nkx-KI compared to control hearts. Jarid2 is also required for proper ES cell differentiation as well as efficient generation of iPS cells. Although Jarid2 interacts with Polycomb Repressor Complex (PRC) in ES cells, the precise function of Jarid2 remains to be determined. We are currently investigating epigenetic roles of Jarid2 via functional interactions with other JMJ factors in cultured cells. All together, our data suggest that Jarid2 regulates target gene expression by interacting with different histone modifiers depending on the cell/promoter context.

Biochemical activities of the plant RNA silencing enzymes, RNA Polymerases IV and V

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RNA polymerases (Pols) IV and V are Pol II-like enzymes that mediate small RNA dependent DNA methylation (RdDM) and transcriptional gene silencing (TGS) of transposons and repeated sequences in plants. During the process of RdDM, Pol IV transcripts generated at target loci are converted into small-interfering RNAs (siRNAs) that associate with longer noncoding RNAs generated by Pol V, and a myriad of other factors, leading to TGS at that locus. Although studied extensively using genetic approaches, very little is known about the biochemical activities of most of the proteins involved in the RdDM pathway, including Pols IV and V. Our lab recently showed that affinity purified Pols IV and V complexes have biochemical activity in-vitro, despite numerous amino acid substitutions at positions that are highly conserved in all other canonical RNA polymerases. Surprisingly, their activity requires an RNA primer. Pols IV and V differ with respect to their ability to displace a downstream non-template DNA strand during transcription. Here we report our efforts to further characterize the RNA primer and template requirements of Pols IV and V.
HP1 Isoforms Are Elongated Molecules with Two Globular Domains Joined by a Flexible Linker, Which Endows Dynamic Flexibility

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Heterochromatin protein 1 (HP1) family members (HP1 α, β, and γ) are non-histone chromatin proteins, which show exquisite primary structure conservation, since they are needed by most eukaryotes for their function. In fact, due to their essential role in assembling heterochromatin, HP1 proteins are key regulators of genome function in health and disease. While several high-resolution structures of the two globular regions of HP1, the chromo- and chromoshadow domains, have been solved, little is currently known about the conformational behavior of the full-length protein. Here, we used threading, homology-based molecular modeling, molecular mechanics calculations, and molecular dynamics simulations for characterizing the dynamic and structural properties of the three full-length human isoforms. We show that the linker region between the N-terminal chromodomain and C-terminal chromoshadow domain contains bipartite nuclear localization signals (NLS) and is highly flexible, endowing these proteins with unrestricted 3D localization of the two functionally competent globular domains to bind chromatin and other protein partners. Our results also provide insights into how HP1 proteins may functionally link nucleosomes during the process of heterochromatin formation. Lastly, the structural models resulting from this work may aid the rational design of drugs to either activate or inactivate these proteins. This is important since recent evidence demonstrates a key role for these epigenetic regulators in the pathobiology of several human cancers. Thus, the results of the current study extend our current understanding of fundamental epigenetic mechanisms, as well as have potential biomedical relevance.

Megabase-scale DNA hypermethylation correlates with chromosome-specific rRNA gene silencing in Arabidopsis

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45S ribosomal RNA (rRNA) genes in eukaryotes are tandemly repeated at chromosomal loci known as nucleolus organizer regions (NORs). RNA polymerase I (Pol I) transcribes rRNA genes to produce 45S pre-rRNA transcripts that are then processed into 18S, 5.8S, and 25S rRNAs that form the catalytic core of ribosomes, the cellular machines that carry out protein synthesis. There are hundreds of rRNA genes present in eukaryotic genomes, but only a subset are active in most cells, a form of ‘dosage control’ that involves changes in DNA methylation and post-translational histone modifications.

Ribosomal RNA genes in Arabidopsis thaliana are organized into two NORs (NOR2 on chromosome 2 and NOR4 on chromosome 4), with each NOR consisting of 350-400 gene copies. Whereas the 18S, 5.8S, and 25S rRNA sequences are identical among these genes, some variation exists in the internal transcribed spacer (ITS) and the external transcribed spacer (ETS) regions. We have mapped four distinct gene variants that differ in their 3’ ETS regions to either NOR2 or NOR4. As a result of dosage control, we find that the rRNA gene variants that map to NOR2 are selectively silenced during development. DNA methylation, histone methylation and deacetylation have been shown to play a role in this epigenetic phenomenon. Using two-dimensional contour-clamped homogeneous electric field (CHEF) gel electrophoresis and methylation-sensitive restriction digestion analyses we show that hypermethylation of silent rRNA genes at NOR2, and hypomethylation of the active rRNA variants at NOR4 occurs on a megabase-scale. We conclude that 45S rRNA regulation in Arabidopsis occurs at a sub-chromosomal level, affecting entire NORs, and not gene by gene.
A Novel Role for the CSN Signalosome in C. elegans Meiosis

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Studies have shown up to 30% of human oocytes are aneuploid and this aneuploidy is the most common cause of birth defects and miscarriages in human reproduction. The synaptonemal complex (SC) is a conserved, protein structure holding homologous chromosome pairs together throughout most of meiotic prophase I. Furthermore, it is essential to the formation of crossovers which allows for the proper segregation of chromosomes into gametes. Little is known about the molecular mechanisms regulating SC assembly. Here, we examine the role of the CSN signalosome in SC assembly in the model organism C. elegans. The CSN signalosome has been shown as a key regulator of the ubiquitin degradation pathway. It also interacts with several meiotic proteins specific to C elegans including: KGB-1 and GLH-1, p-granule proteins; CUL-3 and MEI-1, proteins involved in meiosis to mitosis transition; and MEL-26 which control mitotic spindles in early embryogenesis. Our work shows that CSN mutants exhibit meiotic prophase I defects: decrease in stabilization of pairing interactions between homologous chromosomes, aberrant SC assembly, and decreased crossover formation. We also observe an increase in DNA damage and a decrease in repair of the damage. Furthermore, we also find a marked increase in apoptosis in CSN mutants due to the perturbation of the aforementioned prophase I events which likely contributes to the reduction in maturing oocytes and sterility. To conclude, our work indicates a novel role for the CSN signalosome in chromosome behavior during meiotic prophase I.
Dynamic regulation of distal regulatory elements by Egr2 and Sox10 in Schwann cell myelination and injury

Holly Hung, University of Wisconsin–Madison

The kinetics of histone mark use on active enhancers underlie and help to mediate dynamic changes of gene expression as cells respond to a rapidly changing environment. During nerve injury, Schwann cells show a stem cell-like plasticity in its rapid and reversible transitions between immature proliferating and mature myelination states. Moreover, recent analysis has elaborated specific injury-induced pathways in Schwann cells that promote survival and regeneration of axons. Although regulation of this process has important implications for the successful regeneration of axons, enhancer responses to nerve injury have not been studied on a genome-wide scale. Here, we used ChIP-seq to measure changes in H3K27ac, a mark of active enhancers, to explore the activation of enhancers in myelinating Schwann cells, the changes that occur during demyelination induced by nerve injury, and regulation of the transcription factor network that bind these sites. Sites with dynamic H3K27ac loss are enriched for binding sites of Egr2 (Early Growth Response 2) and Sox10 (SRY (sex determining region Y)-box 10), critical transcriptional regulators of Schwann cell myelination and identity. We show that Sox10 and Egr2 are important factors for defining enhancers controlling myelin gene expression and apoptosis. While Schwann cell dedifferentiation reactivates some properties of its neural crest origins, differential analysis of active enhancers used by Schwann cells in nerve injury and neural crest cells show the reactivated enhancers are associated with genes controlling cell migration and cytoskeleton component expression. This study uses global epigenetic changes in H3K27ac deposition to expand our knowledge of the key transcriptional players that control Schwann cell myelination and play an important role in peripheral nervous system responses to nerve injury.

Characterization of the Chromatin Landscape by Mass Spectrometry—Histone Modifications and Beyond

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Chromatin is a fundamental regulator of gene expression. Understanding the highly dynamic nature of chromatin structure is central to our understanding of epigenetics and ultimately cellular physiology. Chromatin consists of DNA wrapped around core histones. Chemical modifications to these histones regulate local chromatin structure and control transcriptional repression and activation. With the advent of high resolution, high mass accuracy mass spectrometry, the global analysis of histone modifications has become possible. These profiling experiments have led to a more fundamental understanding of how histone modifications impact chromatin structure and ultimately gene expression. These modifications are highly regulated and under the control of a number of agents, including kinases, phosphatases, methyltransferases, methylases, acetyltransferases, and deacetylases, as well as large number of adapter proteins and co-regulators. Understanding the trafficking of these chromatin-associated proteins “on” and “off” the chromatin is essential to extend our understanding of chromatin dynamics. To this end, we have developed novel protocols to simultaneously profile histone modifications as well as the trafficking of chromatin-associated protein complexes. These methods utilize classic nuclear fractionation followed by chemical crosslinking, salt extraction, and analysis by mass spectrometry. Using these profiling techniques, we have built an index of chromatin-associated proteins and histone modifications in cell lines, primary cell culture, and animal tissues, each of which displays a unique signature of histone modifications and chromatin-associated proteins. Using these methods, we have built a preliminary profile of the chromatin landscape that encompasses both histone modifications and the trafficking of protein complexes “on” and “off” chromatin. In the future, we plan to apply this methodology to compare differences in biological conditions and to elucidate physiologically relevant mechanisms of chromatin regulation.
Trimethylated histone H3 lysine 36 (H3K36me3) is predominantly associated with transcribed regions in the genome. Alterations of H3K36me3 distribution are linked to defects in pre-mRNA splicing and DNA mismatch repair. Loss-of-function mutations in SETD2, a H3K36 methyltransferase, have been identified in multiple malignancies including clear cell renal cell carcinoma (ccRCC), which lead to a significant reduction of H3K36me3 level. In order to determine which genes are associated with changes in H3K36me3 occupancy, we performed ChIP sequencing on patient-derived nephrectomy tissue and RCC cell lines. Methods: H3K36me3 ChIP libraries were generated from 12 SETD2-wild-type samples, including three RCC patient tumor samples, their matched normal samples, as well as six RCC cell lines. ChIP DNA was sequenced to 51 base pairs from both ends using the Illumina HiSeq 2000, and the H3K36me3 binding sites were identified using SICER from BWA-mapped reads. To identify the genome-wide distribution of H3K36me3 occupancy, we retrieved the human refGene annotation from UCSC table browser and intersected with the locations of H3K36me3 binding sites. Number of reads mapping to each peak region and to the corresponding region in the input was normalized to a library size of 10 million (FPTM); the input-subtracted FPTM values were used for differential binding analysis. Results: We generated 11.7-48.6 M pairs of reads for each IP and input library. Between 20,861 and 34,674 H3K36me3 binding sites were identified (FDR<=1e-5), covering 10-17% of the mappable regions of the genome. Clustering based on the normalized read count in peak regions identified three distinct clusters corresponding to tumor samples, matched normal samples and cell lines, respectively. As expected, ~90% of the binding sites were identified in gene bodies, with the majority (>80%) of the bound regions representing introns. Further analysis revealed increased peak coverage towards the 3’ end of genes, where 47.5% of the 3’UTRs and 33.5% of the 1kb downstream regions were covered by peaks. In contrast, only 25.1% of the 5’UTRs and 7.6% of the 1kb upstream regions contained significant peaks. About 70% of the 23,298 genes showed H3K36me3 binding in at least one of the 12 libraries, including 7,709 H3K36me3-bound genes identified in all libraries. We identified 440, 558 and 946 H3K36me3-bound genes that were unique to tumor samples, matched normal samples and cell lines, respectively. Comparison across the three tumor-normal pairs identified 105 genes with H3K36me3 binding exclusively in the three tumor samples and 262 genes in the three normal samples. In addition, we identified 2,014-10,285 differential binding sites from each of the three tumor-normal pairs. In two of the comparisons, there were over six times more genes that showed increased binding (>=2-fold) in the tumor samples, while in the third pair, there were about two times more genes with increased binding in the normal sample. Conclusions: Our findings demonstrate distinct H3K36me3 binding profiles in kidney tumors, matched normal tissues and cell lines. We also identified a subset of genes that were enriched with H3K36me3 binding either in kidney tumors or in normal samples. Support: Mayo Clinic Center for Individualized Medicine.
In mammals, DNA methylation is established during early embryogenesis largely by two de novo DNA DNA methyltransferases (MTases), Dnmt3a, and 3b and is maintained semi-conservatively through replication by maintenance MTase Dnmt1. The DNA methylation pattern is regulated by changes in the expression and/or catalytic activity of DNA MTases, which is required for a balanced gene expression. My research, which has largely focused on understanding the mechanisms of DNA methylation, initially involved studies of the mechanism of action of the DNA MTases, and then extended into questions concerning regulation of DNA methylation by changes in gene expression, transcription elongation and splicing.

During my postdoctoral research, we discovered Vezf1 as an insulator binding protein that mediates the barrier function of the chicken β- globin HS4 insulator element by protecting against DNA methylation. This property of Vezf1 binding sites was recapitulated when engineered in aprt CpG island thus confirming their function in protective pathways against DNA methylation (PLoS Genetics Jan 2010). To investigate the mechanism of Vezf1 action, we studied patterns of gene expression and DNA methylation in Vezf1-/- mouse ES cells. We found reduced expression of the full length enzymatically active isoform of Dnmt3b Mtase that led to loss of DNA methylation at many sites throughout the genome. Gene expression analysis revealed changes in the ratio of expressed Dnmt3b isoforms in Vezf1-/- ES cells. The fact that we could identify Vezf1 binding sites near alternatively spliced exons in the Dnmt3b gene (Gowher et al, Genes & Development 2008) suggested some involvement of Vezf1 in the splicing mechanism.

Further investigation of elongating RNA polymerase II revealed that the polymerase is paused at Vezf1 binding sites in the Dnmt3b gene Pausing is known to modulate alternative splicing, consistent with our observations in Vezf1-/- cells. In an extension of this work, our recent data show high correlation between paused Ser2P RNA Pol II and Vezf1 binding sites genome-wide in HeLa cells. To bring the above two sets of observations together, we speculate that the insulator function of Vezf1 is related to its ability to block the advance of Pol II, interfering with a known mechanism for propagating heterochromatic structures. Thus, depending on its genomic localization, the ability of Vezf1 to modulate Pol II action could have diverse effect on transcriptional output and regulatory pathways (PNAS Jan 2012).

Most recent studies in my lab are investigating the mechanism by which Vezf1 functions. We are also investigating molecular mechanisms that regulate the target site specificity and gene expression/splicing of Dnmt3a and 3b. Experiments are designed to identify regulatory mechanisms that govern target site specificity of Dnmt3a or 3b including changes in interacting factors (proteins, non-coding RNA or modifications of histone tails). The long-term goal of our lab is to contribute to a delineation of the underlying epigenetic mechanisms by which normal pathways of cell differentiation and maintenance of cellular homeostasis are disrupted in various human diseases.

In the plant, Arabidopsis thaliana, de novo cytosine methylation is established through the RNA directed DNA methylation (RdDM) pathway. Key steps in this pathway are mediated by two plant specific, multi-subunit RNA polymerases: Pol IV and Pol V. Pol IV is thought to transcribe precursor transcripts for 24nt siRNAs that can be loaded into the argonaute protein, AGO4. Pol V transcribes IncRNAs that can be bound by siRNA-loaded AGO4 at chromatin loci targeted for silencing. AGO4-IncRNA binding then mediates the recruitment of downstream factors that result in DNA methylation and heterochromatin formation. As central components of heterochromatin formation in plants, Pol V produced IncRNAs are currently poorly characterized. In this study, we address this issue by defining complete Pol V transcription units, characterizing IncRNA 5’ and 3’ end modifications, and determining how enzymatic activities of other RdDM pathway components affect transcript length.
Nonsense Suppression to Generate and Utilize Histone H3 Containing Multiple Lysine Acetylations

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In eukaryotic organisms, post-translational modification of histones is a major mechanism for regulating how genomic DNA is utilized. In vitro reconstitution of chromatin model systems can provide a powerful tool for understanding how these modifications affect chromatin structure and function. However, beyond making those histones which contain a single post-translational modification, available techniques for generating multiply modified histones are often either ineffective or arduous. Here we describe our efforts to expand a previously demonstrated nonsense suppression strategy in order to generate H3 histones homogeneously acetylated on lysines: 9, 14, 18, and 23. These marks are usually associated with actively transcribed genes. We show that high levels of exogenous N-ε-Acetyl-L-Lysine are necessary for efficient expression of highly acetylated H3 histone. This finding, coupled with a new purification strategy, allowed us to generate sufficient quantities of pure protein for incorporation into nucleosomal systems. To determine how much lysine acetylation marks on H3 may affect chromatin stability, we are developing a single molecule stability assay using total internal reflection fluorescence microscopy to follow the disassembly of individual fluorescently labeled nucleosomes in real time. To see if lysine acetylation marks on H3 might recruit enzymes to neighboring nucleosomes, we have developed an on-bead di-nucleosome acetylation kinetics assay and are testing how acetylated H3 in one nucleosome will affect enzyme-mediated acetylation on neighboring nucleosomes.

Polyamine mediates sequence- and methylation-dependent chromatin compaction

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All cellular activities, including cell development and differentiation, are consequences of gene regulations. In the conventional view, transcription factors directly regulate gene expressions by activating or repressing the binding of RNA polymerase to specific target genes. However, a more general and global mechanism of gene regulations based on compaction level of chromatin is becoming accepted thanks to high-throughput and high-resolution experimental techniques revealing the chromatin conformations in molecular details. For example, AT-rich heterochromatin segments of human genome are known to cluster one another forming compact spatial topologically associated domains (TADs) whereas GC-rich euchromatin segments form separate relatively less compact TADs. More surprisingly, high level of DNA methylation results in compaction of a specific locus or an entire chromatin, enabling reversible regulation of gene expressions. Although the correlation between the chromatin compaction and genomic sequence (GC and methylation contents) is well established, the underlying principle is poorly understood. Here, we demonstrate that the differential compaction of DNA by sequence can occur only in the presence of sub-mM polyamine using highly optimized computer simulations and novel single-molecule techniques, suggesting that a rather simple physical principle of polyamine-mediated DNA-DNA attractions might govern the global chromatin compaction in the cells. Consistent to the chromatin compaction in vivo, we find that polyamine-mediated DNA-DNA attraction is significantly stronger for AT-rich and methylation-rich DNA segments than GC-rich segments. Further, we show that this sequence-dependent DNA attraction originates from the DNA structure encoded by sequence in atomistic details.

Activation of SIRT 6 by endogenous small molecules

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Sirtuins are a family of NAD+ dependent protein deacylases that are evolutionarily conserved from bacteria to eukaryotes. SIRT6 functions in genomic and metabolic stability and has been reported to harbor NAD+ dependent deacetylase activity towards the H3K9Ac and H3K56Ac histone marks. Utilizing biochemical and kinetic analyses, we previously demonstrated that SIRT6-dependent histone deacetylation produces O-acyl-ADP-ribose in vitro, but at a rate ~1,000 times slower than highly active sirtuins. Structural analysis led us to hypothesize that binding of an activator might stimulate SIRT6 deacetylase activity in vivo. We have identified a number of non-esterified fatty acids, including ω-3 and ω-6 fatty acids that might stimulate SIRT6 under certain metabolic conditions. These observations are the first to describe endogenous small-molecule activators of sirtuins that display low basal deacetylase activity.
The methylcytosine dioxygenase Ten-Eleven Translocase-2 (Tet2) enables elevated GnRH gene expression and the typical development and maintenance of reproductive function.

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Puberty and reproductive function depend on the elevation of gonadotropin releasing hormone (GnRH) neuron activity. We previously found that increased activity of GnRH neurons is accompanied by epigenetic changes (Endocrinology 151:5395, 2010). Specifically, during neuronal maturation, GnRH mRNA levels rise while cytosine residues within the GnRH gene promoter are actively demethylated. Whether active DNA demethylation itself has an impact on neuron development and consequently reproductive function remains unknown. In these studies we aimed to determine whether Tet enzymes, which initiate the process of active DNA demethylation, influence neuronal function and reproduction. First, examining two GnRH neuronal cell lines with immature (GN11) and mature (GT1-7) neuronal characteristics, we found that expression of Tet1 and Tet2 is drastically lower in GN11 compared to the GT1-7 cells. Overexpression of Tet2 in GN11 cells increased GnRH mRNA levels and elevated mean GnRH peptide release in dynamic cultures. While glutamate alone had no impact, exposure of GN11 cells overexpressing Tet2 to glutamate increased GnRH mRNA to levels consistent with mature GT1-7 cells. Second, to evaluate the impact of Tet2 in vivo, we generated two separate sets of mice with selective disruption of Tet2 activity in GnRH (gTKO mice) and kisspeptin (kTKO) neurons. We found minimal impact on the timing of puberty in gTKO mice, perhaps due to developmental activation of the GnRH gene prior to Tet2 disruption. Notably, plasma lutenizing hormone (LH) levels were significantly lower in both male and female adult gTKO compared to WT animals, suggesting Tet2 might be necessary for the maintenance of GnRH neuron function in adulthood. In contrast, disruption of Tet2 in kisspeptin neurons had a more distinguishable influence on pubertal timing in male and female mice. Vaginal opening and regular estrus cycling was significantly delayed in kTKO females and the pubertal rise in plasma LH lagged for both male and female mice compared to WT littermates. Abnormal fecundity was not observed in female kTKO mice, however, for male kTKO animals the interval between pairing with a fertile female and litter birth was significantly longer than for WT males. Together, these studies implicate Tet2 as a mediator of epigenetic control over neuronal maturation and reproductive function. They further suggest that Tet2 activity is necessary for the maintenance of neuroendocrine control of gonadotropin release.

Selective Bioluminogenic HDAC Activity Assays for Profiling HDAC Inhibitors

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Histone deacetylases (HDACs) play critical roles in the regulation of gene transcription and cell signaling events by deacetylating histones and other important non-histone substrates. Aberrant increases in HDAC enzyme activities are therefore implicated in a number of human infirmities, including cancers, metabolic disease and neurodegeneration. Fortunately, HDAC enzymes represent attractive pharmacological targets because they are readily tractable with small molecule inhibitors. In fact, several HDAC inhibitors (HDACi) have recently proceeded through (or are near) the FDA approval process for the treatment of hematologic malignancies. However, the promise of clinical HDACi therapy has been hampered by significant dose-limiting toxicities. These off-target effects have led to a renewed focus on basic HDAC biology and the development of isoenzyme-specific HDAC inhibitors which could avoid off-target effects. To help facilitate the discovery of compounds with better defined selectivity profiles, we have developed lysine deacetylase assays that selectively measure specific isoenzyme activities in cells, extracts, or purified recombinant preparations. These assays are based on substrates that are selective due to a combination of extended peptide sequence and novel chemical modifications. Deacetylase activity is measured by delivering a single, pro-luminogenic, homogeneous assay reagent to assay wells, resulting in luminescence proportional to HDAC activity. In addition to being isoenzyme selective, these novel substrates are cell permeable allowing for lytic and non-lytic cell-based HDAC assays. Lastly, these assays are also fully compatible with fluorescent viability and/or cytotoxicity assays. This provides additional flexibility for multiplexed formats which examine not only selective HDAC inhibition, but the functional consequences they exert on cell health.
Detection of JumonjiC Domain-containing Histone Demethylase activities with homogenous bioluminescent succinate detection assay

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JumonjiC domain-containing histone lysine demethylases (JMJs) play a pivotal role in determining the epigenetic status of the genome by counteracting the activities of histone lysine methyltransferases. These enzymes act as erasers by catalyzing the removal of methyl marks from specific lysine sites in histones, leading to either transcriptional repression or activation of target genes. JMJs are widely studied and because of their implication in cancer, they have become validated drug targets. Thus, assays that monitor JMJC activities are desirable in order to elucidate their mode of regulation, as well as to facilitate the identification of selective and potent inhibitors for drug discovery and as basic research tools. Traditional assays for JMJs are not easily configured for rapid demethylase activity detection because they rely on the use of colorimetric or fluorimetric non-homogenous antibody based assays. Since the conversion of 2-oxoglutarate to succinate is a required step for the activity of these enzymes, an assay that detects succinate as a universal product of these reactions would be suitable for monitoring all JMJC activities as well as other succinate-forming enzymes (i.e.: dioxygenases). We therefore developed a bioluminescent and homogenous succinate detection assay for measuring JMJC activity. The assay is performed in a two-step format that relies on converting the succinate product to ATP, then to light in a robust luciferase reaction. The light output is proportional to succinate concentration from low nM to 20μM. The assay is highly sensitive and robust, two features that are highly desirable and essential for measuring the activity of the majority of JMJC demethylase subfamilies. Therefore, the succinate detection assay is a simple-to-use method that allows significant savings of enzyme usage and does not require antibodies or modified substrates. Examples of various applications of this succinate detection assay will be presented, including studies on specificity of different substrates by diverse JMJs. The development of this succinate detection assay will make it possible to investigate a large number of JMJC demethylases and could have significant impact on diverse areas of Epigenetics research.

Yeast histone demethylase Jhd2 mediates a conserved cross-talk between H3K4 methylation and H3K14 acetylation

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Gene expression has been shown to be regulated through epigenetic modifications to the N-terminal tail of histones. Among these modifications is methylation of lysine residues. In budding yeast the primary sites of methylation are lysines 4, 36, and 79 on histone H3. The enzyme Jhd2 is a histone demethylase that functions to remove H3K4 methylation in S. cerevisiae. Jhd2 is a homologue of the human JARID1 family of histone demethylases, which has four members: JARID1A, B, C and D. JARID1B is of particular interest because it has been shown to be up regulated in 90 percent of primary breast cancers. Furthermore, JARID1A has been shown to be up regulated in gastric cancer. Studies have shown a cross-talk between H3K4 methylation and H3K14 acetylation. A recent report suggests that Jhd2 mediates the cross-talk between these H3K4 trimethylation and H3K14 acetylation; however, the mechanism in which Jhd2 acts to facilitate this cross-talk is unknown. Our study shows that Jhd2 facilitates a cross-talk between H3K4 trimethylation and H3K14 acetylation and that this is mediated through the interaction between Jhd2 and the H3 N-terminal tail in that acetylation disrupts the interaction. By mutating H3K14, H3K4 trimethylation can be modulated in a Jhd2 dependent manner. Additionally, deletion of a histone acetyltransferase (HAT) GCN5, the HAT associated with the SAGA complex, results in a decrease for not only H3 acetylation but also in global and gene specific H3K4 trimethylation. This decrease in H3K4 trimethylation is dependent on Jhd2. We further demonstrate that deletion of GCN5 causes an increase in Jhd2 binding to chromatin. Finally, we were able to show that knock-down of Gcn5 in Drosophila melanogaster also causes a decrease in H3K4 trimethylation levels. Through our study we show that the histone demethylase Jhd2 mediates a cross-talk between H3K14 acetylation and H3K4 trimethylation through modulating its interaction with chromatin, and that this mechanism for maintaining chromatin modifications is conserved through other eukaryotes.
The N-terminal tail domains of histones undergo several post-translational modifications such as methylation, acetylation and ubiquitination. These covalent chromatin modifications form a histone code that is interpreted by distinct proteins to regulate various cellular processes including gene expression. Lysine 4 residues of histone H3 undergo mono, di or trimethylation (H3K4me1/2/3) and these marks are associated with transcriptionally active euchromatin. The human Set1 complexes are responsible for a majority of the H3K4me marks in the genome. The Set1a and Set1b complexes are homologs that differ only at the catalytic component. Recently, it was reported that Set1a and its product H3K4 trimethylation play a significant role in colorectal tumor growth. In a separate study, Set1a was found to be essential for gastrulation in mice. Both Set1a and Set1b complexes contain Cfp1, a protein indispensable for vertebrate development and embryonic stem cell differentiation. Cfp1 is a key regulator of both histone and DNA cytosine methylation. Previously, it has been observed that Cfp1 binds to unmethylated CpG islands located at the transcription start sites of many genes. It was also found that Cfp1 restricts the Set1 complex and its product H3K4me3 to euchromatin. In the absence of Cfp1, the Set1 complex mislocalizes to heterochromatin. Moreover, it was recently found that the DNA binding activity of Cfp1 is not necessary for the appropriate targeting of the Set1 complex. The domain/s of Cfp1 essential for the correct targeting of the Set1 enzymes is/are still unknown. Cfp1 contains plant homeodomains PHD1 and PHD2. The PHD domains are effector domains known to read methylated states of H3K4. The PHD domain of Spp1, a yeast protein homologous to Cfp1, is known to bind H3K4me2/3 marks. We found that the human Cfp1 PHD1 domain possesses sequence similarity to the PHD domain of Spp1 and other methylated H3K4 readers like BPTF, Pygo2 and ING2. Here, we show that the Cfp1 PHD1 domain directly interacts with the unmodified histone H3 and the H3K4me2/3 marks by in vitro pull down assays. Conserved amino acid residues essential for PHD-H3K4me interaction present in other H3K4me readers were identified in Cfp1 PHD1 and were mutagenized. This resulted in the drastic reduction of the binding affinity of the Cfp1 PHD1 domain for H3K4me3 but it did not affect binding towards unmodified H3. Genomic targeting of the Set1 complex was unaffected in embryonic stem cells transfected with these H3K4me3-binding mutants of Cfp1. Efforts to find a Cfp1PHD1 binding mutant unable to interact with unmodified H3 are in place and this mutant will eventually be utilized to understand the functional importance of Cfp1PHD domains for the correct genomic targeting of the Set1 complex.

Mit1 Mobilizes Histone Octamers within Heterochromatin to Suppress Transcription

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Mit1 is the putative chromatin remodeling subunit of the fission yeast Snf2/HDAC repressor complex (SHREC) and is known to repress transcription at regions of heterochromatin. However, how Mit1 modifies chromatin to silence transcription is largely unknown. Here we report that Mit1 mobilizes histone octamers in vitro and requires ATP hydrolysis and conserved chromatin tethering domains including a previously unrecognized chromodomain to remodel nucleosomes and silence transcription. Loss of Mit1 remodeling activity results in nucleosome depletion at specific DNA sequences that display low intrinsic affinity for the histone octamer. We demonstrate that Mit1’s remodeling activity contributes to SHREC function independently of Clr3’s histone deacetylase activity on Lys14 of histone H3. We propose that chromatin remodeling by Mit1 cooperates with the Clr3 and other chromatin modifiers to stabilize heterochromatin structure and to prevent access to the transcriptional machinery.
Complementary pathways synergistically rewire the regulatory network in induced pluripotency

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Transcription factor mediated reprogramming of somatic cells to induced pluripotent stemcells represents a profound change in cell fate. While this process is accompanied by major changes in the epigenome and transcriptome, how these events culminate in the activation of the pluripotency regulatory network remains unknown. Using reprogramming intermediates that are stalled at the stage before achieving full pluripotency, we found that the addition of both ascorbic acid (AA) and 2i (MAP kinase and GSK inhibitors) synergistically allowed the acquisition of a fully reprogrammed state at a very high efficiency. However, minimal conversion occurred with either component alone. When uncoupled, AA treatment had to strictly precede 2i and mediated perturbation of the epigenome. Among the AA-dependent activities, there was critical early requirement for histone demethylase effects while Tet-mediated 5 hydroxymethylation was required throughout the conversion to iPSCs. Transcriptional response to the individual stimuli was almost completely divergent and both components were required for the rewiring of the pluripotency network. AA driven activation of pluripotency genes such as Nanog and Zfp42 was complemented by 2i-mediated activation of Tcfcp2l1 and general transcription machinery, and both stimuli were required for the activation of Esrrb. In addition, 2i was the major driver of repression of cell differentiation and proliferation genes that are downregulated during reprogramming. Taken together our results provide important insights into changes in the epigenome and transcriptome and the pathways that lead to the acquisition of pluripotency.

Analyzing Function of Polycomb Repressive Complex 2 (PRC2) in Peripheral Nervous System

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Peripheral nerve myelin formation by Schwann cells requires coordinate regulation of gene repression as well as gene expression. The EGR2/KROX20 transcription factor is critically required for myelination in the peripheral nervous system, and it mediates not only positive regulation of myelin-associated genes, but also negative regulation of genes expressed in immature Schwann cells. Some of the cofactors that are involved in EGR2/KROX20-mediated repression have been characterized, including the Nab corepressors and the NuRD (Nucleosome Remodeling and Deacetylase) chromatin remodeling complex. However, less is known about the contribution of epigenetic modulators that are involved with this process. We have profiled repressive histone modifications during peripheral nerve myelination in vivo using chromatin immunoprecipitation. These studies have revealed that trimethylation at Lys27 of histone H3 tail (H3K27me3) is the most abundant repressive histone mark in genes that are repressed in myelinating Schwann cells. We found that this repressive mark was established relatively early during myelination. To determine the role of the H3K27me3 repressive mark in peripheral nerve myelination, we have generated a mouse model that has a Schwann cell-specific knockout of Eed (Eed cKO), which is an essential subunit of the polycomb repressive complex 2 (PRC2) that catalyzes H3K27 trimethylation. Surprisingly, our findings indicate that Eed activity is not absolutely required during the active period of myelination, but its loss eventually leads to impaired gene expression patterns and structural defects including progressive hypermyelination and accelerated age-related morphology changes affecting both myelinating and non-myelinating Schwann cells. Using microarray analysis of Eed cKO nerve, PRC2 target genes were identified by integrated analysis with H3K27me3 ChIP-seq. Target genes include important transcriptional regulators of neuronal or other neural crest lineages, such as Pax6, Shh, and Isl1. Additionally, Brn2 and Tbx2 genes that normally become repressed prior to myelination were upregulated in Eed cKO nerves. Moreover, some activated genes are also induced after nerve injury-induced dedifferentiation of Schwann cells. Our results identify an integral role for PRC2 as an epigenetic regulator important for peripheral nerve homeostasis and maintenance of lineage integrity through its repression of tissue- and stage-inappropriate gene expression.
Androgens are crucial for prostate development and regeneration, and also mediate benign prostatic enlargement, a significant risk factor for urinary dysfunction in aging men. The role of DNA methylation in androgen-induced prostate growth and its influence over urinary function has not been examined. We hypothesize that throughout life, the DNA methylation landscape shapes the prostate's growth response to androgen and thereby impacts urinary function. We first tested the requirement for global DNA methylation in fetal prostate development. 14 days post coitus (dpc) mouse prostate explants were pulse-treated with media containing the DNA methylation inhibitor 5-aza-2'-deoxycytidine (5AzadC) or control media, and then grown in media without 5AzadC and containing dihydrotestosterone (androgen). 5AzadC increased AR protein abundance and the number, rate, and androgen sensitivity of prostatic duct formation in vitro. We then tested whether DNA methylation influences androgen-dependent adult prostate ductal regeneration. Mice were castrated to induce prostate involution, pulse treated with 5AzadC or vehicle control, and prostate regeneration was then stimulated by testosterone administration. 5AzadC increased the number of prostate cells with detectable AR protein and augmented androgen-induced prostate epithelial cell proliferation and regrowth. The role of DNA methylation in adult urinary function was assessed in a mouse model of urinary dysfunction, in which mice are implanted with testosterone+estradiol (T+E2) pellets to mimic the hormone environment of aging men. T+E2 alone decreased prostate global DNA methylation and caused urinary retention. Feeding mice with a methyl donor-enriched diet (folic acid) during in utero development and adulthood prevented the T+E2-induced loss of prostate global DNA methylation and improved voiding function in T+E2 treated mice. Thus, DNA methylation restricts prostate androgen responsive growth throughout life, and a deficiency in prostate DNA methylation may be an underlying basis for urinary dysfunction in aging men. Supported by NIH grant DK096074 and NSF grant DGE-0718123.

Transposable elements (TEs) and their remnants make up a large part of eukaryotic genomes. As a result, eukaryotes must use a variety of mechanisms to control transposable elements and protect their genomes. In Drosophila the piRNA system acts in the germ line and early embryo to repress transposons, but much about how it functions is not known. Although part of the piRNA pathway appears to work through post-transcriptional silencing, as is the case for other RNAi systems, the Argonaute protein Piwi requires nuclear localization to silence transposons. We have previously identified a set of chromatin sites where the presence of a transposon results in variegation of an adjacent hsp70-white reporter, similar in appearance to Position Effect Variegation (PEV). This effect disappears and one observes a full red eye when the transposon is excised from the site. Mutations in the piRNA pathway also cause a loss of silencing. Using these reporter sites, we are testing what DNA elements are necessary for silencing to be targeted to these loci, in particular testing elements found in piRNA populations. Transcription at the reporter site occurs, and may be necessary for silencing; this would suggest an RNA-RNA pairing mechanism for targeting silencing. We will test this hypothesis by using a reporter flanked by transcription termination elements. We hope to learn more about how the piRNA system targets specific loci for silencing.
The nuclear proteome is intimately linked with gene regulation and may also be closely coupled to metabolism. N-terminal tails of histone proteins are post-translationally modified and are a major mechanism for regulating transcription. These modifications (PTMs) can be quantified via liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Currently, two acquisition methods are being used to quantify histone PTMs: data-dependent acquisition mode (DDA) and targeted proteomics (also known as selected reaction monitoring, SRM). Both of these modes have limitations. DDA mode limits detection to only the most highly abundant peptides, yielding stochastic results that do not consistently generate the same set of peptides from run to run. Isobaric peptides, which have the same molecular weight but different PTMs, also present a problem for DDA. For example, the peptide spanning histone H3 residues 9-17, KSTGGKAPR, may be acetylated at either K9 or K14, but both peptide variants will have the same mass and similar retention times, making the two variants impossible to distinguish by DDA alone. Additionally, targeted proteomics queries only a pre-specified set of peptides, thus limiting detection to a predefined list. Data-independent acquisition mode (DIA), however, allows users to avoid these limitations and yields time-resolved MS2 ion spectra for all analytes detected within a specific precursor mass range and retention time window. We have developed a novel method to quantify histone PTMs via DIA mass spectrometry. Using human breast cancer MCF-7 cells treated with the histone deacetylase inhibitor SAHA (suberoylanilide hydroxamic acid) as a model system, we demonstrate that using a combination of DDA- and DIA-generated data yields consistent and accurate quantitation of histone modifications. In our workflow, we generate a library of peptide spectra from a collection of DDA data sets. Using a collection of DDA data sets rather than the results of a single LC-MS/MS run eliminates issues related to the stochastic nature of these data, since a peptide variant need only appear in the collective data once to be included in the library. We then match spectra from raw DIA data to the library. Since DIA data generates high quality MS2 ion fragments, this additional level of information improves our accuracy of peptide peak identification within the spectra, especially for isobaric peptides. Using a combination of DDA- and DIA-generated data in this manner allows us to benefit from existent tools developed for DDA data while gaining accuracy and resolution from DIA data. This method enables higher resolution and more accurate studies of the chromatin state in both health and disease.

We used the GoldenGate template-assisted ligation assay to follow heterozygosity in developing FVB X BALB-NeuT F1 breast tumors to visualize somatic genetic changes. The detected changes revealed a strong bias for retention or accumulation of alleles on chromosome 4 derived from the FVB parent and sporadic, apparently unbiased, loss or gain of other chromosomes within the analyzed tumor cell populations. Cytogenetic analysis confirmed both the high incidence of loss of chromosome 4 and sporadic loss or gain of other chromosomes on an individual cell basis. Remarkably, the ligation assay also showed pervasive, patchy, random allelic deviations from heterozygosity which differentiated tumors from normal tissue. Molecular analytical strategies which readily detected skewing in allelic ratios within tumors for alleles on chromosomes where gross chromosomal changes were evident, found normal heterozygosity at loci where evidence suggested retention of normalF1 chromosomal composition, even though skewing of allelic ratios was more extreme than in cases associated with gross chromosomal-change. Remarkably, the examined regions of the genome where these putative epigenetic modifications were evident contained no canonical CpG target sites for epigenetic methyl modification of the DNA. These results give rise to the hypothesis that pervasive non-canonical epigenetic modifications of DNA occurs in the developing breast tumors.
Phosphoinositide levels modulate sperm chromatin packaging
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Sperm chromatin packaging into a highly condensed and mostly transcriptionally inert structure during spermatogenesis is essential for accurate transmission of genetic information and successful reproduction. Phosphoinositides (or PIPs) play important roles in regulating various aspects of sperm development in Drosophila. Here, we show that PIPs are also required during late stages of spermiogenesis to regulate the shape and size of the sperm head, and the state of chromatin condensation. During spermiogenesis, the nuclei elongate, becoming leaf-, canoe- and then needle-shaped. As they elongate, the perinuclear microtubules form the dense body, a rigid structure which gives support to the long and thin nucleus, and which is disassembled in mature nuclei. At the same time, the chromatin inside the nucleus is reorganized to fit into a much smaller space. This reorganization involves replacing the histones with transition proteins and then with protamines. Through immunofluorescence and immunoelectron microscopy experiments we show that phosphatidylinositol 4,5-bisphosphate (PIP2) localizes to the nuclei, in regions associated with dense body, nuclear membrane, and chromatin. Spermatids in which levels of PIP2 have been reduced show profound defects in nuclear shaping; the nuclei do not mature and the males are sterile. Posttranslational modification of histones is impaired and their removal is delayed. Protamines get incorporated into nuclei despite histones not being completely removed, whereas transition proteins are missing. Marker analysis revealed that these defects in chromatin condensation occur early during nuclear elongation and that localization of inner nuclear membrane proteins, sumoylation and repair of double-stranded DNA breaks are PIP2 dependent. Our data suggest that normal levels of PIP2 are required to coordinate interactions between the nuclear membrane, chromatin and the cytoskeleton to drive chromatin and nuclear remodeling.

Identification of Hairy Mediated Repression Mechanisms in the Drosophila Embryo
Kurtulus Kok, Michigan State University

Precise regulation of gene expression is essential for animal development. Context-specific switching between activation and repression is a central process in cell fate determination, and is orchestrated by transcriptional repressors through changes in chromatin structure. The nonpermissive chromatin environment in gene silencing is created by multi-subunit protein complexes, involving histone modifying enzymes and chromatin remodelers that are recruited by DNA binding repressors through co-repressors. In order to elucidate distinct repression mechanisms, we assessed chromatin changes on a genome-wide scale using Drosophila Hairy repressor as a paradigm in the blastoderm embryo. Hairy is a member of Hairy/Enhancer of Split (HES) family proteins and retains multiple repression domains through which it interacts with co-repressors Gro, dCtBP and dSir2. Misexpressing Hairy or mutant versions of Hairy that lack specific motifs required for co-repressor interaction allowed us to correlate dynamic changes in various histone marks with the modes of repression. We found that Hairy induces wide-spread histone deacetylation, especially in the distal regulatory regions of target genes, consistent with its long-range repression mechanism. Strikingly, local loss of acetylation marks is also associated with repression, more commonly at nearby promoters, indicating Hairy may employ short-range repression mechanism in some cases. The mutation in the CtBP interaction domain partially impairs repression ability of Hairy on some genes but completely abolishes its activity on others. These results support the model that Hairy acts in a context-dependent manner utilizing different repression mechanisms, and CtBP contributes to repression in a quantitative and gene-specific fashion. Comprehensive characterization of chromatin states and cofactor interactions mediated by Hairy help us understand the repression systems in Drosophila, and more generally, pathways of transcriptional repression in metazoans. In addition to providing mechanistic insights, lessons from this study will be constructive to interpret genome-wide transcription factor binding, chromatin modifications and understand the activity of cis-regulatory elements.
The nuclear lamina protein Otefin prevents DNA Damage Response activation to promote adult stem cell survival

Lacy Barton, University of Iowa

The nuclear lamina is a filamentous network that underlies the nuclear envelope. Lamina components include LEM domain (LEM-D) proteins, named for LAP2, emerin and MAN1. LEM-D proteins organize chromatin at the nuclear periphery through interactions with lamins and the DNA and histone binding protein Barrier-to-Autointegration-Factor. Despite global expression, several highly tissue-restricted, age-enhanced diseases result from mutations in individual LEM-D genes, including bone density disorders, cardiomyopathies and muscular dystrophies. Emerging evidence suggests that stem cells are compromised in affected tissues, yet how LEM-D proteins support stem cell maintenance and function in unknown. To gain insights into critical LEM-D protein functions, we study the Drosophila emerinhomologues, Bocksbeutel (Bocks) and Otefin. While loss of Bocks causes no developmental defects, loss of Otefin causes both male and female sterility due to germ line stem cells (GSCs) depletion. We find that GSC depletion is due to stem cell death, not inappropriate differentiation. Strikingly, loss of the DNA Damage Response (DDR) proteins Ataxia telangiectasia and Rad3 related (ATR) or Checkpoint kinase 2 (Chk2) suppresses GSC death, fully restoring gametogenesis in both male and female animals. Surprisingly, Chk2 activation in ote mutants occurs in the absence of detectable DNA damage or other canonical triggers of Chk2 activation. We are investigating whether ATR and Chk2 activation in ote mutant GSCs is caused by changes in chromatin structure and organization. These data suggest that Ote promotes survival of two different stem cell populations by preventing the activation of a unique ATR and Chk2-dependent checkpoint that is DNA-damage independent. Further, our findings suggest that DDR activation within stem cell populations may be a critical, primary defect associated with LEM-D associated diseases.

SIN3 is critical for stress resistance and modulates adult lifespan

Lori Pile, Wayne State University

Coordinate control of gene activity is critical for fitness and longevity of an organism. The SIN3 multi-subunit histone deacetylase (HDAC) complex functions as a transcriptional repressor of many genes. SIN3-regulated genes include those that encode proteins affecting multiple aspects of mitochondrial function, such as energy production and stress responsiveness, important for health maintenance. Here we used Drosophila melanogaster as a model organism to examine the role of SIN3 in the regulation of fitness and longevity. Because ubiquitous knockdown of Sin3A is lethal during embryogenesis, we used a conditional system to activate RNA interference (RNAi) induced knockdown during adulthood. Adult flies with decreased expression of Sin3A have reduced climbing ability; an activity that likely requires fully functional mitochondria and utilizes ATP. Additionally, compared to wild type counterparts, adult Sin3A knockdown flies were more sensitive to oxidative stress. Interestingly, media supplementation with the antioxidant glutathione largely restored fly tolerance to oxidative stress. Consistent with those results, we found that Sin3A knockdown corresponded to a significant reduction in expression of genes encoding proteins involved in the de novo synthesis of glutathione, including the key enzyme glutamate–cysteine ligase (GCL). Although Sin3A knockdown flies exhibited decreased longevity compared to wild type flies, no significant changes in expression of a number of well-categorized “aging” genes were observed. The decreased longevity in Sin3A knockdown flies, having reduced expression of GCL, is consistent, however, with previous reports demonstrating that overexpression of the GCL catalytic component extends lifespan. Taken together, the data support a model whereby SIN3 regulates pathways required for proper mitochondrial function and effective stress response during adulthood. These findings suggest that SIN3 is critical for regulation of a gene expression program required after the majority of developmental cell proliferation and differentiation has occurred.
Early life stress results in adult behavioral deficits that are associated with a sex-specific loss of 5-hmC in the brain

Ligia Papale, University of Wisconsin–Madison

The hypothalamic-pituitary-adrenal (HPA) axis is a dynamic metabolic system that regulates homeostatic mechanisms, such as the ability to respond to stressors, and is highly sensitive to early life adversities. This axis governs the activity of gender-specific endocrine mechanisms and responds to stress by altering the neuronal epigenome. Indeed, early life stressors such as abuse/neglect increase physiological sensitivity to even mild adversities later in life and are associated with alterations of epigenetic marks (e.g. DNA methylation) and gene expression. Together, these data suggest that DNA methylation has a role in gender-specific susceptibilities to psychiatric disease. However, despite growing knowledge of the link between DNA methylation and stress responses, improved understanding of DNA methylation has revealed that previous interpretations should be reconsidered, due to the inability of standard DNA methylation detection methods to distinguish between 5-methylcytosine (5-mC) and 5-hydroxymethylcytosine (5-hmC), the newly identified mark enriched in neurons. Recently, we and others have developed genome-wide methods capable of providing a distinction between 5-mC and 5-hmC. These studies found that unlike 5-mC, 5-hmC is mediated by members of the ten-eleven translocation (TET) family of proteins, which are influenced by environmental cues such as oxidative stress. In addition, 5-hmC associates with active transcription of neuronal genes. To preliminarily examine the role of methylation in response to stress, we subjected adult mice (N=4 for each gender) to a 30-minute restraint stress followed by a one-hour recovery and found a significant reduction of 5-hmC levels compared to controls in the hippocampus, amygdala, and cortex (p < 0.05; Figure 1). Since these differences were found in all brain regions investigated, we hypothesized that the 30-minutes restraint may affect overall arousal levels and thus alter more than just the stress pathway. Thus, to investigate whether restraint stress and concomitant loss of 5-hmC have long-term effects on behavior, we subjected adult mice (N=6 for each gender) to a 2-hour restraint stress followed by a ten-day recovery and found that only males spent significantly more time in the closed arms of an elevated-plus maze (t-test, p < 0.05; Figure 1). In contrast, when young mice (pre-weaning; N=10 for each gender) were subjected to seven days of established variable stressors, and behaviorally tested as adults, only the early-life stressed females exhibited anxiety-like behaviors compared to controls in elevated-plus maze and light/dark box tests (p < 0.05). Together, these studies are consistent with a role for DNA methylation (i.e. 5-hmC) in response to stress and suggest that the developmental timing of the stress exposure results in a clear gender-specific response.

The Molecular Mechanism of PRC2 Regulation by PHF1

Lynne Dieckman, University of Iowa

The maintenance of gene repression is crucial for the prevention of cancer development and progression. PRC2 (Polycomb repressive complex 2) regulates the transcriptional repression of oncogenes through the tri-methylation of histone H3 at lysine 27 (H3K27me3), which is accomplished by the PRC2 catalytic subunit EZH2. Aberrant expression of EZH2 can lead to the onset of several highly aggressive cancers including prostate, breast, lung, melanoma, lymphoma, and pancreatic cancer. Efficient methyltransferase activity of EZH2 in vivo is dependent on the presence of the PHD finger protein 1 (PHF1). PHF1 has been shown to differentially regulate PRC2 activity, however the interaction between PHF1 and EZH2 and the molecular mechanism by which PHF1 alters PRC2 activity remains poorly understood. We hypothesize that PHF1 interacts with EZH2 and directly alters its H3K27 methylation activity, and that this regulation is dependent upon the local epigenetic environment. Here we show the structure of the PHF1 Tudor domain bound to a peptide containing the H3K36me3 mark and show that this interaction inhibits the activity of PRC2 to methylate H3K27. In addition, we show that the direct interaction between EZH2 and PHF1 requires the PHD2 of PHF1.
Methylation of MED12 by CARM1 as a predictor for breast cancer chemosensitivity

Lu Wang, University of Wisconsin–Madison

CARM1 belongs to the type I protein arginine (R) methyltransferase (PRMT) family and asymmetrically di-methylates proteins on arginines. CARM1 was shown to transactivate cancer-relevant transcription factors including ER1, NF-kB, p53 and E2F1, and it might be an essential factor in the DNA damage signaling pathway. CARM1 requires its enzymatic activity for the majority of in vivo functions. However, in our previous studies, we found that knocking down 90% of endogenous CARM1 is unable to eliminate substrate methylation in breast cancer cells. To fully characterize the functions of CARM1 in breast cancer, we knocked out CARM1 in several breast cancer cell lines using Zinc-Finger Nuclease (ZFN) technology. Knocking out of CARM1 dramatically changed the cell morphology and significantly decreased breast cancer cell growth both in vitro and in vivo. Western blot using an asymmetric di-methylated R antibody revealed striking differences between parental and CARM1 knockout MCF7 cell lysates, indicating that CARM1 methylates a significant number of substrates in breast cancer cells. By mass spectrometry, mediator of RNA polymerase II transcription subunit 12 (MED12), was identified as a substrate of CARM1. Biochemical analyses further validated that MED12 was methylated by CARM1 in vivo and in vitro and the methylation sites were mapped Arginine 1862 and 1912 in its C-terminus, a critical domain which also mediates interaction between MED12 and other transcriptional factors such as SOX9, -catenin, Nanog, and G9a. By DNA microarray, we found methylated MED12 controls the expression of different downstream genes, comparing with non-methylated MED12. My preliminary result showed that methylation of MED12 affected the sensitivity of breast cancer cells to some chemo-drugs. The proposed research will directly address whether MED12 methylation predicts sensitivity of breast cancer cells to chemo-drugs in cell culture and xenograft tumor models, and whether the MED12 R1862/R1912 mutations exists in human breast tumors and correlates with chemo-sensitivity in patients treated with neoadjuvant chemotherapy. Additionally it will identify targets regulated by methylated MED12 that determines chemo-sensitivity. The intent is to identify a potential biomarker for predicting resistance of breast cancer to commonly used, different classes of chemo-drugs and determine novel targets for future therapeutic interventions.

synMuv B regulation of chromatin states at high temperature

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Most organisms experience variations in temperature throughout their lifespan and thus require mechanisms to buffer gene expression in response to fluctuations in temperature. Understanding epigenetic response to temperature is important in determining the mechanisms organisms use to control gene expression during times of temperature stress. We are investigating changes in the chromatin state at high temperature through the study of the synMuv B chromatin regulator proteins in Caenorhabditis elegans. synMuv B proteins include members of the DRM complex, such as lin-35 the sole worm homolog of Rb, LIN-15B, and HPL-2, a worm homolog of HP1. Mutations in many synMuv B genes demonstrate a high temperature larval arrest phenotype that can be rescued by loss of germline chromatin modifiers. This suggests that changes in chromatin states underlie the temperature-sensitive gene misexpression in synMuv B mutants. To investigate changes in chromatin modifications in the synMuv B mutants we have performed ChIP-seq experiments in synMuv B mutants at both low and high temperature on H3K36me3 and H3K9me2. Methylation of H3K9 has generally been found throughout the coding region of repressed genes and is highly associated with heterochromatin. However, we have found enrichment of H3K9me2 in a novel location: over the promoter of genes whose expression is enriched in the germ line in wild type animals. This H3K9me2 promoter enrichment is lost in lin-15B mutants. Because the loss of synMuv B proteins affect critical histone marks at high temperatures, we hypothesize that chromatin environment may be impacted as well. In order to investigate this we are using the lacO/LacI system to visualize changes in chromatin compaction at elevated temperatures. Preliminarily, we see a decrease in chromatin compaction at high temperatures across all strains, although compaction is more severely compromised in synMuv B mutants compared to wild type. These results lead us to believe that synMuv B proteins are important in regulating chromatin environment especially under conditions of temperature stress.
SIN3: a junction to connect histone acetylation to histone methylation

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SIN3 controls histone acetylation through association with the RPD3 histone deacetylase. Our laboratory has found that a histone H3K4 demethylase named LID also co-immunoprecipitates with SIN3. These results indicate that SIN3 may regulate histone methylation in addition to acetylation. SAM synthetase (SAM-S) generates the major methyl donor s-adenosylmethionine (AdoMet) from methionine and influences histone methylation. We have found that RNAi knockdown (KD) of Sam-S suppressed the Sin3A KD curved wing phenotype in adult Drosophila. These data led us to examine the connection of SIN3 to SAM-S in Drosophila embryonic S2 cells. We measured cellular proliferation and observed that loss of SIN3 or SAM-S resulted in a lower cell density. Unlike the effect in adult Drosophila, RNAi KD of Sam-S did not suppress the Sin3A KD phenotype in S2 cells. The difference implies that histone acetylation and methylation may regulate specific developmental genes that are not influenced during embryogenesis. Moreover, we checked the global histone methylation levels in S2 cells. Loss of SIN3 led to increased H3K4me3, although the effect was not strong. Interestingly, compared to decreased H3K4me3 in Sam-S KD or Set1 KD S2 cells, the global H3K4me3 level was restored to the control level in Sam-S+Sin3A KD and Set1+Sin3A KD S2 cells. These results support earlier findings from our laboratory and others suggesting that SIN3 controls histone methylation. To understand how SIN3 affects histone methylation, we performed differential gene expression analysis in Sin3A KD, Sam-S KD and Sin3A+Sam-S KD S2 cells by RNA-seq. We found that expression of some methionine metabolic genes, not major histone methyltransferases or demethylases were regulated by SIN3. These data indicate that SIN3 may control histone methylation via influencing the methionine pathway. Given the interaction between SIN3 and LID, we are testing whether SIN3 influences histone methylation through affecting LID binding or enzyme activity. Results from these analyses are anticipated to provide insight into the cross-talk between histone acetylation and methylation in the regulation of cellular proliferation, histone methylation and development.

Analysis of plant development through chromatin profiling focusing on phase transition

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In plants, as in other eukaryotes, trimethylation of histone H3 at lysine 4 (H3K4me3) and lysine 36 (H3K36me3) mark transcriptional activity, whereas trimethylation of histone H3 at lysine 27 (H3K27me3) is linked to transcriptional repression, especially of developmentally regulated genes. In this study, we are analyzing the association between chromatin structure and gene expression across the spectrum of developmental processes, using Malus (apple) species as a model for woody perennial plants. We mapped domains of H3K27me3 across the Malus genome for a variety of developmental states and linked these with transcriptional activity. We analyzed the phase transition from juvenile to adult growth, a critical developmental switch that enables a plant to flower. This project generated 24 sequence data sets totaling ~500 million reads produced by chromatin-immunoprecipitation (ChIP)-seq, using antibodies directed against non-modified H3 and H3K27me3, to identify changes in chromatin structure accompanying this switch. An additional 60 RNA-seq data sets representing nearly 2 billion sequence reads were generated and processed with bioinformatics tools to predicted transcript models and changes in gene expression at unprecedented resolution. Results of these studies will be presented.
DNA Methylation Changes underlie Hyperglycemia and the Metabolic Memory

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Studies from human cells, rats, and zebrafish have documented that hyperglycemia induces the demethylation of specific cytosines throughout the genome. Using a zebrafish diabetic model, we previously documented that a subset of these changes become permanent and may provide, in part, a mechanism for the persistence of diabetic complications referred to as the metabolic memory phenomenon. Clinically, MM is defined as the persistence of diabetic complications even after glycemic control has been pharmacologically achieved. In our zebrafish model, MM occurs following pancreatic regeneration which returns fish to a euglycemic state. We reported that specific changes in DNA methylation are associated with altered gene expression patterns underlying the secondary complications seen in DM/MM fish. In this report we present two sets of findings: 1) elucidating the molecular machinery that is responsible for the hyperglycemia induced DNA demethylation observed and 2) a detailed bioinformatics analysis of DNA methylation patterns that result from the hyperglycemic state and are maintained into the MM state. In regard to the molecular machinery of de-methylation, RNA expression analyses and enzymatic activity assays indicate that the ten-eleven translocation family of enzymes (Tet) were activated by hyperglycemia resulting in the conversion of 5mC back to the unmethylated form via the Tet-dependent iterative oxidation pathway. In addition, evidence is provided that the Poly-ADP ribose polymerase enzyme’s (Parp) activity is required for activation of Tet activity as the use of a Parp inhibitor prevented the accumulation of the Tet induced intermediates. Remarkably, this inhibition was accompanied by a complete restoration of the tissue regeneration deficit that is also induced by hyperglycemia. In regard to the second set of findings, we focused on the diabetic limb (caudal fin) and analyzed 1) what functional gene groups are prominent during DM, 2) which genes of these groups persist into MM, and 3) what is the positional relationship of altered DNA methylation to these genes. We found that the DM/MM states are associated with changes in the expression of genes of the DNA replication and DNA metabolism groups. Aberrantly methylated DNA regions (MRs) were 6-13 kb upstream of the transcription start site of a subset of functionally important genes within these groups. Lastly, MRs were associated with potential transcription factor (TF) binding sites whose methylation is known to perturb TF binding. Translatability of this data to the human genome was also investigated.

Mitotic regulatory function of histone H3 and chromatin modifying enzymes Gcn5p and Rpd3p

Min-Hao Kuo, Michigan State University

Faithful segregation of the duplicated chromosomes during mitosis is essential to maintain cellular function and species perpetuation. Bipolar attachment of mitotic spindles to the kinetochores aligns chromosomes in the metaphase midplate before cells enter the anaphase when sister chromatids are irreversibly segregated. Prior to segregation, the poleward pulling force by spindles generates tension between sister chromatids. Tension thus is a key criterion for the spindle assembly checkpoint (SAC) to ensure proper alignment of metaphase chromosomes. We showed previously that the budding yeast histone H3 at pericentromeres plays an essential role in tension sensing. This novel mitotic function of H3 is carried out by the Tension Sensing Motif, TSM, of H3 that recruits the Shugoshin protein, Sgo1p, to the pericentromeres. Unlike other eukaryotes in which heterochromatic marks are enriched at pericentromeres and are responsible for Shugoshin recruitment, the budding yeast pericentromeres are euchromatic and lack appreciable epigenetic marks that distinguish them from chromosomal arms. Thus, how Sgo1 is selectively recruited to pericentromeres in budding yeast remains an enigma. Our recent data strongly suggest that the Gcn5p histone acetyltransferase and Rpd3p histone deacetylase play antagonistic roles in the H3 TSM-Sgo1p pericentric interaction. Furthermore, although TSM is not part of the flexible N' tail domain of H3, our genetic and ChiP experiments suggest that Sgo1p may use the tail domain as a secondary docking site when the integrity of TSM becomes impaired. Overall, our discoveries reveal a network involving different domains of H3, multiple histone modifying enzyme, and the Shugoshin protein in maintaining the function of the SAC to ensure faithful segregation during mitosis.
Functional characterization of the Arabidopsis RNA silencing enzyme, RDR2
(RNA-DEPENDENT RNA POLYMERASE 2)

Mishra Vibhor, Indiana University

RNA DEPENDENT RNA POLYMERASE 2 is an enzyme involved in biogenesis of double stranded RNAs (ds-RNA) that serve as precursors for siRNAs involved in transcriptional gene silencing of transposons and repeated elements. Genetic and biochemical evidence suggests that RDR2 physically associates with DNA dependent RNA polymerase IV, suggesting that their activities are coupled for the synthesis of the precursors for 24nt small RNAs that guide RNA-directed DNA methylation (RdDM). The RDR2 protein consist of a N-terminal uncharacterized domain as well as a RNA dependent RNA polymerase domain. To characterize the structural and functional features of RDR2 we have overexpressed and purified the protein to homogeneity. The purified recombinant protein exist as a monomer of molecular mass about 130 kDa, under physiological conditions. It possess primer independent RNA polymerase activity on single stranded RNA and DNA templates. Recombinant RDR2 specifically interacts with RNA polymerase IV under in vitro conditions. Point mutation studies suggest that the metal binding aspartate residues present in the active site are required for the functional activity of the RDR2. These findings provide novel bio-chemical/physical insights into the dsRNA synthesis especially in context with the RdDM pathway.

Vitamin D Receptor Loss in Lamin A/C-Deficient Cells Causes DNA Repair Defects

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Laminopathies are diseases caused by mutations of proteins of the nuclear lamina. Over 300 mutations have been identified in the LMNA gene (encoding lamin A/C), and associated with a dozen of diseases, including muscular dystrophies, lipodystrophies, neuropathies, and premature aging disorders such as Hutchinson Gilford Progeria Syndrome. HGPS is the most devastating, with patients exhibiting accelerated aging phenotypes, and dying in their teens from atherosclerosis and cardiovascular complications. Cells with altered lamin A/C show nuclear perturbations, epigenetic alterations, nuclear stiffness and fragility, altered signaling pathways, and genomic instability.

Our studies identified molecular mechanisms contributing to genomic instability in laminopathies. In particular, loss of lamin A/C leads to activation of cathepsin L-mediated degradation of 53BP1, a key factor in DNA repair by non-homologous end-joining (NHEJ), and downregulation of BRCA1 and RAD51, two critical factors in homologous recombination (HR)-mediated repair. Consequently, lamin A/C-deficient cells exhibit DNA repair deficiencies and genomic instability. Interestingly, vitamin D treatment restores the levels of 53BP1, BRCA1, and RAD51, and reduces DNA repair defects in these cells. These studies revealed an unprecedented role for vitamin D regulating DNA repair. However, the molecular mechanisms behind the effect of vitamin D rescuing DNA repair defects remained unknown.

Here, we show that depletion of lamin A/C in a variety of cells or expression of progerin in HGPS fibroblasts results in downregulation of Vitamin D Receptor (VDR), a nuclear receptor that mediates most of the genomic actions of vitamin D. Importantly, reduced expression of VDR is responsible for the loss of BRCA1 and RAD51 in both contexts. Interestingly, treatment of HGPS fibroblasts or lamin A/C-depleted cells with vitamin D rescues VDR expression, and as a consequence increases BRCA1 and RAD51 levels, ameliorating DNA repair defects. Based on these data, and the fact that VDR knockout mice exhibit premature aging, atherosclerosis, and cardiovascular disease, we propose that VDR deficiency could be a major factor in the phenotype of laminopathies such as HGPS that are characterized by DNA repair deficiencies and genomic instability. Moreover, vitamin D emerges from these studies as an exciting new therapeutic possibility to ameliorate genomic instability and other altered signaling pathways in cells with altered lamin A/C function.
Differential Regulation of HP1 Isoforms by Aurora Kinases During Mitosis

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Previous elegant studies performed in the fission yeast Schizosaccharomyces pombe have identified a requirement for Heterochromatin Protein 1 (HP1) in spindle pole formation and appropriate cell division. However, the regulation of HP1 isoforms by kinases critical for supporting mitotic progression remains to be fully characterized. We report for the first time the phosphorylation of both HP1γ and HP1α by the mitotic kinase Aurora A and B, respectively. Our results demonstrate that EGF, working through the RAF-MEK-ERK pathway, ultimately activates Aurora kinases, which in turn phosphorylate HP1γ at serine 83 and HP1α at serine 92. Notably, these phosphorylation events occur within Aurora consensus sites located within the linker region of these proteins. Using double-thymidine block in HeLa cells, we define that the phosphorylation of both HP1 isoforms takes place during mitosis, concordant with the time that the phosphorylated serine10 mark is deposited on Histone H3. Congruently, the phosphorylated form of these proteins localize to the mitotic apparatus. Experiments employing pharmacological inhibitors, dominant negative proteins, and siRNA-based knockdown of Aurora A and B confirm that these enzymes are the responsible HP1 kinases. Utilizing HP1γ as a model for mechanistic studies, we demonstrate that the Aurora-dependent phosphorylated form of this protein is necessary for cell proliferation. Combined, these results demonstrate that posttranslational modifications in histone code readers serve as distinct signals to endow these proteins with specific functions.

Architectural proteins modulate the higher order chromatin structure of the CFTR locus

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Cystic fibrosis is an autosomal, recessive disease that results from mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which encodes a chloride ion channel with a complex expression pattern. Tissue-specific CFTR expression is regulated by multiple cis-acting elements, including intronic enhancers, which interact with the promoter. The mechanisms that organize higher order chromatin structure to establish and maintain gene expression are not fully understood; however, data suggest they may involve CTCF and cohesin. These factors have important roles in the three-dimensional organization of loci and at insulators, which are critical barriers for preventing inappropriate activation or repression of genes. To determine the functions of these proteins at the CFTR locus, we used an siRNA-mediated knockdown approach. ChIP for CTCF and the cohesin components, RAD21 and SMC1, after CTCF or RAD21 depletion shows loss of all three factors at several sites across the region and retained occupancy at others. After RAD21 knockdown, quantitative chromosome conformation capture (q3C) data reveal a partial loss of interactions across the locus between the gene promoter, known enhancers such as the one in intron 11, and CTCF/cohesin binding sites. In contrast, after CTCF depletion no changes in enhancer-promoter interactions are observed; however, interactions between the -20.9 kb insulator element and CTCF/cohesin binding elements 3’ to CFTR are suppressed. These data suggest that cohesin and CTCF have distinct roles in the chromatin organization of CFTR. We also show that additional components of the chromatin looping complex at CFTR include tissue-specific transcription factors such as FOXA1 and FOXA2, which are responsible for some of the enhancer-promoter associations. Furthermore, depletion of CTCF or the cohesin component, RAD21, leads to an increase in CFTR mRNA levels, and analysis of histone modifications suggests that a mechanism of repression may involve changes in the chromatin landscape of CFTR.

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Emerging evidence has linked photoreceptor cell-specific receptor (PNR/NR2E3), an orphan nuclear hormone receptor, to human breast cancer. PNR was shown to be a transcriptional activator of ERα and high-level expression of PNR correlates with favorable response of ERα positive breast cancer patients to tamoxifen. Previously, gene expression microarray study showed that PNR regulates distinct genes from those regulated by ERα, suggesting that PNR could have ERα-independent functions. Herein, we investigated the function of PNR in ERα negative breast cancer cells. Our results showed that PNR induced cell migration and metastasis of ERα negative breast cancer cells both in vitro and in vivo, and the effect was attributed to the up-regulation of IL-13Rα2, a high affinity receptor for IL-13 that regulates tumor growth, invasion and metastasis of various human cancers. Using luciferase reporter assay and chromatin immunoprecipitation assay, we found that PNR activated transcription of IL-13Rα2 through direct recruitment to IL-13Rα2 promoter, which was associated with increased levels of global acetylation of histone H3, H3K4me3 and Pol II binding at IL-13Rα2 promoter region. Mutation of PNR in the conserved DBD or PNR consensus sequence on IL-13Rα2 promoter abolished the transcriptional activation. Upon stimulation with IL-13, IL-13Rα2 increased the ERK1/2 phosphorylation, which led to breast cancer migration and metastasis. The IL-13 triggered signal cascade was specific to IL-13Rα2 as the closely related IL-13Rα1 was not regulated by PNR. Our finding presents the first evidence that PNR could promote ERα negative breast cancer metastasis through activation of IL-13Rα2-mediated signaling pathway. Our results suggest that targeting IL-13 signaling in combination with inhibiting the activity of PNR may impose synergistic beneficial effects on triple negative breast cancer patients. Ongoing experiments are focused on what coactivators are involved in the transcriptional induction of IL-13Rα2 by PNR and how PNR mediates the crosstalk between breast cancer cells and the immune cells in the microenvironment.

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Identification of Replication-dependent and Replication-independent Linker Histone Complexes

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There are at least 11 different variants of linker histone H1 in mammalian cells: somatic replication-dependent types (H1.1 to H1.5), somatic replication-independent types (H1.0, H1.x), and germ cell types (H1t, H1T2m and HILS1 for testicular cells, and H1oo for oocytes). The sequences of these H1 variants vary greatly in their C-terminal domains.

The individual functions of H1 variants are not fully understood now. It was believed that H1 served as a global gene regulator by binding to chromatin unspecifically. However, although partially redundant in function, some H1 variants play distinctive roles in gene silencing and histone modification. While knocking out a single H1 variant might not lead to any observable phenotype, triple knockouts of H1 variants in mice has shown developmental defects and embryonic lethality. The expression levels and activities of the H1 variants seem to be highly regulated during cell differentiation and tumorigenesis as well. These observations suggest the hypothesis that each H1 variant has its individual function in the cells in addition to its roles as global chromatin modifiers. For core histones, replication-dependent and replication-independent variants have distinctive functions and regulations. For instance, histone H3.1 is assembled to chromatin in coordination with DNA replication during S phase, while another variant H3.3 is exchanged throughout the cell cycle. The dynamics of H3.1 and H3.3 are mediated by distinctive protein complexes that contain different chaperones for each of the H3 variants. It's not known whether the dynamics of replication dependent and independent variants of H1 are also regulated by their own chaperones in a similar manner.

To answer this question, we are identifying the proteins associated with soluble forms of replication-dependent and replication-independent H1 variants. Tetracycline-inducible U2OS cell lines were generated to express 6xHis-tagged human histone H1.1 and H1.2 (replication-dependent variants), and H1.0 and H1.x (replication-independent variants). Soluble whole cell extracts made from those cell lines were purified by ion exchange chromatography and then affinity chromatography. Proteins associated with these H1 variants were identified by mass spectrometry. We found that some of the H1 variants form two separate complexes while others only exist in one complex. Importantly, we found that some linker histone associated factors associate with both replication-dependent and replication-independent H1 variants while other factors are specific for one type of histone H1. These findings will contribute to the understanding of the non-redundant functions of histone H1 variants, and to the possible new discoveries of proteins that impact chromatin structure by interacting with linker histones.

Experimental design issues in ChIP-seq experiments: the effects of sequencing parameters on the downstream analysis

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Chromatin immunoprecipitation followed by sequencing (ChIP-seq) is currently the dominant high throughput assay in functional genomic and epigenomic research, especially for mapping transcription factor-DNA interactions and histone modifications. Read length and sequencing depth are two important experimental design parameters for ChIP-seq experiments. These parameters have direct impact on the downstream analysis, such as alignment, peak calling, motif analysis, allele specific analysis, and differential analysis.

In this study, we hypothesize that different designs might be more optimal for specific goals. We quantitatively and systematically investigate the impact of multiple designs on the downstream analysis. Specifically, we contrast short and long paired-end (PE) and single-end (SE) read designs and their impact to detecting signal from repetitive regions of the genome with data-driven computational experiments. While we identify setting in which specific designs perform better than the others, our results, overall, suggest that performance of the experimental designs heavily depend on the choice of the analysis tools and their specific parameters.
Silent chromatin varies across the ribosomal DNA array in Saccharomyces cerevisiae

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The ribosomal DNA locus (rDNA) in Saccharomyces cerevisiae contains ~200 tandem repeats of the ribosomal RNA genes. Despite being one of the most transcriptionally active regions in the genome, the rDNA contains silent chromatin that represses transcription of Pol II genes and limits recombination between rDNA repeats. Because the rDNA is a series of identical repeats, silent chromatin at the rDNA has been assumed to be uniform. However, we have obtained striking evidence to the contrary. Analysis of recombination and expression of a HIS3 gene in different rDNA repeats has revealed that rDNA silent chromatin is not uniform. We refer to variation in the strength of silent chromatin at the rDNA as rDNA position effects (rDNA-PE). Possible mechanisms for regulation of rDNA-PE are being tested, including differences in Pol I activity, chromatin structure at the HIS3 promoter, and the role of the well-known silencing protein Sir2. The role of Hmo1, a high-mobility group protein associated with actively transcribed 35S rRNA genes, is being analyzed to examine the relationship between Pol I activity and rDNA-PE. Preliminary ChIP experiments indicate that Pol I activity is not influencing rDNA-PE. Modified histones at the HIS3 gene promoter are being examined to assess chromatin structure. Sir2 at HIS3-containing repeats is being analyzed to determine if different amounts of Sir2 are associated with different repeats. Additionally, a tetracycline regulatable system is being used to assess the role of Sir2 in rDNA-PE. Preliminary data show that upon removal and subsequent reestablishment of Sir2, silencing is lost and reestablished to pre-treatment levels. These results indicate that while Sir2 is required for silencing it is not the sole factor determining the silencing capacity of a repeat. This work has uncovered a novel epigenetic characteristic of rDNA silent chromatin and is likely to shed light on mechanisms that regulate chromatin function in repeated DNA sequences.

Analyzing the gene regulatory roles of SIN3 isoforms

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SIN3 acts as the master transcriptional adapter protein that interacts with the deacetylase RPD3 and other accessory proteins to form the SIN3/HDAC complex. In fruit flies, a single gene codes for different isoforms of SIN3, of which SIN3 220 and SIN3 187 are the major isoforms. While SIN3 220 is predominantly expressed in proliferative tissues, SIN3 187 is expressed during differentiated phase of the fly development. Previously, we have shown that SIN3 isoform specific complexes are non-redundant in function however, their functional differences in regulating gene transcription remain unclear. Our hypothesis is that the isoform specific SIN3 complexes are targeted to both similar and distinct regions of the Drosophila genome, thereby affecting different biological processes. ChIP-qPCR analysis of some of the putative genomic targets of SIN3 show that both the SIN3 isoforms are enriched equivalently. The enrichment of SIN3 187 over the putative binding sites of SIN3 220 is likely due to the loss of SIN3 220 upon overexpression of SIN3 187. Transcriptome analysis in cells in which SIN3 187 is ectopically expressed or in cells with reduced levels of SIN3 220,suggest differential regulation of gene expression by the SIN3 isoforms. The protein components that preferentially interact with the SIN3 220 complex may modulate the function of the SIN3 220/HDAC complex. CAF1/p55 has been shown to preferentially interact with the SIN3 220 complex. Based on the functional and structural properties of p55, we predict that p55 might play a role in targeting or stabilizing SIN3 220 to chromatin. Here, we found that ectopic expression of a mutant variant of p55 results in the reduction of SIN3 220 at putative SIN3 target sites. Currently, we are examining the role of p55 in the SIN3 220 complex by biochemical approaches. Results of these studies are anticipated to provide insight into the regulation of transcription by the SIN3 isoform specific HDAC complexes.
Considerable evidence demonstrates that children with an anxious temperament (AT) are particularly sensitive to new social experiences, showing increased freezing behavior, decreased communications, and increased pituitary-adrenal and autonomic activity. However, despite substantial knowledge of these behavioral phenotypes, the molecular mechanisms governing them remain unclear; in part due to the difficulty in accessing human brain tissues for molecular analysis. Recently, we validated a nonhuman primate model of AT that demonstrates structural and functional similarities between rhesus monkeys and humans in the neural circuits mediating increased freezing, decreased vocalizations, and increased cortisol levels. We further identified that while AT-associated metabolic brain activity is heritable, it also is greatly influenced by the environment. Notably, AT-related brain metabolism in the central nucleus of the amygdala (CeA) and the anterior hippocampus (aHIP) significantly differ in their heritability (aHIP > CeA), suggesting that even though these structures are closely linked, and both involved in AT, they are differentially governed by genetic and environmental influences. Since epigenetics marks (e.g. DNA methylation) are mediated by the environment, these data led us to hypothesize a role for DNA methylation in the development of AT. To test this hypothesis, we profiled 5-mC levels in genomic DNA from the CeA region of monkeys (N = 24) using the ‘reduced representation bisulfite sequencing’ (RRBS) detection method. Data passing a stringent quality control (N = 419,507 CpG dinucleotides) were subjected to a fixed-effects linear model and an algorithm that takes into account the underlying dependence between adjacent CpG sites. This analysis identified 994 CpG sites (corresponding to N = 109 genes) with methylation levels that are associated with AT (false discovery rate (FDR) p-value < 0.01). However, these results do not survive a more strict multiple testing correction (bonferroni), leading us to investigate correlations between methylation and gene expression in these monkeys to identify candidate loci associated with AT. While we do not find a significant overlap of the 109 differentially methylated genes and 371 genes with significant AT-associated expression levels (uncorrected p < 0.05), there is a significant overlap between the gene ontological (GO) terms associated with each of these gene sets (p = 0.001). In addition, these overlapping terms (N = 17) are significantly enriched for neuronal GO terms (p = 0.001), which include nervous system development and behavior. Consistent with the role of these terms in anxiety and depression, several genes that derive them are of high interest; such as ribosomal protein S6 kinase polypeptide 3 (RPS6KA3), which has been implicated in AT, and several members of the Semaphorin gene family, which have been implicated in autism and schizophrenia. Together, these data indicate that despite the inability of the methylation and gene expression data to directly identify consistent genes contributing to AT, their overlapping GO terms provide insights into the neuronal pathways associated with the AT phenotype and reveal novel risk targets of anxiety and depression.
DNA methyltransferases (DNMTs) are responsible for establishing (DNMT3A, 3B, 3L) and maintaining (DNMT1) DNA methylation genome-wide. Aberrant DNA methylation is frequently observed in cancer; however, little is known about how regulation of this modification goes awry. In this study, we aim to understand how DNA methylation is regulated by the DNMTs throughout the genome by identifying specific and broad changes in methylation patterning upon depletion of the DNMTs. We utilize siRNA technology to acutely deplete NCCIT embryonic carcinoma cells of DNMT mRNA (individually and in combination), and then assay the impact on genome-wide DNA methylation patterns using the HumanMethylation450 Bead Chip (450K array). Depletion of DNMT1 (individual/combination) resulted in widespread hypomethylation, most notably in gene bodies, 3'UTRs, and intergenic sequences. DNMT3 knockdown resulted in more specific changes in DNA methylation, but surprisingly, more hypermethylation (predominately in gene bodies) than hypomethylation events occurred. These specific hypermethylation events, particularly in samples with DNMT3B KD, significantly overlapped with sites hypomethylated in DNMT1 KD conditions, indicating a potential cross-regulatory role for DNMT1 and DNMT3B in regulating DNA methylation across gene bodies. To gain a more comprehensive genome-wide view of DNA methylation in the absence of DNMT3B, we performed Methyl-CpG-Binding-Domain (MBD)-seq on DNMT3B KD cells. DNA methylation patterns observed by MBD-seq were dynamic across the genome, and overall displayed trends towards hypomethylation. However, analysis of the most significant methylation changes (> 4-fold) revealed that more hypermethylation events occur in intronic sequences, consistent with results obtained using the 450K array. Notably, these hypermethylation events predominately occur in high-expressing genes that are marked by H3K36me3. To further investigate the overlap between DNMT1 hypomethylated and DNMT3B hypermethylated sites, we examined DNA methylation in HCT116 colorectal carcinoma cells lacking (KO) or over-expressing (KI) DNMT1/DNMT3B. Interestingly, a marked number of CpG sites that gained methylation in the DNMT3B KO overlapped significantly with sites that became hypermethylated in DNMT1 and DNMT3B KI, and hypomethylated in DNMT1 KO. Additionally, these HCT116 hypermethylated CpG sites gained methylation in NCCIT DNMT3B KD (individual/combination) and lost methylation in DNMT1 KD. Taken together, these results suggest that DNMT1 and DNMT3B co-regulate DNA methylation at conserved loci across cell types in an opposing fashion, providing novel insight into a potential regulatory mechanism for DNA methylation patterning. Further elucidation of this DNMT1 and DNMT3B co-regulation holds the potential to yield novel therapeutic strategies for correcting aberrant methylation events in cancer.
4C-seq identifies long range chromosome interaction and regulatory elements

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The cystic fibrosis transmembrane conductance regulators (CFTR) gene encodes a cAMP-activated chloride ion channel, which when mutated, causes the genetic disease cystic fibrosis. Cis-regulatory elements play an important role in tissue-specific regulation of CFTR. Some of these elements locate far away from the gene promoter and regulate gene expressions through chromosome looping which establishes physical interaction. In order to identify these regulatory elements and understand the molecular mechanism of CFTR gene regulation, 4C-seq (chromosome conformation capture combined with high-throughput sequencing) technique has been established in the lab. This new technology aims to identify and quantify interactions between selected genomic sites (viewpoints) and unknown regulatory sequences. The 4C-seq experiment was carried out in Caco2 cells which express high level of CFTR. CFTR promoter, CTCF insulator sites and previously known enhancers were chosen as viewpoints. Results highly correlate with previous 3C data. 4C data indicates that in Caco2 cells, CFTR gene locus has a very complex 3D structure, which contributes to CFTR gene regulation. 4C-seq is also extremely useful in finding unknown disease associated genes and regulatory elements. Recently, Wright et al. identified genomic loci which modify lung disease severity in CF patients by genome-wide association study (GWAS). Several SNPs identified in this study reside at 11p13 region, surrounded by ELF5, EHF, APIP, and PDHX genes. DNase-seq data in primary airway cells previously published by our lab identified several DNaseI hypersensitive sites (DHS) in this region. Other lab members have shown that some of these DHS exhibit enhancer activities by luciferase assay. To investigate how the surrounding genes are regulated and how they modify the lung disease severity in CF patients, EHF promoter, APIP promoter and two DHS are selected as viewpoints and 4C-seq is performed with Calu3 cells. Sequencing data is going through analysis and results will be out soon.

Universal Homogenous Bioluminescent Assay to monitor the Effect of Modulators on Various Classes of Methyltransferases in vitro

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Methylation/demethylation of DNA and Proteins play major roles in modulation of the epigenome and has been implicated in a wide variety of human diseases. Recent biochemical and biological data suggest that the enzymatic activities of several of these enzymes have pathogenic roles in cancer, inflammation, and neurodegenerative diseases. Thus, pharmacological modulation of these enzymes by small molecules will be beneficial in developing novel therapeutics for multiple unmet medical needs. Towards this goal of searching for activators/inhibitors of these enzymes for the development of next generation of drugs, screening assays for these modulators are urgently needed. To address these unmet needs, we have developed a novel assay that monitors the activities of these enzymes and their modulation by small molecules. The assay is bioluminescent based, HTS formatted and highly sensitive. The assay is universal since it is based on monitoring the formation of the universal product S-adenosylhomocysteine (SAH), i.e., capable of detecting changes inactivity of a broad range of methyltransferases such as DNA, protein, and small molecules methyltransferases. In addition, the assay has been validated for all classes of protein methyltransferases (Lysine and Arginine), and with different types of substrates (small peptides, large proteins, or even nucleosomes). This enables determining the specificity of these enzymes and their substrate requirements. The assay has high signal to background and low C.V. The assay is robust (Z’ value > 0.7) and has been validated using various plate densities such as 96-, 384, and 1536-well plates. A strong feature of this assay is its utility with broad range of substrates with no limitations on the use of high concentrations of substrates or the composition of the substrates (short vs. long peptides), thus enabling the generation of kinetic data and determining the mechanism of action of various modulators of methyltransferases of interest.
Interstitial cells of Cajal (ICC) are mesenchymal cells that express the receptor tyrosine kinase (RTK) Kit and function as electrical pacemakers and neuromodulators of the gastrointestinal (GI) tract. ICC are reduced or their functions are dysregulated in GI motor disorders and aging. ICC depletion can be reproduced by inhibiting Kit signaling in vivo and in vitro and may reflect phenotypic changes including loss of Kit expression rather than cell death. We hypothesized that epigenetic repression of gene transcription underlies ICC loss and dysfunction in aging and these changes may be reversible. To track the fate of ICC in vivo, we indelibly labeled Kit+ cells in KitCreERT2/+;R26mT-mG/mT-mG mice by inducing mG (membrane-targeted EGFP) expression by tamoxifen administration on postnatal days (PD) 7-10. By flow cytometry, phenotypically identified (Kit+CD34–) and genetically tracked (mG+Kit+CD34–) gastric ICC declined by 57% and 50%, respectively, between PD11 and PD163. These changes were accompanied by a concomitant increase in mG+ cells in the Kitlow/–CD34– fraction that accounted for 49% of the decline in mG+Kit+CD34– ICC. The age-related decline in Kit was accompanied by a >13-fold increase in the frequency of mG+CD34– cells expressing the related RTK Pdgfra. We also observed a 2.3-fold increase in the proportion of KitlowCD34–Pdgfra+ cells in mixed-background mice between 6 and 104 weeks of age. Together, these data indicated that aging-associated ICC depletion reflects a phenotypic transition involving reduced Kit and increased Pdgfra expression. To further study these changes and their mechanisms, we used FACS to establish clonal, conditionally immortalized ICC from the stomach of PD12 Immortomice (e.g., ICL2A). Initially, ICL2A cells displayed ICC-like light microscopic and ultrastructural morphology, expressed Kit and generated regular electrical pacemaker activity at frequencies characteristic of the mouse antrum. With extended culturing, Kit expression declined, pacemaker activity became sporadic or nonexistent, and the cells were found to express Pdgfra. Microarrays, RNA-seq and RT-PCR showed that 28% and 34% of the 542 and 403 genes uniquely expressed in freshly purified neuromodulator and pacemaker ICC, respectively, were downregulated in ICL2A cells along with 43% of the genes specifically implicated in ICC function. Thus, cultured ICC replicated the phenotypic changes and dysfunction seen in aging. ICL2A cells showed elevated expression of the epigenetic regulators Ehmt2, Suv39h1, HP1 isoforms, Hdac8 and Dnmt1, as well as members of polycomb repressive complexes PRC1 and 2 including Ezh2. ChIP-seq revealed that compared to the entire genome, repressed ICC genes had a ~2.24-fold reduction of promoter occupancy by the activating histone mark H3K4me3; whereas binding of the inhibitory H3K27me3 and H3K9me3 marks increased 1.43- and 1.18-fold, respectively. Inhibition of Ezh2 with adenosine dialdehyde (Adox) reduced occupancy of the Kit promoter by H3K27me3 and H2AK119ub1 and increased Kit mRNA and protein expression. The latter effect could be reproduced with the specific Ezh2 inhibitor EPZ-6438 and siRNA-mediated Ezh2 knock-down, but not with the Ehmt2 inhibitor BIX-0129, the Hdac inhibitor SAHA or the Dnmt blocker RG-108. 2-week exposure to Adox increased the proportion of cells displaying electrical pacemaker activity from 8.5% (6/71) to 62.5% (10/16). We conclude that polycomb-mediated epigenetic repression plays a key role in aging-associated ICC depletion and dysfunction. Inhibition of these repressive mechanisms may help restore ICC function in GI motor disorders and aging. Support: NIH R01 DK58185, Mayo Clinic Center for Individualized Medicine.
Diverse etiologic-specific effects on the epigenome during liver disease

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Aberrant DNA methylation landscapes are a ubiquitous feature of human carcinogenesis that are manifested by global hypomethylation leading to genomic instability and promoter-specific hypermethylation resulting in tumor suppressor gene silencing. Alterations to the normal DNA methylation pattern are particularly apparent in hepatocellular carcinoma (HCC), a disease that manifests itself in over three quarters of a million new patients per annum [1]. Indeed, a variety of tumor suppressors (e.g. p53, E-cadherin) are well characterized as hypermethylated and silenced in HCC [2-4]. An important attribute of tumorigenesis in HCC is the presence of the premalignant stage of cirrhosis in 80% of cases. One substantial confounder to interpretation of the methylome in liver carcinogenesis is the occurrence of multiple etiologies acting across all stages of disease to varying degrees, including Hepatitis C virus (HCV), Hepatitis B virus (HBV), and chronic alcoholism. To address this unique issue, we profiled the methylation landscape in more than 170 primary human samples, resulting in the most comprehensive genome-wide methylation panel in liver cancer to date. Our results demonstrate a definitive impact of specific etiologies on the DNA methylation landscape in cirrhosis and HCC. Interestingly, HCV alone demonstrated a profound impact during cirrhosis, which was overtaken in HCC by the effects of chronic alcoholism. Furthermore, these CpG dinucleotides that were aberrantly methylated during HCV-cirrhosis and conserved in HCC were enriched for genes involved in tumorigenesis, such as AKT1, MTOR, EGFR, and CDK6, suggesting a potential pathogenic role. Additionally, we unveiled large domains encompassing many genes that showed stepwise hypermethylation, as well as hypomethylated regions in HCC-HCV that were exacerbated in HCC-EtOH. This study emphasizes etiologic-specific effects which may provide pathogenic/therapeutic biomarkers for downstream clinical applications. Research performed thus far is our first step toward unveiling the epigenome in cirrhosis and hepatocellular carcinoma, which will be juxtaposed with future genomic and epimutational screenings (e.g. transcription, histone modifications) to determine the true interplay between epigenetic marks in liver disease. Altogether, this research has significant potential to result in diagnostic, prognostic, and therapeutic epigenetic signatures in cirrhosis and hepatocellular carcinoma.

Targeted repression of AXIN2 and MYC gene expression using designer TALEs

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Designer TALEs (dTALEs) are chimeric transcription factors that can be engineered to regulate gene expression in mammalian cells. Whether dTALEs block gene transcription downstream of signal transduction cascades, however, has yet to be fully explored. Here we tested whether dTALEs can be used to target genes whose expression is induced by the canonical Wnt/ß-catenin signaling pathway. TALE DNA binding domains were engineered to recognize sequences adjacent to Wnt responsive enhancer elements (WREs) that control expression of axis inhibition protein 2 (AXIN2) and c-MYC (MYC). These custom DNA binding domains were linked to the mSin3A interaction domain (SID) to generate TALE-SID chimeric repressors. The TALE-SIDs repressed luciferase reporter activity, bound their genomic target sites, and repressed AXIN2 and MYC expression in HEK 293 cells. We generated a novel HEK293 cell line to determine whether the TALE-SIDs could function downstream of oncogenic Wnt/ß-catenin signaling. Treating these cells with doxycycline and tamoxifen stimulates nuclear accumulation of a stabilized form of ß-catenin found in a subset of colorectal cancers. The TALE-SIDs repressed AXIN2 and MYC expression in these cells, which suggests that dTALEs could offer an effective therapeutic strategy for the treatment of colorectal cancer.
Beyond division, differentiation and death: A hitherto unknown role of the Drosophila retinoblastoma tumor suppressor protein 1

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First identified as a cell cycle regulator, the evolutionarily conserved retinoblastoma tumor suppressor protein (Rbf in flies) is also a key regulator of developmental gene expression programs that control cell differentiation, metabolism, and apoptosis. Our comprehensive profiling for Rbf1 using genome wide ChIP sequencing technique revealed novel genomic targets for Rbf1 encoding signaling components of the Insulin, Hippo, Wnt and the Jak/Stat pathways. Intriguingly, Rbf1 also associated with core components of the less understood –apical-basal and planar cell polarity (PCP) pathway.

To define a possible role for Rbf1 in planar polarity, we reduced the level of Rbf1 in the developing wing imaginal discs of Drosophila third instar larvae through RNAi and tested for PCP pathway effects in the emerging adult fly wings. PCP in the adult flies is visualized as a regular array of distally pointing hairs on the apical surface of wing; reducing the level of Rbf1 resulted in a deviation from the normally observed pattern. Furthermore, reducing Rbf1 levels by RNAi altered the mRNA levels of key PCP genes in wing imaginal discs, suggesting that Rbf1 may directly control these genes. Analysis of the effect of overexpression of Rbf1 on the cloned promoter fragment of a core PCP gene, strabismus / van gogh in S2 cells indicated that vang is one physical and a direct functional target of the retinoblastoma protein.

Significance: PCP plays a critical role in collective cell movement during embryonic development (observed during body axis elongation, neural crest development invertebrates, and dorsal closure in flies) and in processes such as wound healing. The disrupted regulation of PCP has been additionally connected to abnormal ability to invade during metastasis. The asymmetric localization of the PCP proteins is imperative for establishing polarity, but we lack knowledge about how transcriptional regulation of PCP genes may influence cell polarity. This study points to a hitherto unsuspected link between Rbf1 protein function and this conserved signaling pathway; of particular interest will be determining whether the connection is conserved in higher eukaryotes. Given the spectrum of mutations associated with the retinoblastoma protein observed in virtually all cancer cell types, we propose that RB family inactivation contributes to enhanced metastatic potential of cancer cells.

DNA Sequence and Modifications Control Nucleosome Mechanical Stability

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The fundamental unit of genome compaction in eukaryotic cells is the nucleosome particle, where 147 base pairs of DNA wrap around a protein core. Understanding the physical basis of how DNA sequence and modifications affect nucleosome dynamics and nucleosomal DNA exposure will help elucidate how genomic and epigenetic modifications regulate cellular functions, cell differentiation and cancer development. We used single-molecule force fluorescence spectroscopy and single-molecule DNA cyclization measurement to investigate local conformational dynamics of the nucleosome under tension and its modulation by DNA sequence and modifications. First, we observed that the nucleosome can unwrap asymmetrically and directionally under force. Second, we show that the nucleosome unwrapping process is controlled by local DNA flexibility. We demonstrated the correlation between DNA flexibility and unwrapping force by varying DNA sequence, DNA methylation and DNA mismatches. DNA methylation decreases DNA flexibility and reduces nucleosome mechanical stability while DNA mismatches have opposite effects. Our work elucidates the fundamental physical principles for regulation role of DNA sequence and modifications on DNA metabolism pathway.
Characterization of a gene modifier locus for cystic fibrosis lung disease severity

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Cystic fibrosis (CF) is a disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. The major cause of death in this disorder is lung pathology, which is partially due to epithelial cell dysfunction. EHF is an Ets family transcription factor expressed in the epithelial cells lining the trachea and bronchi. It can act as either a transcriptional activator or repressor, but its targets in the lung epithelium are largely unknown. EHF maps to chromosome 11, in the 11p13 region. A genome-wide association study (GWAS) identified single nucleotide polymorphisms (SNPs) in an intergenic region of 11p13 immediately downstream of EHF that associate with lung disease severity in CF patients. We hypothesize that EHF is an important regulator of lung epithelial function in health and disease states, and that it controls a network of genes critical for normal processes in these cells. We used a combination of high throughput methods to determine direct targets of EHF. Chromatin immunoprecipitation followed by sequencing (ChIP-seq) was used to identify genome-wide binding sites of EHF. EHF depletion with siRNA followed by RNA-sequencing identified genes regulated by this transcription factor. We found that the majority of EHF binding sites were intergenic or intronic, as expected for binding to tissue-specific cis-regulatory elements. EHF knockdown altered expression of genes involved in epithelial differentiation, cell proliferation, and cell motion. EHF binding sites were found near differentially regulated genes, implicating them as direct targets. These results suggest that EHF regulates pathways important for epithelial function. We identified multiple putative enhancers in the intergenic section of 11p13 using ChIP-seq for modified histones and will perform further analyses to determine which genes they regulate. These results suggest that EHF regulates pathways important for epithelial function, and further studies will determine the role of the 11p13 region and EHF in lung epithelial function.

Novel regulatory control for the Retinoblastoma (RB) co-repressor: implications for transcriptional mechanisms in development and disease.

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The Retinoblastoma (RB) family of proteins play critical roles in normal development through their governance of genes involved in cell fate. During normal growth RB family activity is tightly regulated through Cdk-dependent phosphorylation, resulting in their dissociation from E2F family transcription factors. In addition, the RB pathway is also governed through the ubiquitin-proteasome system, with deregulated degradation of RB proteins frequently associated with human cancer. Recent studies from our labs have shown in Drosophila that the Retinoblastoma family (Rbf) proteins are subjected to proteasome mediated turnover during embryonic development and this process enhances Rbf engagement in transcriptional repression. This positive linkage between Rbf1 activity and its destruction indicates that repressor function is governed in a manner similar to that described by the degron theory of transcriptional activation. To understand the relationship between RB family stability and their repressor function during early mammalian development, we initiated studies in mouse embryonic stem (ES) cells. Our studies suggest that differentiation of mouse ES cells is associated with the establishment of a functional RB pathway and simultaneous changes in stability of RB family members. As pluripotent ES cells are characterized by unrestrained cdk activity which plummets at the onset of differentiation, we speculated that the observed changes in protein stability upon ES cell differentiation reflects an intimate relationship between RB phosphorylation and stability. Indeed, we show that phosphorylation dependent turnover of RB, p107 and p130 is mediated by an evolutionarily conserved and autonomous instability element (IE) located in their C-terminal regulatory domain. Moreover, stabilizing mutations within the IE elements also debilitate them for transcriptional repression. We conclude that the overlap of degron sequences and repression modules is a conserved feature shared among the RB homologues, and represents a novel mode of transcriptional repression. Together, these findings implicate Retinoblastoma family IE region as a regulatory nexus linking repressor potency to the ubiquitin-proteasome system in development and disease.
Determining interaction partners of the transcriptional repressor Snail in cancer cells
Shawn Krueger and Archana Dhasarathy, University of North Dakota

Metastasis is a critical event in cancer progression that involves the spread of tumor cells from a primary cancer to secondary sites in the body. A process known as 'Epithelial to Mesenchymal Transition' (EMT), which causes cells to change their shape and migrate during development, is hypothesized to play an important role in metastasis. The Snail and Slug transcription repressor proteins regulate the expression of genes that are essential for EMT. Histone modification changes (such as histone deacetylation) are known to be associated with Snail repression, but the exact mechanism behind Snail regulation is unknown.

The Snail family of zinc-finger transcription factors consists of Snail 1 (Snail), Snail2 (Slug) and Snail3 (Smuc). These molecules are composed of highly conserved carboxy terminal region containing four to six C2H2-type zinc fingers, which mediate sequence-specific interactions with DNA promoters containing an alternate E-box sequence, CAGGTC. Overexpression of Snail or Slug is associated with metastasis, tumor recurrence, and poor prognosis. Our preliminary data indicated that while Snail and Slug repress many genes in common, they also regulate unique sets of genes, despite binding to the same sequence.

We hypothesize that Snail and Slug can regulate unique sets of genes based on (a) their interactions with different sets of proteins that can target them to different regions of the genome, (b) additional sequence specificity, or (c) some combination of (a) and (b). To this end, we propose to use Co-immunoprecipitation (Co-IP) experiments to pull down protein complexes that associate with Snail and Slug and identify them by Mass Spectrometry. In addition, we plan to use genome-wide Chromatin Immunoprecipitation sequencing (ChIP-seq) to determine genome-wide binding sites of Snail and Slug. We have currently cloned Snail into 3xFLAG-tagged adenoviral vectors and infected MCF-7 breast cancer cell line. We further successfully immunoprecipitated Snail from MCF-7 lysates. Currently, we are preparing to use Mass spectrometry to identify binding partners of Snail. Additionally, we are also currently making adenoviral vectors of other EMT proteins such as Slug, Zeb1, Zeb2 and Twist. We predict that our method will find unique interaction partners of Snail and Slug.

Inhibition of chondrocyte differentiation by Histone H3 K36M mutation
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The highly recurrent Lys27Met (K27M) mutation of histone H3.3 (H3F3A) and H3.3 (HIST3H1B) genes in human Diffuse Intrinsic Pontine Gliomas (DIPG) leads to significantly lower levels of genome-wide K27 trimethylation on histone H3. Previously, we demonstrated that the H3 K27M protein interacts with- and inhibits the catalytic activity of the SET domain-containing lysine methyltransferase EZH2 subunit of the PRC2 complex. Recently, an exome sequencing study identified the Lys36Met (K36M) mutation of the H3.3 (H3F3B) gene in 95% of chondroblastomas. Like H3K27, H3K36 is methylated by SET domain-containing methyltransferases including SETD2. We investigated the molecular mechanisms through which H3K36M may drive tumorigenesis of this rare pediatric cancer. We found that expression of H3K36M transgenes lead to a global loss of K36 methylation (K36me1/2/3), and to increased H3K27me1/2/3 levels. Additionally, an H3K36M transgene inhibited differentiation of mesenchymal lineages (adipocyte, chondrocyte, and osteocyte) in a mouse cell culture model. Moreover, aberrant expression of chondrocytes-specific genes was correlated with reduced level of K36 trimethylation in gene bodies determined by ChIP-qPCR analysis. This correlation was further supported by partial block of chondrocytic differentiation in SETD2 knockout cells. Our data support a model where the H3K36M mutation may drive tumorigenesis through impaired chondroblast differentiation by global loss of H3K36 methylation.
Sequencing of First-strand cDNA Library Reveals Full-length Transcriptomes

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Massively parallel strand-specific sequencing of RNA (ssRNA-seq) has emerged as a powerful tool for profiling complex transcriptomes. However, many current methods for ssRNA-seq suffer from underrepresentation of both 5' and 3' ends of RNAs, which can be attributed to second-strand cDNA synthesis. 5' and 3' ends of RNA harbor crucial information for gene regulation, namely transcription start sites (TSSs) and polyadenylation sites, respectively. A lack of information about RNA ends hampers genome-wide studies of gene regulatory mechanisms. Here, we report a novel ssRNA-seq method that does not involve second-strand cDNA synthesis, as we directly ligate adaptors to the first-strand cDNA (DLAF).

Sequencing of DLAF libraries followed by a novel analysis pipeline enables the profiling of both 5' ends and polyadenylation sites at near-base resolution. Furthermore, the significantly simplified workflow of the DLAF method with fewer enzymatic reactions results in higher yield, mappability, and complexity of sequence reads compared to the conventional method. DLAF offers the first genomics tool to obtain the “full-length” transcriptome with a single library.

Studies of a Brain-enriched Epigenetic Mark Reveals Sex Differences and a Genome-wide Loss in Response to Acute Stress

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Background: Posttraumatic stress disorder (PTSD) is often the result of a single acute stressor and shows evidence of epigenetic influences. Elucidating the molecular mechanisms by which an acute stressor contributes to PTSD is critical for understanding it and other stress-related psychiatric disorders. Stressors such as abuse/neglect increase the risk of developing PTSD and are associated with alterations of DNA methylation (5-methylcytosine; 5-mC). The recent identification of another modified form of cytosine, 5-hydroxymethylcytosine (5-hmC), which is enriched in post-mitotic neurons and is mediated by environmental cues such as oxidative stress, raises new questions on the role of this base in neuropsychiatric outcomes. Here we investigated the role of 5-hmC during an acute stress response.

Methods: Adult C57BL/6 mice (n = 4) were subjected to a 30-minute restraint stress followed by a one-hour recovery. Brain tissue were sectioned and labeled with antibodies against 5-hmC and examined by confocal microscopy to measure the raw intensity density of 5-hmC.

Results: Control females have significantly less genome-wide 5-hmC in hippocampus, cortex, and thalamus brain regions compared to males (p < 0.05). Exposure to acute stress resulted in a further significant reduction of 5-hmC in these brain regions of both males and females compared to controls (p < 0.05).

Conclusion: These studies reveal a clear sex difference in genome-wide 5-hmC levels in adult brain tissues and that acute stress results in a genome-wide reduction of 5-hmC in the brain. These data suggest a role for 5-hmC in response to a single acute stress, and perhaps PTSD.
Long non-coding RNAs (lncRNAs) are a newly recognized class of molecules that impinge on the expression of protein-coding genes through a poorly understood mechanism. Previous studies have established the GAL cluster-associated lncRNAs of S. cerevisiae as a model lncRNA-dependent gene regulatory system that repress expression of the protein-coding GAL genes. Recently, however, by utilizing strains lacking the RNA helicase, DBP2, or RNA decay enzyme, XRN1, we uncovered a previously unrecognized role for the GAL lncRNAs in activating gene expression. Furthermore, we also showed that the GAL lncRNAs promote induction in wild type cells. In characterizing the mechanism by which GAL lncRNAs promote gene activation, we found that the GAL lncRNAs function through formation of RNA-DNA hybrids. In fact, overexpression of Ribonuclease H1, which specifically digests the RNA strand in RNA-DNA hybrids, restores repression in dbp2Δ and this restoration is dependent on the presence of GAL lncRNAs. Furthermore, we conducted a chromatin immunoprecipitation assay and detected a higher level of RNA-DNA hybrids at the GAL gene cluster in dbp2Δ. We suggest that the GAL lncRNAs activate gene induction by changing chromatin status through formation of RNA-DNA hybrids. This enables cells to more effectively trigger new transcriptional programs in response to cellular cues. Interestingly, many mammalian lncRNAs are involved in the regulation of genes involved in cell differentiation and pluripotency, cellular processes that require precise temporal control. Our study could provide a novel mechanism for how these lncRNAs function in the proper timing of gene expression.

Recombinant antibodies to trimethylated lysine marks

Antibodies to histone marks are essential tools in epigenetics research. However, most currently available antibodies to histone marks are polyclonal, whose lack of reproducibility and uneven quality have been a widely recognized problem. We aim to address this “antibody bottleneck” by generating high-quality recombinant antibodies to histone marks. Recombinant antibodies generated from antibody repertoires (libraries) in vitro, are inherently monoclonal and renewable, thus fundamentally eliminate lot-to-lot variation. We first identified a low-affinity antibody that was highly specific to trimethylated lysine but lacked sequence specificity. We designed a second-generation library based on structure-function analysis of the lead antibody, from which we successfully generated a recombinant antibody to H3K9me3 with high specificity and affinity. This recombinant antibody performed very well in common epigenetics applications including ChIP, and it exhibited no lot-to-lot variation as expected (Hattori et al., Nature Methods 10, 992-995 (2013)).

Based on this success, we are expanding our effort to generate recombinant antibodies to other trimethylated lysine marks. Iterative processes of library design and selection enabled us to evolve specificity and affinity of the lead antibody, resulting in successful generation of high-quality recombinant antibodies directed to H3K4me3, H3K36me3 and H4K20me3 as well as a “pan” antibody to trimethylated lysine. Together, these results demonstrate the feasibility of generating recombinant antibodies to a range of histone marks, which will accelerate epigenetics research.

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In fruit flies, as in mammals, males have one X chromosome while females have two. This imbalance in gene dosage is potentially lethal, and is corrected by a process termed dosage compensation. Male flies achieve dosage compensation by upregulating expression from their single X chromosome approximately twofold. This process requires the Male Specific Lethal (MSL) complex, which binds transcribed genes on the X chromosome and modifies chromatin to increase expression. It is currently unclear how the extraordinary selectivity of MSL complex for X-chromatin is achieved. One hypothesis is that sequences limited to the X chromosome contribute to X-identification. To test this idea we have designed a functional test for the ability of X-limited DNA sequences to recruit dosage compensation to an autosomal region. Large autosomal deficiencies that make part of the 2nd chromosome hemizygous were generated. The dose of genes within the deleted region is reduced, leading to lethality. DNA sequences to be tested for recruitment of dosage compensation were then inserted on a wild type second chromosome opposite the deficiency. Our expectation is that recruitment of dosage compensation to the hemizygous portion of the second chromosome will increase expression and rescue males to adulthood. Preliminary tests of candidate X-linked sequences show partial rescue, as a few deficiency males are recovered as pharate adults. Optimization of this system will enable its use as a quantitative measure of the ability of specific sequences to attract compensation. In the future, this system will be used to dissect the genetic factors that enable X-linked DNA sequences to attract dosage compensation.

Reprogramming of somatic cells to induced pluripotent stem cells (iPSC) using the four transcription factors Oct4, Sox2, Klf4 and c-Myc is not well defined. Recent advances in understanding the reprogramming process have focused on the changes in the transcriptional and epigenetic landscape of single cells or isolated population subsets. However, while useful in defining various stages that occur throughout the reprogramming process, these studies are also limited by their use of a single cell type of origin, restricting their ability to describe universal molecular cornerstones of reprogramming. In this project we will utilize different primary cell types, isolated from a mouse with the four reprogramming factors under the control of a doxycycline inducible promoter, to identify molecular changes common to cells of varying origin. To accomplish this goal we have isolated cells from a variety of ectodermal tissue (neural stem cells, astrocytes and keratinocytes) from newborn mice. While we have confirmed successful reprogramming for each cell type by analyzing Nanog and Esrrb expression, they each reprogram with a varying degree of efficiency. The inhibition of the methyltransferase (Dot1L) responsible for H3K79 methylation results in increased reprogramming efficiency in each of these cell types. Future experiments will aim to identify the transcriptional changes unique to each cell type and changes that occur in common regardless of starting cell type. Further, we hope to link the transcriptional changes to altered levels of H3K79 methylation to better understand the underlying cell dynamics that lead to the successful reprogramming of somatic cells into iPSC.
The Role of the N-terminal Region in EED Recognition of H3K27me3 Nucleosomes

Tyler Weaver, University of Iowa

Histone post-translational modifications (PTMs) are key determinants of the local chromatin landscape and critical for regulation of eukaryotic gene expression. These histone marks are deposited by a vast number of histone modifying enzymes and preferentially recognized by specific associated reader domains. The Polycomb repressive complex 2 (PRC2), is a multisubunit methyltransferase complex consisting of EZH1/2, Suz12, EED1/2/3/4 and RbAp46/48 which is essential for developmental gene repression. PRC2 catalyzes the trimethylation of lysine 27 on the histone 3 tail (H3K27me3) through the catalytic component, EZH1/2. Recognition of H3K27me3 by the EED subunit leads to allosteric activation of PRC2 activity, which is important for the maintenance and propagation of this repressive mark. Despite recent advances, our understanding of the structural basis of chromatin association by EED is incomplete. The canonical EED protein consists of seven C-terminal WD40 repeats that fold into a structured β-propeller domain and a disordered N-terminal region. A previously reported crystal structure of an N-terminally truncated EED in complex with H3K27me3 peptide revealed interaction between the trimethyl lysine and an aromatic cage consisting of Phe 97, Phe 148 and Tyr 365 resting on top of the EED propeller. However, it was found that the WD40 domain alone is insufficient for binding H3K27me3 nucleosomes, suggesting a critical role of the N-terminal region in chromatin targeting. Moreover, the four isoforms of EED (EED1/2/3/4) vary only in their N-terminal region suggesting that each isoform of EED may differentially target PRC2 to H3K27me3 nucleosomes and/or differentially regulate PRC2 activity. Herein, we present preliminary data for the interaction between H3K27me3 nucleosomes and the N-terminal regions of the four EED isoforms describing their role in recognition of H3K27me3 using a combination of molecular and biophysical techniques. Ultimately, these data help further our understanding for the structural basis of EED bound to H3K27me3 and how recognition of a histone PTM may be mediated through interactions outside of the histone mark itself.
Enzymatic Regulation of a DEAD-box RNA Helicase Promotes Efficient mRNP Assembly During Transcription

Wai Kit Ma, Purdue University

Proper RNA structure and ribonucleoprotein (RNP) complex formation are critical for gene expression. The dynamic changes in RNP composition occur co-transcriptionally and can be coupled to splicing, RNA export, and efficient translation. A class of RNA helicases called DEAD-box proteins plays a fundamental role in RNA and RNP structure in every aspect of RNA metabolism. However, it is not currently known how RNA structural changes within mRNA influences gene expression. Our laboratory recently demonstrated that the S. cerevisiae DEAD-box protein Dbp2 is an active, double-stranded RNA-directed helicase in vitro. Furthermore, we have found that Dbp2 is associated with actively transcribing genes and is required for efficient assembly of mRNA-binding proteins Yra1, Nab2 and Mex67 with mRNA. We now provide evidence that the recruitment of Dbp2 to actively transcribing genes is RNA-dependent. Moreover, we show that Dbp2 interacts directly with Yra1 and that this interaction is necessary for removal of Dbp2 from mRNA in vivo. Finally we show that Yra1 regulates the helicase activity of Dbp2 by promoting ATP hydrolysis and release of Dbp2 from single-stranded RNA. We present a model whereby Dbp2 is recruited to nascent RNA during transcription, unwinds secondary structure in the RNA to facilitate RNA-binding protein assembly, and is then released from the single-stranded RNA product by Yra1. Thus, a sequential order of events involving regulation of a DEAD-box RNA helicase is required for efficient mRNP assembly in the nucleus. The fact that the mammalian ortholog of Dbp2 and Yra1, termed p68 and Aly, respectively, also directly interact suggests that this activity and regulatory mechanism is conserved in multicellular eukaryotes.
Genomic Position Effects on Double-Strand Break Repair Are Correlated With Chromatin Environment

William Engels, University of Wisconsin–Madison

Cells possess an array of alternative methods to repair double-stranded DNA breaks. Some methods are error-prone but rapid and have few prerequisites while others are more accurate but with other disadvantages. We measured the relative usage of two of these methods, end-joining (NHEJ) and single-strand annealing (SSA), in Drosophila germ cells as a function of the genomic location of the break. We find that the choice between these two repair processes is highly dependent on where the break lies. Several sites had exceptionally low NHEJ frequency with a compensatory increase of SSA. Homologous repair also increases in these cases suggesting that the effect is driven by a reduction in NHEJ rather than a stimulation of SSA. These position effects are closely correlated with the chromatin domain types previously defined on the basis of how strongly each genomic site is associated with various histone modifications and other chromatin binding factors. We also identified a large number of individual chromatin factors that are specifically correlated with the relative usage of NHEJ and SSA. We propose that the cell’s choice of double-strand break repair method is highly sensitive to which chromatin proteins are present in the vicinity of the break.

Delayed development induced by toxicity to the host can be inherited by a bacterial-dependent, transgenerational effect

Yael Fridmann Sirkis, Weimann Institute of Science

Commensal gut bacteria in many species including flies are integral part of their host, and are known to influence its development and homeostasis within generation. We report an unexpected impact of host–microbe interactions, which mediates multi-generational, non-Mendelian inheritance of a stress-induced phenotype. We have previously shown that exposure of fly larvae to G418 antibiotic induces trans-generationally heritable phenotypes, including a delay in larval development, gene induction in the gut and morphological changes. We now show that G418 selectively depletes commensal Acetobacter species and that this depletion explains the heritable delay, but not the inheritance of the other phenotypes. Notably, the inheritance of the delay was mediated by a surprising trans-generational effect. Specifically, bacterial removal from F1 embryos did not induce significant delay in F1 larvae, but nonetheless led to a considerable delay in F2. This effect maintains a delay induced by bacterial-independent G418 toxicity to the host. In line with these findings, reintroduction of isolated Acetobacter species prevented the inheritance of the delay. We further show that this prevention is partly mediated by vitamin B2 (Riboflavin) produced by these bacteria; exogenous Riboflavin led to partial prevention and inhibition of Riboflavin synthesis compromised the ability of the bacteria to prevent the inheritance. These results identify host–microbe interactions as a hitherto unrecognized factor capable of mediating non-Mendelian inheritance of a stress-induced phenotype.

Evolution of multi-subunit RNA polymerases and co-evolution of Eukaryote complexity with the RNAP II CTD

Zachary Burton, Michigan State University

One class of Eukaryotic interfering RNA polymerase and multi-subunit RNA polymerases (RNAPs) each have two double psi beta barrel (DPBB) motifs (a distinct pattern for compact 6-beta sheet barrels) at their active site. Between beta sheets 2 and 3 of the beta subunit type DPBB of multi-subunit RNAPs is a sandwich barrel hybrid motif (SBHM) that interacts with conserved initiation and elongation factors in the three domains of cellular life that diverged from the last universal common ancestor (LUCA). Analysis of RNAP core protein motifs, therefore, indicates that RNAP evolution can be traced from the RNA-protein world to LUCA to the present day, spanning about 4 billion years. In the Eukaryotic lineage, I posit that splitting RNAP functions into RNAPs I, II and III and innovations developed around the carboxy terminal domain (CTD) of RNAP II and the extensive CTD interactome may largely describe how greater structural, cell cycle, epigenetic and signaling complexity co-evolved in Eukaryotes relative to Eubacteria and Archaea. Essentially, this is a story of genesis told from the point of view of multi-subunit RNAPs including an Old and New Testament of gene transcription and regulation.
Transcriptional regulation of the Insulin Receptor gene in Drosophila melanogaster

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The insulin-signaling pathway is a conserved signaling cascade that plays a major role in regulating metabolism and growth in diverse metazoan species. The insulin receptor (IR) functions to direct insulin to specific target tissues, and to initiate the response to the hormone. Deregulation of the IR gene has been found in type-II diabetes and in a number of mammalian tumors. Drosophila has one insulin-like receptor (InR) that is 35% identical to human IR. It is essential for development and is required for the formation of the epidermis and nervous system during embryogenesis. Natural variation of the InR gene is also associated with regional size variation and stress tolerance. The InR gene has three transcription start sites, and nearly 40 Kb intron regions that may serve as binding sites for transcription factors. Previous studies have shown that Drosophila forkhead protein FOXO (dFOXO) and ecdysone receptor (EcR) directly target the InR gene, and effectively regulate its gene transcription in response to nutrient and steroid hormone. Our research identified, for the first time, that retinoblastoma family members, Rbf1 and Rbf2, strongly associate with the promoter-proximal region of the InR gene, and Rbf1 functionally represses the InR promoter in vitro. To identify the cis-regulatory elements (CREs) for Rbf1, dFOXO, and EcR, and systematically analyze their roles in controlling the InR gene expression, we generated a luciferase reporter library by dissecting the InR gene regulatory regions into 1.5 Kb fragments and fusing to the basal promoter of the InR gene. By in vitro assays, we found that those reporters had distinct basal expression levels, and differential behaviors in response to dFOXO/EcR over-expression or ecdysone treatment. In addition, some fragments also drove tissue specific expression in vivo. These suggest that the CREs in the InR gene control both the spatial and temporal patterns of the gene expression. In cooperate with bioinformatics, we will deciphering the “cis-acting code” of the InR gene, and identify the roles of the CREs in vivo. Our study will offer molecular insights into the nutrient growth-maturation coordination in Drosophila development and the deregulation of insulin receptor in multiple human tumors.

H3 deviants in nature - an in silico study

Yokiko Hiromoto-Ruiz, Michigan State University

In higher eukaryotes, a conserved mechanism for silencing of developmentally important genes involves methylation of lysine-27 (K27) of nucleosomal histone H3s by the Polycomb-group (PcG) protein, Polycomb Repressive Complex 2 (PRC2). In humans, somatic mutation leading to substitution of K27 with methionine (M) in H3F3A, one of the two genes encoding the replication-independent histone variant H3.3, is strongly associated with pathogenicity in pediatric cases of diffuse intrinsic pontine glioma (DIPG), a type of malignant brain tumor. This effect is potentially mediated through inhibition of the catalytic subunit of PRC2, Ezh2. Based on the observed ability of H3F3A K27>M to globally alter gene expression in human cells, we hypothesized that a similar mechanism may have evolved in nature, potentially as a mechanism to help reprogram terminally differentiated cells. In this ‘in silico’ study, we identified 762 H3-like protein sequences, representing 4,799 distinct genes, from the current NCBI reference protein sequence dataset, and assessed these for K27 variation. Among these, we identified only two such H3 ‘deviants’ (H3K27>R, H3K>Q), suggesting that the hypothesized mechanism has not been widely deployed during evolution. However, surprisingly, one of the proteins is encoded in the genome of Arabidopsis thaliana, an extensively studied model for plant biology. To our knowledge, this variant has not been examined in the context of its ability to influence chromatin functions. This protein is especially interesting because it also exhibits a substitution at K9, another lysine that is methylated associated with silencing, and because it is strongly expressed in rapidly dividing and/or undifferentiated cells. We will present this data along with results of our ongoing in silico search for H3 deviants harboring substitution at other conserved residues of the amino-terminal tail subject to regulatory, post-translational modification (R2, T3, R8, S10, T11, K14, R17, K18, K23, R26, K36), within and beyond NCBI data sets.
Illumina 450K microarray vs. RRBS in genome-wide methylation study: which one to choose?

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DNA methylation is one of the main epigenetic events in normal physiology and diseases. Abnormal DNA methylation can be used for biomarkers of disease diagnosis and prognosis. Illumina HumanMethylation450 BeadChip (450K array) and reduced representation bisulfite sequencing (RRBS) are the most commonly used platforms for genome-wide methylation research. Performance comparisons have been conducted between the two platforms but are mostly limited to overall correlation. It is not clear which one has better accuracy and precision for DNA methylation measurement or what the impact is on downstream differential methylation analyses. This leads to uncertainty for investigators to choose which method to use in their study design and experiment. In this study we performed in-depth comparisons for datasets with both 450K array and RRBS (one with WGBS) and tried to answer the above questions. We compared their respective genomic coverage, accuracy of methylation measurement and sensitivity to technical variability. The results showed that although 450k array interrogated more genes, the number of CpGs per genes was 3-4 times fewer than RRBS. A higher percentage of CpGs was in CpG islands and intergenic regions for RRBS than the 450K array while the CpGs in exon, intron and around transcription start site were similar. For un-normalized data (Probe design bias correction), the 450K array had significantly higher overall methylation levels than RRBS (mean >16% and median >21%) but was less sensitive in measuring the methylation at very low (totally unmethylated) and high (totally methylated) range with deviation about 10%. The 450K array data showed higher variance across samples than the RRBS data. Probe design bias correction or normalization alleviated the probe II bias and moved the data closer to RRBS data although the overall correlation changed little. The normalization increased the sensitivity of detecting differentially methylated CpGs (DMCs). The lower precision of methylation measurement in the 450K array lead to a higher false positive call for DMCs while the compression of the value gave rise to a smaller difference between comparison conditions. About 35-38% of DMCs were commonly detected between the two platforms while 40% of DMCs detected by either platform did not change in the same direction. Understanding the differences and potential issues is essential in analysis and interpretation of the results from the platforms.

Towards a fluorescence based single-molecule epigenetic assay

Vasudha Aggarwal, University of Illinois–Urbana-Champaign

Chromatin Immunoprecipitation and bisulphite sequencing have many limitations, primarily, in being ineffective to allow single nucleosome level studies. Single-molecule view of chromatin can enable novel insights about the distribution of epigenetic modifications on each histone of an individual nucleosome. This will be particularly important to study the role of “bivalent nucleosomes” in different cellular processes and establish the “real” histone code. Keeping these goals in mind, I am working towards designing a single-molecule epigenetic assay to allow absolute quantification of histone modifications in different cells and tissues. I am currently testing many possible strategies to specifically pulldown single nucleosomes, directly from cells, on microscopic slides for visualization by total internal reflection microscopy (TIRF). I will discuss the details of this assay and our results on different histone modifications showing the specificity, resolution, and other strengths of our method. I will also present our data on how the distribution of histone modifications varies in various kinds of cell lines.
Defects in chromatin assembly during DNA replication can cause misregulation of epigenetic processes, including altered silencing phenotypes in Saccharomyces cerevisiae. One of the key factors involved in replication-coupled chromatin assembly is the histone H3/H4 chaperone Chromatin Assembly Factor 1 complex, CAF-1. CAF-1 promotes the deposition of histones H3/H4 onto newly replicated DNA during replication and localizes to chromatin via interactions with the DNA replication processivity factor PCNA. When CAF-1 activity is disrupted by either deletion of CAC1 or mutation of POL30, silencing is restored at a HMR locus containing a mutated E silencer, HMRae** (Miller, JBC, 285:35142, 2010). A similar phenotype is observed when the cell cycle-regulated kinase Cdc7, which is required for replication initiation, is mutated (Axelrod A, Mol Cell Biol, 11:1080, 1991). In addition to functioning during replication initiation, Cdc7 also phosphorylates H3 Thr45 in S. cerevisiae. However, in contrast to cdc7 mutants, we have found that silencing at HMRae** is not restored in H3 T45A mutants that mimic the unphosphorylated state at this residue. In humans, Cdc7 can phosphorylate p150, the largest subunit of CAF-1, and regulate CAF-1’s activity (Gerard A, EMBO Rep, 7:817, 2006). Here, we report that Cdc7 and CAF-1 interact in vivo in S. cerevisiae, and that CAF-1 is phosphorylated in a cell cycle-regulated manner. These and additional results support a model in which Cdc7 can influence silencing via a CAF-1-dependent chromatin assembly pathway during DNA replication.
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