

April 4–5, 2013

Natcher Conference Center, NIH Campus

*NCI Symposium on*

# **CHROMOSOME BIOLOGY**

## **Epigenetics in Development**

**Program and Abstract Book**



# **NCI Symposium on Epigenetics in Development**

April 4-5, 2013

**PROGRAM AND ABSTRACT BOOK**

**Natcher Auditorium, NIH Campus  
Bethesda, Maryland**

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Welcome to the National Cancer Institute (NCI) Symposium on Chromosome Biology. On behalf of the NCI Center for Cancer Research, Center of Excellence in Chromosome Biology, it is our great pleasure to welcome you to this symposium.

The mission of the Center of Excellence in Chromosome Biology (CECB) is to achieve a comprehensive understanding of the mechanisms involved in chromosome function, how aberrations in chromosomes and chromatin lead to disease, and how these defects can be corrected.

Towards achieving our mission, this symposium brings together internationally renowned experts in the fields of chromosome structure and function, with the focus on Chromatin Modifications, Stem Cells and Reprogramming, Epigenetics in Disease, Transcription and Non-Coding RNAs.

We hope this symposium offers you an opportunity to learn more about the current status of chromosome structure and function in development and disease, to share your research, and to discuss the use and implications of these advances for clinical applications.

Sincerely,

Center of Excellence in Chromosome Biology Steering Committee  
Center for Cancer Research  
National Cancer Institute

Kathrin Muegge, M.D., Symposium Co-Chair  
Shalini Oberdoerffer, Ph.D., Symposium Co-Chair  
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Susan Gottesman, Ph.D.  
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Mikhail Kashlev, Ph.D.  
David Levens, M.D., Ph.D.  
Michael Lichten, Ph.D.  
Tom Misteli, Ph.D.  
Andre Nussenzweig, Ph.D.  
Thomas Ried, M.D.  
Carl Wu, Ph.D.



# NCI Symposium on Epigenetics in Development

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# Agenda



NCI Symposium on  
Epigenetics in Development  
Natcher Auditorium, NIH, Bethesda, MD  
April 4-5, 2013

**Thursday, April 4, 2013**

7:30 a.m. Registration

8:30 a.m. **Welcome**  
*Gordon Hager, Ph.D., Chair of the CECB, National Cancer Institute*

**SESSION 1: CHROMATIN MODIFICATIONS I**  
Chair: *Dinah Singer, Ph.D., National Cancer Institute*

8:45 a.m. **“Chromatin Assembly And Disassembly”**  
*Jessica Tyler, Ph.D., University of Texas, M.D. Anderson*

9:15 a.m. **“Manipulating Long-Range Genomic Interactions To Reprogram The Beta Globin Locus”**  
*Gerd Blobel, M.D., Ph.D., University of Pennsylvania*

9:45 a.m. **“The 3D Genome Landscape And Long Range Control Of Gene Expression”**  
*Bing Ren, Ph.D., University of California-San Diego*

10:15 a.m. Break

**SESSION 2: CHROMATIN MODIFICATIONS II**  
Chair: *Tom Misteli, Ph.D., National Cancer Institute*

10:45 a.m. **“Regulating mRNA Levels Globally: The Roles Of Myc And Of Promoter DNA Melting”**  
*David Levens, M.D., Ph.D., National Cancer Institute*

11:15 a.m. **“Chromatin And Cell Fate Specifications In *C. Elegans*”**  
*Oliver Hobert, Ph.D., Columbia University*

11:45 a.m. **“Epigenetic Regulation In The *C. Elegans* Germ Line”**  
*William Kelly, Ph.D., Emory University*

12:15 p.m. **LUNCH BREAK AND POSTER VIEWING**

### **SESSION 3: STEM CELLS AND REPROGRAMMING**

Chair: *Kathrin Muegge, M.D., National Cancer Institute*

- 2:00 p.m.     **“Remodeling The Epigenome Through Reprogramming”**  
*George Daley, M.D., Ph.D., Children’s Hospital Boston, Harvard University*
- 2:30 p.m.     **“Hdac6 Is A Stem Cell-Specific Modulator Of Tip60-P400 Function”**  
*Thomas Fazzio, Ph.D., University of Massachusetts Medical School*
- 3:00 p.m.     **“Function Of Histone Variant H2A.Z In ESC Self-Renewal And Differentiation”**  
*Keji Zhao, Ph.D., National Heart, Lung and Blood Institute*
- 3:30 p.m.     Break

### **SESSION 4: EPIGENETICS IN DISEASE**

Chair: *Paul Meltzer, M.D., Ph.D., National Cancer Institute*

- 3:50 p.m.     **“Epigenetic Regulation Of Aging”**  
*Anne Brunet, Ph.D., Stanford University*
- 4:20 p.m.     **“Epigenetic Heterogeneity In Cancer”**  
*Bradley Bernstein, M.D., Ph.D., Massachusetts General Hospital, Harvard Medical School and Broad Institute*
- 4:50 p.m.     **“Histone Methylation In Leukemia Development And Maintenance”**  
*Scott Armstrong, M.D., Ph.D., Memorial Sloan-Kettering Cancer Center*
- 5:20 p.m.     **“DNA Methylation And The Regulation Of Normal And Malignant Hematopoiesis”**  
*Margaret Goodell, Ph.D., Baylor College of Medicine*
- 5:50 p.m.     Adjourn

### **Friday, April 5, 2013**

### **SESSION 5: TRANSCRIPTION I - MECHANISMS**

Chair: *Shalini Oberdoerffer, Ph.D., National Cancer Institute*

- 8:30 a.m.     **“Direct Measurement Of Transcription Initiation And Elongation In Living Human Cells”**  
*Daniel Larson, Ph.D., National Cancer Institute*
- 9:00 a.m.     **“Mechanisms And Evolution Of Transcriptional Regulation In Mammals”**  
*Duncan Odom, Ph.D., Cancer Research UK, Cambridge Research Institute*
- 9:30 a.m.     **“Mediator And The Regulation Of RNA Polymerase II Transcription”**  
*Joan Conaway, Ph.D., Stowers Institute for Medical Research*
- 10:00 a.m.    **“Fungal Chromatin Structure And Function”**  
*Oliver Rando, M.D., Ph.D., University of Massachusetts Medical School*

10:30 a.m. Break

**SESSION 6: TRANSCRIPTION II - CONSEQUENCES**

Chair: *Mikhail Kashlev, Ph.D., National Cancer Institute*

11:00 a.m. **“RECQL5, Suppressor Of Transcription-Associated DNA Recombination”**

*Jesper Svejstrup, Ph.D., London Research Institute*

11:30 a.m. **“Driving RNA Polymerase II Through Chromatin On The Many Roads To Splicing Regulation”**

*Maria Carmo-Fonseca, M.D., Ph.D., Institute of Molecular Medicine, University of Lisbon*

12:00 p.m. **“Epigenetic Regulation Of Alternative Pre-mRNA Splicing”**

*Shalini Oberdoerffer, Ph.D., National Cancer Institute*

12:30 p.m. **LUNCH BREAK AND POSTER SESSION**

**SESSION 7: NON-CODING RNAS**

Chair: *Susan Gottesman, Ph.D., National Cancer Institute*

2:00 p.m. **“Noncoding Function For mRNA In Chromatin Insulator Activity”**

*Elissa Lei, Ph.D., National Institute of Diabetes and Digestive and Kidney Diseases*

2:30 p.m. **“Long Noncoding RNAs In Epigenetic Regulation”**

*Jeannie Lee, M.D., Ph.D., Massachusetts General Hospital, Harvard Medical School*

3:00 p.m. **“RNA-Mediated Transcriptional Silencing In Plants”**

*Craig Pikaard, Ph.D., Indiana University*

3:30 p.m. **“Epigenetic Genome Control By RNAi And Heterochromatin Machinery”**

*Shiv Grewal, Ph.D., National Cancer Institute*

4:00 p.m. **“Multi-Generational Epigenetic Inheritance And Germline Immortality”**

*Scott Kennedy, Ph.D., University of Wisconsin-Madison*

4:30 p.m. Adjourn

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# Poster Abstracts

## P-1

### **GENOME-WIDE MAPPING OF UV LESIONS REVEAL RELATIONSHIPS BETWEEN CHROMATIN, TRANSCRIPTION, DNA DAMAGE SUSCEPTIBILITY, AND REPAIR**

Adar, S.<sup>1</sup>, Garrison, P.<sup>1</sup>, Sancar, A.<sup>2</sup> and Lieb, J.D.<sup>1</sup>

<sup>1</sup>Department of Biology, Carolina Center for Genome Science, and Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC; <sup>2</sup>Department of Biochemistry and Biophysics, University of North Carolina School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC

Chromatin has strong influence on the susceptibility of DNA to damage and the efficiency of its repair. The relationship between nucleosome binding, transcription and DNA damage has been difficult to study in living cells. The fact that all three influence each other makes the relative contribution of any one factor difficult to isolate. Nucleosome binding can both protect DNA from damage, and hinder the access of repair enzymes. Lesions in the DNA can affect nucleosome stability and block RNA polymerases, but at the same time, induce a transcriptional stress response. Finally, active transcription is associated with a more open chromatin structure, but is also known in itself to enhance repair. To decipher this complex network of relationships, we developed a genome-wide DNA-damage detection assay based on the isolation of UV-damaged genomic DNA followed by high throughput sequencing. We employ this assay in the yeast *S. cerevisiae*. We show that UV-induced cyclobutyl pyrimidine dimers (CPDs) are widespread throughout the genome, are unaffected by nucleosomes, and are dictated mostly by the underlying DNA sequence. We follow the repair rates of these damages by two repair mechanisms, nucleotide excision repair (NER) and photoreactivation (PHR). As expected, our preliminary results show faster repair by photoreactivation, faster repair at expressed genes, and delayed repair at sites packaged in nucleosomes. In combination with existing high throughput genomic methods for nucleosome and RNA mapping, this approach will be a powerful tool to decipher the relationship between gene expression, chromatin structure, DNA damage formation, and DNA repair.

**DNase2TF: AN EFFICIENT ALGORITHM FOR FOOTPRINT DETECTION**

Baek, S., Sung, M. and Hager, G.L.

Laboratory of Receptor Biology and Gene Expression, CCR, NCI, NIH, Bethesda, MD

By deep sequencing of DNase-seq data and analyzing the nucleotide-resolution DNase cleavage profiles, it is possible to achieve digital footprinting of transcription factors. The DNA regions that are bound by proteins and relatively protected from enzymatic cutting are termed footprints. Decreasing costs and higher yields of improved sequencing methods make digital footprinting more feasible, making de novo discovery of relevant transcription factors possible. However, reliable and fast computational methods must be widely available to enable footprint detection from DNase-seq data. Here we present DNase2TF, a new detection algorithm that scans DNase I hypersensitive sites for putative footprints. The algorithm is implemented in MATLAB and C. Source codes are provided as online supplementary information. When compared to previous methods, DNase2TF is 100 times faster and more accurate in predicting actual transcription factor binding sites. We also assess a limitation of using footprints for binding prediction that may be caused by insufficient sequencing and/or certain binding events that do not produce footprints. DNase2TF allows rapid identification of footprint candidates, but care should be taken when inferring transcription factor binding through footprints.

### P-3

## **CORE PROMOTER ELEMENTS ARE NOT ESSENTIAL FOR TRANSCRIPTION IN MAMMALS**

Barbash, Z.S., Weissman, J.D., Mu, J. and Singer, D.S.

Experimental Immunology Branch, CCR, NCI, NIH, Bethesda, MD

Core promoter elements are thought to be the docking site for transcription factors binding, and thereby essential for transcription initiation. The role of core promoter elements has been studied for decades in reporter systems. The central model claim that each cis sequence has an epigenetic role in navigating transcription initiation. We use here the MHC class I gene as model to study transcription in a transgenic mouse. MHC class I genes are ubiquitously expressed and subject to both tissue-specific and hormonal regulation. The core promoter contains four conventional elements: CCAAT, a TATAA-like element, an Sp1 binding site and a canonical Inr. The in vivo function of these elements was determined by mutating each individually within the context of the native gene. Surprisingly, none of the elements was essential for transcription since no single mutation eliminated transcription. Indeed, mutation of any one of the elements resulted in increased promoter activity, indicating that these elements function as transcriptional modulators. Further, each of the elements was found to have a distinct function, contributing uniquely to tissue-specificity, hormonal responses or both. The core promoter elements do not affect start site selection, demonstrating that they do not invoke a cryptic promoter. However, they do modulate relative start site usage. The patterns of chromatin modification reflect the expression status of the different promoters. In tissues where the different promoters constructs support active transcription, histone H3K4 trimethylation is high and H3K9 trimethylation is low. Conversely, H3K9 trimethylation is high and H3K4 trimethylation low across the gene in tissues where the promoter constructs are less active. The wild type promoter is activated by interferon, while the Inr and Sp1 mutants repress transcription in response to interferon treatment. Finally, the CAAT element was found to have dual function, both as a transcriptional regulator and as a barrier element. The barrier function correlated with the binding of C/EBP, CTCF and Cohesin to the wild type but not CAAT element, In addition to variegated expression across generations when the CAAT element was mutated. 3C analysis revealed that the CAAT expression pattern is affected by chromosome conformation along the PD1 gene. Remarkably, these results demonstrate that none of the elements homologous to canonical core promoter elements are necessary for promoter activity. However, they do contribute to the fine-tuning of the tissue specific patterns of expression, extracellular signaling, overall promoter activity and chromatin modifications

**IDENTIFICATION OF EARLY REPLICATING FRAGILE SITES REVEALS A NOVEL SOURCE OF DNA REARRANGEMENT LEADING TO B CELL LYMPHOMAS**

Barlow, J.H.<sup>1</sup>, Faryabi, R.B.<sup>1</sup>, Callen, E.<sup>1</sup>, Wong, N.<sup>1</sup>, Malhowski, A.<sup>1</sup>, Chen, H.T.<sup>1</sup>, Gutierrez-Cruz, G.<sup>2</sup>, McKinnon, P.<sup>3</sup>, Wright, G.<sup>4</sup>, Robbiani, D.<sup>5</sup>, Staudt, L.<sup>4</sup>, Fernandez-Capetillo, O.<sup>6</sup> and Nussenzweig, A.<sup>1</sup>

<sup>1</sup>Laboratory of Genome Integrity, CCR, NCI, NIH, Bethesda, MD; <sup>2</sup>Laboratory of Muscle Cells and Gene Regulation, NIAMS, NIH, Bethesda, MD; <sup>3</sup>Department of Genetics, St. Jude Children's Research Hospital, Memphis, TN; <sup>4</sup>Metabolism Branch, CCR, NCI, NIH, Bethesda, MD; <sup>5</sup>Laboratory of Molecular Immunology, Rockefeller University, NY, NY; <sup>6</sup>Genomic Instability Group, Spanish National Cancer Research Centre (CNIO), Madrid, Spain

DNA double strand breaks (DSBs) in B lymphocytes are thought to arise stochastically during replication or as a result of targeted DNA damage by activation induced cytidine deaminase (AID) in G1. Lymphocytes are susceptible to replication-stress-inducing agents such as hydroxyurea (HU) since they undergo several bursts of replications during their development. Using genome-wide profiling of DNA repair proteins RPA, SMC5,  $\gamma$ -H2AX, and BRCA1 in primary murine lymphocytes treated with HU, we identify a novel class of recurrent DNA lesions at early replicating sites, termed Early Replication Fragile Sites (ERFS). ERFS regions are enriched for repetitive DNA elements, including LINE and SINE DNA, and occur at transcriptionally active, gene-rich 'open' chromatin regions. Both HU treatment and inhibition of the major S phase checkpoint kinase ATR induce DNA damage at ERFS, while treatment with low doses of the polymerase inhibitor aphidicolin does not, indicating that ERFS fragility is distinct from common fragile sites (CFS). Interestingly, the IKZF1, BACH2, and BCL2 genes are among the strongest ERFS hits, and are frequently rearranged in B cell lymphoma. Moreover, ERFS can translocate to AID-induced breaks at the Immunoglobulin Heavy chain gene (IgH). Moreover, greater than 50% of common amplifications/deletions observed in human diffuse large B cell lymphoma map to ERFS. We propose that replication damage occurring at ERFS early in S phase generates genome rearrangements affecting lymphoma progression.

**DISTINCT RECURRENT DNA METHYLATION ABERRATIONS IN INDUCED PLURIPOTENT STEM CELLS MADE WITH DIFFERENT REPROGRAMMING FACTORS**

Batada, N.<sup>1</sup>, Ji, J.<sup>1</sup>, Sharma, V.<sup>1</sup> and De Carvalho, D.<sup>2</sup>

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The dedifferentiation of somatic cells into induced pluripotent stem (iPS) cells due to expression of reprogramming factors results in a genome-wide change in DNA methylation. Methylation at a significant proportion of CpG sites in iPS cells differs from that of embryonic stem (ES) cells. Failure to erase parental DNA methylation has been shown to influence differentiation propensity of iPS. In addition, time in culture is known to influence the methylome of iPS cells, with extended culture increasing resemblance of iPS epigenetic state to that of ES cells. Here, we isolated the tissue of origin and time in culture to determine the extent to which the DNA methylation aberrations in iPS cells depend on the reprogramming factors used. We used Illumina HumanMethylation450 platform to assess DNA methylation levels in 15 iPS cell lines made from a common fibroblasts cell source with either the Yamanaka factors (OCT4, SOX2, KLF4 and MYC) or the Thomson factors (OCT4, SOX2, NANOG and LIN28) and cultured for the same amount of time. This specific experimental setting allowed us to identify reprogramming factors-independent differences between iPS cells and ES cells. Strikingly, it also allowed us to identify reprogramming factors-dependent differences. The pattern of aberrations revealed that reprogramming with Yamanaka factors mainly resulted in DNA demethylation failure while reprogramming with Thomson factors mainly resulted in DNA methylation failure. Aberrantly methylated genes in Yamanaka iPS were enriched for NANOG targets while the aberrantly methylated genes in Thomson iPS were depleted for OCT4 targets. Despite the presence of c-Myc, aberrantly methylated genes in Yamanaka iPS were not differentially methylated in cancer, while spuriously methylated genes in Thomson iPS were also spuriously methylated in cancer cells. Our study reveals that the choice of reprogramming factors influences the extent of DNA methylation aberrations in iPS cells and should be taken into consideration in disease modeling and regenerative medicine.

**Pc2, THE POLYCOMB GROUP PROTEIN RECRUITS THE c-Myb TRANSCRIPTION FACTOR INTO POLYCOMB BODIES AND INHIBITS ITS ACTIVITY**

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The transcription factor c-Myb plays a critical role in development of virtually every lineage in the hematopoietic system by direct regulation of genes controlling cell cycle, lineage commitment during cellular differentiation, and apoptosis. Essential role for c-Myb in definitive hematopoiesis was convincingly showed in mouse embryos with homozygous disruption of the c-myb gene where mice lacking functional c-Myb died around day 15 in utero due to severe anemia. More recently it was shown that even modulation of c-Myb expression levels and/or its activity in mice has a strong impact on the proper balance of hematopoietic cell production and differentiation. Therefore, strict regulation of c-Myb activity is crucial for balanced production of hematopoietic cells. Dynamic post-translational modifications of c-Myb, such as phosphorylation, acetylation, ubiquitylation, and SUMOylation, play an important role in modulation of its activity. Conjugation of the SUMO proteins dramatically decreases the transactivation capacity and the proteolytic turnover of c-Myb. The importance of this modification is underscored by the fact that SUMOylated lysines are located in the conserved region of the negative regulatory domain frequently lost during oncogenic activation of c-Myb protein. Two c-Myb-specific SUMO-1 E3 ligases PIASy and TRAF7 were identified recently. PIASy enhances SUMOylation of c-Myb in the nucleus, while TRAF7 sequesters and SUMOylates c-Myb in the cytosol. Interestingly, homozygous deletion of Piasy in mice did not affect the SUMOylation of nuclear c-Myb fraction, suggesting the existence of an additional c-Myb-specific nuclear SUMO-E3 ligase. Here we demonstrate that the Polycomb group protein Pc2 is a novel interaction partner for c-Myb. Pc2, which has been shown to have a SUMO E3-ligase activity for the co-repressors CtBP and CtBP2, increases conjugation of SUMO-1 to Lys499 and Lys523 located in NRD of c-Myb. Co-expression of Pc2 also has a strong negative effect on the c-Myb-induced reporter gene activation. Interestingly, we detect a similar inhibition of transactivation activities of the wild type (cMybwt) and the SUMOylation-deficient (cMyb2KR) mutant of c-Myb. Thus, covalent conjugation of the SUMO-1 protein to the negative regulatory domain of c-Myb is not a prerequisite for down-regulation of c-Myb activity by Pc2. Fluorescent confocal laser microscopy showed that Pc2 recruits the c-Myb transcription factor into specific subnuclear speckles called Polycomb bodies, where it co-localizes with another member of the PRC1 repressive complex, the Bmi-1 protein. The localization in these Polycomb repressive structures seems to be sufficient for downregulation of the c-Myb activity exerted by Pc2, since both, the wild-type c-Myb and the SUMOylation-deficient mutant, are very effectively recruited by Pc2. In conclusion, we have identified Pc2 as a novel interaction partner of c-Myb that suppresses the c-Myb's transcriptional activity not only through increased SUMOylation of its NRD, but also through sequestration of this oncoprotein into specific sub-nuclear Polycomb repressive structures.

**PHOSPHORYLATION OF CENTROMERIC HISTONE H3 VARIANT REGULATES CHROMOSOME SEGREGATION IN *S. CEREVISIAE***

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The centromeric histone H3 variant (CenH3) is essential for chromosome segregation in eukaryotes. In this study, we have identified post-translational modifications of *S. cerevisiae* CenH3, Cse4. Using a phosphoserine-specific antibody we showed that the association of phosphorylated Cse4 with centromeres is increased in response to defective microtubule attachment or reduced tension. ChIP analysis revealed that evolutionarily conserved Ipl1/Aurora B contributes to phosphorylation of Cse4, as levels of phosphorylated Cse4 were reduced at centromeres in an *ipl1* strain in vivo. This phosphorylation of Cse4 by Ipl1 was confirmed by in vitro assays. To elucidate the function of Cse4 phosphorylation we generated mutants to mimic the non-phosphorylated or phosphorylated state of Cse4, respectively. Analysis of these mutants revealed that a phosphomimetic *cse4-4SD* mutation suppresses phenotypes of *ipl1-2* and Ipl1 substrate mutants *dam1 spc34* and *ndc80* that are defective for chromosome bi-orientation. Cell biology approaches using a GFP labeled chromosome showed that *cse4-4SD* suppresses the chromosome segregation defects in *dam1 spc34* strains. Further we observed reduced growth and enhanced chromosome segregation defects in *cse4* phosphorylation mutants when combined with kinetochore COMA-complex mutants *okp1* and *ame1*. Based on these results we propose that phosphorylation of Cse4 may destabilizes defective kinetochores to promote bi-orientation to ensure faithful chromosome segregation.

**TARGETING THE CHD1 CHROMATIN REMODELER TO HISTONES RATHER THAN EXTRANUCLEOSOMAL DNA CONFERS SWI/SNF-LIKE CHARACTERISTICS**

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Nucleosome organization plays a fundamental role in all aspects of DNA metabolism and gene expression in eukaryotes, yet the mechanisms by which particular arrangements of nucleosomes are achieved is unclear. Here we show that the direction and outcome of nucleosome sliding by the Chd1 chromatin remodeler change dramatically depending on how it is targeted to nucleosomes. Using a Chd1-streptavidin fusion remodeler, we found that targeting via biotinylated DNA resulted in directional sliding toward the recruitment site, whereas targeting via biotinylated histones produced a distribution of nucleosome positions. Remarkably, the fusion remodeler shifted nucleosomes with biotinylated histones up to 50 bp off the ends of DNA and was capable of disrupting nucleosome wrapping within nucleosome arrays, similar to remodeling characteristics observed for SWI/SNF-type remodelers. These data suggest that forming a stable attachment to nucleosomes via histones, and thus lacking sensitivity to extranucleosomal DNA, appears to be sufficient for allowing a chromatin remodeler to possess SWI/SNF-like disruptive properties.

**EPIGENOMIC PLASTICITY ENABLES HUMAN PANCREATIC A- TO B-CELL REPROGRAMMING**

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Insulin-secreting  $\beta$ -cells and glucagon-secreting  $\alpha$ -cells maintain physiological blood glucose levels, and their malfunction drives diabetes development. Precise profiling of the epigenomic and transcriptional landscape of human pancreatic cells will yield to important insights of cell-type specific epigenomic landscapes. Human pancreatic islets were dispersed, stained, and subjected to FACS analysis to obtain cell populations highly enriched for  $\alpha$ -,  $\beta$ -, and exocrine cells. Using chromatin-immunoprecipitation sequencing (ChIP-Seq) and RNA-Seq analysis, we determined the H3K4me3 and H3K27me3 profiles and the transcriptional landscape of human pancreatic  $\alpha$ -,  $\beta$ -, and exocrine cells. We found that compared to exocrine and  $\beta$ -cells, differentiated  $\alpha$ -cells exhibited many more genes bivalently marked by the activating H3K4me3 and repressing H3K27me3 histone modifications. This was particularly true for  $\beta$ -cell signature genes involved in transcriptional regulation. Remarkably, thousands of these genes were in a monovalent state in  $\beta$ -cells, carrying only the activating or repressing mark. Our epigenomic findings suggested that  $\alpha$ - to  $\beta$ -cell reprogramming could be promoted by manipulating the histone methylation signature of human pancreatic islets. Indeed, we show that treatment of cultured pancreatic islets with a histone methyltransferase inhibitor lead to co-localization of both glucagon and insulin, and glucagon and insulin promoter factor 1 (PDX1) in human islets. Thus, human pancreatic islet cells display cell-type-specific epigenomic plasticity, suggesting that epigenomic manipulation could provide a path to cell reprogramming and novel cell replacement-based therapies for diabetes.

**THAILANSTATINS: NEW PRE-mRNA SPLICING INHIBITORS AND POTENT ANTIPROLIFERATIVE AGENTS DISCOVERED FROM BURKHOLDERIA THAILANDENSIS MSMB43**

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More than 90% of human genes undergo alternative splicing, which results in protein variants far greater than the number of the encoding genes. While this processing inherently provides for transcriptome diversity, aberrant alternative splicing has been implicated in numerous diseases conditions such as cancer and neurodegeneration. The cellular machinery of alternative splicing has thus become a valid drug target, and dozens of small molecule effectors interrogating the alternative splicing process have been identified and evaluated as drug candidates. The aim of our research is to discover new natural products from rare bacterial species that target eukaryotic epigenetics and gene transcriptional regulation including alternative splicing. Mining the genome sequence of *Burkholderia thailandensis* MSMB43 revealed a cryptic biosynthetic gene cluster highly resembling that of FR901464, a prototype pre-mRNA splicing inhibitor produced by *Pseudomonas* sp. No. 2663. Transcriptoal analysis identified a cultivation condition in which a key gene of the cryptic gene cluster is adequately expressed. Consequently, three new compounds, named thailanstatins A, B and C, have been isolated from the fermentation broth of *B. thailandensis* MSMB43 through natural product chemistry. Thailanstatins belong to the FR901464-family of microbial products biosynthesized by a hybrid polyketide synthase-nonribosomal peptide synthetase pathway. They have an overall structural similarity with FR901464, but differ by lacking an unstable hydroxyl group and by having a carboxyl moiety which together endow the compounds with a significantly greater stability than FR901464 under physiologically relevant conditions. In vitro assays showed that thailanstatins inhibit pre-mRNA splicing as potently as FR901464, with half-maximal inhibitory concentrations in the single to sub  $\mu$ M range, causing pre-mRNA to accumulate and preventing the production of mRNA and splicing intermediates. In vitro cell culture assays indicated that thailanstatins also possess potent antiproliferative activities in representative human cancer cell lines, with half-maximal growth inhibitory concentrations in the single nM range. This work provides new chemical entities as reagents for research and as drug candidates for development, and validates the *Burkholderia* species as an exciting new source of bioactive natural products.

**P-11**

**IN VIVO TRANSCRIPTION AND SPLICING KINETICS REVEALED BY FLUCTUATION ANALYSIS OF SINGLE-RNA MEASUREMENTS IN LIVING HUMAN CELLS**

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Eukaryotic transcription involves the coordination of many multi-subunit complexes, including the pre-initiation complex, the polymerase, the spliceosome and elongation and termination factors. Most knowledge in the field is inferred from ensemble and/or in vitro assays, giving a detailed but static picture. How these macromolecular machines coordinate in vivo remains unknown. Recently, we were able to observe transcription in live yeast cells by monitoring fluorescently-tagged nascent transcripts [Larson et al. 2011, *Science*, 322:475]. Here, we extend this method to a dual-color system in human cells, allowing one for the first time to resolve the kinetics of initiation, elongation, splicing and termination at the same gene in a living human cell. Using the orthogonal RNA-binding MS2 and PP7 bacteriophage coat proteins, we fluorescently labeled the largest intron and the 3'UTR of a stably integrated reporter gene. Fluorescence fluctuations recorded simultaneously in both channels at the transcription site reflect the initiation of pre-mRNA synthesis, the elongation kinetics of the polymerase, and the kinetics of intron and transcript release. We developed an approach based on cross-correlation to reveal the relative timing of these events for single transcripts. To that end, we derived a mathematical model that predicts the correlation functions depending on the timing of the underlying processes, allowing us to test hypotheses about polymerase progression and pausing at the single-molecule level in vivo. We observe an elongation speed of 1.5 kb/min, which is measured independently of initiation and termination. No pausing was detected in the body of the gene, but termination took an average of ~200 sec, during which co-transcriptional splicing was observed for a fraction of transcripts. These results raise the possibility that co-transcriptional RNA processing may result in a kinetic checkpoint at termination rather than pauses during elongation.

**DISTINCT NUCLEOSOME ORGANIZATION AROUND P53 RESPONSE ELEMENTS ASSOCIATED WITH CELL CYCLE ARREST AND APOPTOSIS**Cui, F.<sup>1</sup> and Zhurkin, V.B.<sup>2</sup><sup>1</sup>Thomas H. Gosnell School of Life Sciences, College of Science, Rochester Institute of Technology, Rochester, NY; <sup>2</sup>Laboratory of Cell Biology, CCR, NCI, NIH, Bethesda, MD

DNA is severely deformed in the complexes with p53 tetramer as revealed by crystal structures. The deformations occur not only at the CWWG motif in a p53 half site, but also in the central region of a full site. The overall curvature of p53-bound DNA resembles that found in nucleosomal DNA, suggesting that p53 may recognize its cognate sites in nucleosomes. Two recent papers established unambiguously that p53 is a nucleosome-binding protein. That is, the site in a nucleosome is accessible if it is bent in the direction similar to that found in the p53-DNA co-crystals; the site becomes inaccessible if the orientation is changed by  $\sim 180^\circ$ . This implies that the rotational positioning of a p53 site is critical for its accessibility, which may have direct impact on how p53 selectively activates its target genes in the chromatin context. We illustrate the functional importance of these findings by comparing the p53 sites associated with cell cycle arrest (CCA-sites) with those associated with apoptosis (Apo-sites), the two extreme cellular outcomes after p53 activation. To elucidate the rotational setting of p53 sites in nucleosomal DNA, we developed computational approaches based on well-established DNA sequence patterns related to nucleosome positioning. Unexpectedly, we found that the CCA-sites are oriented in such a way that they tend to be 'open' and 'exposed' on the nucleosomal surface. This predication is corroborated by just-published human nucleosomes mapped in high resolution. The nucleosome dyad positions are out of phase with the CCA-sites, at positions 4, 17, 34/35, 55/57, 65 and 73 from the sites, resembling the score profile we obtained. Interestingly, the CCA-sites reside in genomic regions with high nucleosome occupancy, indicating that the nucleosomes embedding CCA-sites are well positioned. Together, our data suggest that the CCA-sites are in a correct rotational setting, presenting themselves to p53, facilitating p53 recognition and subsequent gene induction. This assessment is consistent with in vivo data for the CCA-sites such as p21 5' RE. By contrast, our results show that Apo-sites, on average, have different rotational setting from the CCA-sites, consistent with the high-resolution human nucleosomes data. It suggests that the Apo-sites in general are likely to be 'closed' in the chromatin context. This prevents p53 binding and may require factors like chromatin remodeling complexes to expose the sites, which may be related to the 'delayed' kinetics of induction of apoptotic genes in vivo. In our opinion, the distinct nucleosome organization around the CCA- and Apo-sites is related to the difference in pyrimidine-purine (YR) dinucleotides in the central region of the sites. Unlike YCGR in the center of Apo-site, flexible YCAR is predominant in the center of the CCA-sites. These YCAR tetramers tend to bend into minor groove with large changes in Twist and Slide, forming so-called 'Kink-and-Slide' deformations critical for nucleosome positioning. These structural interpretations, if true, can explain why the CCA-sites are likely to occur in the regions with high nucleosome occupancy and why they are 'exposed' on the nucleosomes.

**POLYCOMB DETERMINES RESPONSES TO SMAD2/3 SIGNALING IN STEM CELL DIFFERENTIATION AND IN REPROGRAMMING**

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A central goal of developmental biology is to understand the remarkable ability of cells to balance robustness to maintain their identity with the plasticity to change upon specific developmental cues. Changing cellular identities requires signaling pathways to regulate expression of specific genes and epigenetic factors to stabilize the new expression patterns. So clearly, understanding how robustness and plasticity is balanced during development requires identification of the connections between signaling and epigenetics. We have recently discovered a mechanism that integrates the developmental signaling pathway Nodal-Smad2/3 and the epigenetic regulator Polycomb. In this mechanism, Nodal activated Smad2/3 was found to counteract Polycomb repression of specific target genes by recruiting the de-methylase Jmjd3 to remove the histone methylation mark H3K27me3, which is essential for Polycomb's function. Polycomb plays an essential role in both controlling dynamic gene expression changes during early development and in maintaining identity of robust cell types. We therefore decided to investigate if Polycomb determines the distinct responses to Smad2/3 signaling in robust versus dynamic cell states. Indeed, we found that Smad2/3 signaling maintained expression of the central pluripotency gene Oct4 during initiation of stem cell differentiation, but not in the robust ground state stem cells. This cell type specific role of Smad2/3 was dependent on Polycomb because in Polycomb deficient stem cells, Oct4 expression was signaling independent during differentiation. We noted that this context dependent role of Smad2/3 in controlling Oct4 expression might explain why Smad2/3 inhibition does not reduce, but in fact enhance the efficiency of reprogramming fibroblasts to induced pluripotent cells (iPS cells), since the robust stem cells does not need Smad2/3 for Oct4 expression. We found that this enhancement by Smad2/3 inhibition was abolished by shRNA-knockdown of Polycomb or Jmjd3, so reprogramming related responses to Smad2/3 depended on Polycomb as well. These data implied that Polycomb determines the responses to Smad2/3 signaling when cells undergo dynamic changes in stem cell differentiation or in reprogramming to induced pluripotency, but not in the robust terminally differentiated fibroblasts or ground state stem cells. So the Smad2/3-Polycomb interplay is one mechanism by which the cells can balance robustness and plasticity.

## **TRANSDIFFERENTIATION FACTORS ARE DIFFERENTIALLY POLYCOMB REPRESSED**

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Polycomb group (PcG) proteins play a critical role in establishing and maintaining the identity of differentiated cells by repressing the expression of genes that regulate alternate cell fates (Holmberg and Perlmann, 2012). We recently observed that several transcription factors (TFs) regulating the identity of Kenyon cell neurons in the adult *Drosophila* brain are expressed in these cells and are PcG-repressed in another neuronal population, the octopaminergic neurons (Henry et al., 2012). Based on our and others' findings, we hypothesized that TFs important for cell identity can be identified in pairwise comparisons of two cell types as being more highly expressed in one cell type and more strongly H3K27me3 modified in another cell type. Repressing these key TFs in other cell types is critical, because ectopic expression of TFs that regulate cell identity has the potential to convert, or "transdifferentiate", adult cells of one type to another (Vierbuchen and Wernig 2011). Recent reports describe small sets of TFs that can, typically at low efficiency, transdifferentiate one adult cell type (the "source" cell type) to another ("target" cell type) by reprogramming the nucleus to express gene batteries characteristic of the target cell type. These factors have been discovered empirically by testing pools of factors (known to play a role in the maintenance or development of the target cell type) for the smallest combination (typically 3-4 factors) that induces transdifferentiation. Here we explore whether comparison of gene expression and PcG repression profiles between a pair of source and target cell types can help identify TFs that can convert one to the other. We show by reanalysis of published datasets that most transdifferentiation factors exhibit the same genomic signature we previously observed for regulators of *Drosophila* neuronal identity -- higher expression in one cell type and stronger PcG repression in another -- whereas this is not true for transcription factors in general. Furthermore, we find that the combined criteria of (i) greater H3K27me3 modification in the source cell and (ii) higher expression in the target cell is an effective genome-wide screen that significantly enriches for transdifferentiation factors. This finding suggests that candidate transdifferentiation factors can be identified using genome-wide expression and chromatin profiles and without prior knowledge of their functional or developmental role. Our results suggest that barriers between adult cell types, as depicted in Waddington's "epigenetic landscape" (Waddington, 1957), consist in part of differentially Polycomb-repressed transcription factors that can be identified by standard genomic methods. This genomic model of cell identity helps rationalize a growing number of transdifferentiation protocols and may help facilitate the engineering of cell identity for regenerative medicine. Henry GL, Davis FP, Picard S, Eddy SR. *Nucleic Acids Res* 2012. doi:10.1093/nar/gks671 Holmberg J, Perlmann T. *Nat Rev Genet* 2012. doi:10.1038/nrg3209 Vierbuchen T, Wernig M. *Nat Biotechnol* 2011. doi:10.1038/nbt.1946 Waddington CH. *The Strategy of the Genes*. George Allen & Unwin. 1957.

**USF1 AND hSET1A MEDIATED EPIGENETIC MODIFICATION REGULATES LINEAGE DIFFERENTIATION AND HOXB4 TRANSCRIPTION**

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Interplay between polycomb and trithorax complexes has been implicated in embryonic stem cell (ESC) self-renewal and differentiation. However, it remains unknown how and where these complexes are targeted to specific gene loci to specify lineage-specific transcription patterns. Here, we report that the cooperation of hSET1 and USF1 governs the activation of hematopoietic associated genes and facilitates ESCs hematopoietic fate. Knockdown of hSET1 or inhibition of USF DNA binding specifically suppresses mesoderm differentiation and blocks differentiation of hematopoietic stem and progenitor cells (HS/PCs). Although hSET1 depletion or inhibition of USFs has minimal effects on ESC self-renewal, KD led to a block in HS/PC differentiation by decreased H3K4me3 levels and transcription preinitiation complex formation at the hematopoietic associated genes, for example HoxB4 and TAL1. Transcription factor USF1 maintains the hematopoietic potentials by mediating H3K4me3 modifications at HSC-associated bivalent genes in ES cells. Further, enforced expression of USF1 in ESCs promotes mesoderm differentiation and enforces the endothelial-to-hematopoietic transition by inducing hematopoietic-associated transcription factors, HoxB4 and TAL1. Thus, our data reveal that the guided-recruitment of hSET1 and its H3K4 methylations by DNA-binding factor USF1 are essential for establishing and maintaining the hematopoietic transcription patterns.

**P-16**

**HMGN1 MODULATES NUCLEOSOME OCCUPANCY AND DNASE I HYPERSENSITIVITY AT THE CpG ISLAND PROMOTERS OF EMBRYONIC STEM CELLS**

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Chromatin structure plays a key role in regulating gene expression and modulating embryonic stem cell differentiation; however the factors that determine the organization of chromatin around regulatory sites are not fully known. We find that HMGN1, a nucleosome binding protein ubiquitously expressed in vertebrate cells, preferentially binds to CpG island-containing promoters, and affects the organization of nucleosomes, DNaseI hypersensitivity, and the transcriptional profile of mouse embryonic stem cells and neural progenitors. Loss of HMGN1 alters the organization of an unstable nucleosome at transcription start sites, reduces the number of DNaseI hypersensitive sites genome wide, and decreases the number of Nestin-positive neural progenitors in the SVZ region of mouse brain. Thus, architectural chromatin binding proteins affect the transcription profile and chromatin structure during embryonic stem cell differentiation.

**THE IMPRINTED KCNQ1OT1 NON-CODING RNA HAS DIRECTIONAL SILENCING ACTIVITY**

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Although the existence genomic imprinting has been known for decades, the phenomenon of tissue-specific imprinting or loss-of-imprinting has been underappreciated and remains unaccounted for. The *Kcnq1* imprinted domain is highly conserved between human and mouse, and exhibits complex tissue-specific expression patterns co-existing with a domain-wide cis-acting control element. Imprinting defects in the region are responsible for a subset of Beckwith-Wiedeman fetal overgrowth syndrome. Transcription of the paternally expressed antisense 90 kb non-coding macro-RNA, *Kcnq1ot1*, silences some neighboring genes in the embryo, while others are unaffected. *Kcnq1*, encoding a potassium channel protein, from which the ncRNA emerges in antisense direction, is monoallelic in early cardiac development but becomes biallelic after midgestation, apparently overcoming the repression by the ncRNA. To explore the mechanism of this transition, we used allele-specific assays and chromosome conformational studies in wild-type mice and mice with a premature termination mutation for *Kcnq1ot1*. We show that *Kcnq1* imprinting in early heart is established and maintained independently of *Kcnq1ot1* expression. Thus, in the embryo, *Kcnq1ot1* is only required for silencing of downstream genes, including *Cdkn1c*, a cell-cycle inhibitor. In later developmental stages, however, *Kcnq1ot1* does have a role in modulating *Kcnq1* levels, since its absence leads to overexpression of *Kcnq1*, an event accompanied by an aberrant three-dimensional structure of the chromatin. Thus, our studies reveal a novel mechanism by which an antisense non-coding RNA affects transcription through regulating chromatin flexibility and access to enhancers. Our data underscore the value of tissue- and stage-specific studies of imprinted domains in elucidating how competing transcriptional mechanisms are resolved in the context of tissue-specific needs.

**CENP-A NUCLEOSOMES ADOPT AN UNCONVENTIONAL SHAPE**

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The centromere is the locus that ensures proper segregation of chromosomes through generations. In all eukaryotes, except for budding yeast, centromere location is maintained by an epigenetic mechanism. The most attractive candidate to provide the centromere-specifying epigenetic mark is the histone variant centromere protein A (CENP-A) that replaces histone H3 in centromeric nucleosomes. The (CENP-A/H4)<sub>2</sub> heterotetramer crystal structure (Sekulic et al., 2010, *Nature* 467:347-351) led to our proposal that CENP-A could distinguish centromeric chromatin from the rest of the chromosome via structural deviation from within the folded octameric core of the nucleosome. Our model is based on the intranucleosomal rigidity conferred by CENP-A and rotation at the CENP-A/CENP-A interface. If preserved upon nucleosome formation the rotation would alter the radius of curvature of nucleosomal DNA and histone subunit packing. However, in the subsequent crystal structure of the CENP-A nucleosome (Tachiwana et al., 2011, *Nature* 476:232-235), nucleosomal DNA curvature and histone subunit packing adopt the same conformation as the conventional nucleosome but the DNA termini unwrap 13 bp at each end of the nucleosome core particle. To investigate the conformation of CENP-A nucleosomes, we used intranucleosomal FRET and to examine the extent of DNA wrapping in solution we used micrococcal nuclease (MNase) digestion. We find CENP-A nucleosomes more sensitive to MNase digestion than H3-containing nucleosomes, indeed indicating more frequent events of DNA unwrapping. Using FRET, we find that CENP-A nucleosomes heavily populate an atypical conformation where the H2A/H2B dimers are 5 Å further apart at steady state relative to conventional nucleosomes. These data suggest that CENP-A nucleosomes in solution exist in equilibrium between several structural conformations: one that is similar to conventional nucleosomes and one that is altered by rotation at the CENP-A/CENP-A interface and/or unwrapping of the terminal DNA. To further interrogate the structure of CENP-A nucleosomes in solution we are also employing SAXS (small-angle X-ray scattering) and SANS (small-angle neutron scattering). SANS experiments exploit contrast variation schemes to provide information on DNA and protein subunits independently. Our ongoing biophysical studies promise to provide critical insight into how CENP-A nucleosomes distinguish centromeric chromatin in the context of mammalian chromosomes.

**USE OF GENOME-WIDE EPIGENOMIC DATA IN THE CURATION OF THE VERTEBRATE REFSEQ DATASET: CURRENT APPLICATIONS AND FUTURE PROSPECTS**

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The Reference Sequence (RefSeq) database at NCBI represents a curated set of non-redundant sequences, including genomic DNA, transcripts and proteins. These sequences provide a stable reference for genome annotation, gene identification and gene characterization, and they are widely used in basic, biomedical and bioinformatics research. The RefSeq collection includes sequences from a range of organisms, including bacteria, viruses and eukaryotes. Among the vertebrate RefSeq collection, curation is carried out using a combination of data submitted to International Nucleotide Sequence Database Consortium databases, publication data and bioinformatics analysis, with a curation emphasis on higher vertebrate species for which a high quality sequenced genome assembly exists. At present, the dataset is comprised mainly of genes and their products, with little or no representation of gene regulatory or other functional genomic regions. The represented genes, which are included in NCBI's Gene database, can be protein-coding, non-coding or pseudogenes (either transcribed or non-transcribed), and current curation attempts are largely dependent on available transcript, protein or publication evidence. Recently, however, the increasing availability of genome-wide epigenomic data has become a useful tool for certain aspects of RefSeq curation and gene determination. Due to the large volume of data and the readily available display of individual cell type and combined summary tracks on genome browsers, two major contributors of epigenomic data are primarily used in RefSeq curation, namely data from the NIH Roadmap Epigenomics Mapping Consortium (REMC) and the ENCODE (ENCyclopedia Of DNA Elements) Project. Current RefSeq applications of these data include the verification of promoter presence and 5' completeness of a gene or a transcript variant based on histone H3 tri-methylated lysine-4 (H3K4Me3) signals combined with DNase I hypersensitivity or other modification signals that are correlated with an active promoter, as well as epigenomic evidence that suggests an active gene when the gene being considered either lacks or has insufficient transcript support. Examples of how epigenomic data can be combined with other data types in the RefSeq curation process will be shown. In addition, limitations of using epigenomic data in RefSeq curation will be discussed, including problems with epigenomic data resolution, cell type specificity and the interpretation of these data for multi-copy genes. Future prospects of using epigenomic data to curate and annotate functional genomic regions will also be discussed, and advice will be sought from the chromatin research community to gauge interest in and applicability of such annotations.

**A DNA METHYLATION ELEMENT RESPONSIBLE FOR TSA EFFECT ON CYCLIN D1 EXPRESSION**

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Cyclin D1 is a key regulator in cell cycle progression and its high expression was found in so many tumor cell lines. Trichostatin A (TSA) is an organic compound that serves as an antifungal antibiotic and mammalian histone deacetylase (HDAC) inhibitor which shows some potential as an anti-cancer drug. In our research, from tumor cell line, we found cyclin D1 mRNA level decreased under TSA treatment. By DNA methylation analysis we found a functional DNA methylation pattern in cyclin D1 promoter. The 15bp (GCGCGAGGGAGCGCG) long sequence is GC enriched and shows palindrome sequence which then we called it an element. No putative transcriptional factors were found to bind this element by computer searching which suggested that it could be a new element for gene regulation through DNA methylation. By DNA sequence alignment analysis, we found the 15bp sequence evolutionary conserved between human and macaque (*Macaca mulatta*). Only one bp difference between human and mouse, and it locate in the middle position not in the GCGC methylation site. In *Gallus gallus*, the element lost including most of first exon of *CCND1*. Interestingly the methylation element is half mutant (the second GCGC mutated to GTGT) in *Monodelphis domestica*. The conversion from C to T may express the methylated C was mutated to T which can give clue for the species evolution. The 15bp long methylation element has two potential methylation sites. Mutation of first GCGC sequence can reverse the decreased promoter activity and the second GCGC methylation site has no effect. To verify the methylation element, we insert only the 15bp and its mutant fragment into pGL3-promoter-LUC vector to investigate the effect on TSA treatment. Data showed that the first GCGC sequence mutation in the element can decrease the TSA effect on the reporter activity driven by SV40 promoter. In conclusion, we found an element which can regulate gene expression by DNA methylation modification, although the mechanism need further study including binding factors identification.

## P-21

### **CONTRIBUTION OF NUCLEOSOME BINDING PREFERENCES AND CO-OCCURRING DNA SEQUENCES TO TRANSCRIPTION FACTOR BINDING**

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Chromatin plays a critical role in regulating transcription factors (TFs) binding to their canonical transcription factor binding sites (TFBS). Recent studies in vertebrates show that many TFs preferentially bind to genomic regions that are well bound by nucleosomes *in vitro*. Co-occurring secondary motifs sometimes correlated with functional TFBS. We used a generalized linear model (GLM) to evaluate how well the propensity for nucleosome binding and co-occurrence of a secondary motif identify which canonical motifs are bound *in vivo*. We used ChIP-seq data for three transcription factors binding to their canonical motifs: c-Jun binding the AP-1 motif (TGAGTCA), GR (glucocorticoid receptor) binding the GR motif (G-ACA---TGT-C), and Hoxa2 (homeobox a2) binding the Pbx (Pre-B-cell leukemia homeobox) motif (TGATTGAT). For all canonical TFBS in the mouse genome, we calculated intrinsic nucleosome occupancy scores (INOS) for its surrounding 150-bps DNA and examined the relationship with *in vivo* TF binding. In mouse mammary 3134 cells, c-Jun and GR proteins preferentially bound regions calculated to be well-bound by nucleosomes *in vitro* with the canonical AP-1 and GR motifs themselves contributing to the high INOS. Functional GR motifs are enriched for AP-1 motifs if they are within a nucleosome-sized 150-bp region. GR and Hoxa2 also bind motifs with low INOS, perhaps indicating a different mechanism of action. Our analysis quantified the contribution of INOS and co-occurring sequence to the identification of functional canonical motifs in the genome. This analysis revealed an inherent competition between some TF and nucleosomes for binding canonical TFBS. GR and c-Jun cooperate if they are within 150-bps. Binding of Hoxa2 and a fraction of GR to motifs with low INOS values suggesting they are not in competition with nucleosomes and may function using different mechanisms.

**DIRECT ASSESSMENT OF TRANSCRIPTION FIDELITY BY RNA SEQUENCING**

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Cancerous and aging cells have long been thought to be impacted by transcription errors that cause genetic and epigenetic changes. Until now a lack of methodology for directly assessing such errors hindered evaluation of their impact to the cells. We report a high-resolution Illumina RNA-seq method that can analyze non-coded base substitutions in mRNA at  $10^{-4}$ - $10^{-5}$  per base frequencies in vitro and in vivo. A combination of the RNA-seq and biochemical analyses of the positions for the errors revealed that increased backtracking of RNA polymerase represents a major sequence-dependent mechanism to increase transcription fidelity. Backtracking decreases a chance of error propagation to the full-length transcript and provides an opportunity to proofread the error. This method is adoptable to a genome-wide assessment of transcription fidelity.

**P-23**

**PAPILLOMAVIRUS GENOMES ASSOCIATE WITH THE CELLULAR PROTEIN BRD4 TO REPLICATE AT FRAGILE SITES IN THE HOST GENOME**

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Papillomaviruses are small DNA viruses that establish a persistent infection in the epithelia of the host. To ensure long-term replication and maintenance of the extrachromosomal viral genome, the viral E2 protein tethers it to host chromatin, often in complex with the cellular bromodomain protein, Brd4. To determine whether the E2-Brd4 complex interacts with specific regions of host chromosomes, we performed ChIP-chip analysis on the entire human genome to detect binding targets on chromatin derived from interphase and mitotic cells. We identified 54 large regions of chromatin (ranging in size from several hundred Kb to over 1.6Mb) that are highly occupied by E2 and Brd4 throughout the cell cycle. These regions have a shared signature of chromatin modification in that they are highly acetylated and have a distinctive pattern of H3K4 methylation. Combined FISH-immunofluorescence analysis demonstrated that these regions correspond to the E2-Brd4 foci observed by microscopy. We have named these regions Persistent E2 and Brd4 - Broad localized enrichments of chromatin or PEB-BLOCs.

Papillomavirus E1 and E2 proteins form nuclear replication foci that induce a DNA damage response and recruit DNA repair proteins. Even E2 proteins that do not bind tightly to Brd4 will recruit Brd4 to the replication foci in the presence of E1. Therefore we questioned, and confirmed by whole genome ChIP-chip, that E1-E2-Brd4 replication foci also form at sites that overlap PEB-BLOCs. In addition, we show that replicating HPV genomes are recruited to PEB-BLOCs. Further analyses showed that PEB-BLOCs overlap with many known common fragile sites. Notably, oncogenic HPVs are often found integrated in the vicinity of fragile sites. Similar to fragile sites, PEB-BLOCs frequently contain deletions and have high rates of asynchronous DNA replication. To confirm that PEB-BLOCs correspond to fragile sites, C33A cells (human cervical cells) were treated with aphidicolin and analyzed for binding of a fragile site marker (FANCD2) by whole genome ChIP-chip. There was a large overlap among known fragile sites, PEB-BLOCs, the new C33A fragile sites and HPV integration sites. Furthermore, RNA seq confirmed that many PEB-BLOCs contained long, transcriptionally active genes, consistent with the recent findings that common fragile sites occur because of a conflict between replication and transcription of long genes. We propose that replication of papillomavirus genomes, which involves hijacking the host DNA damage and repair response, occurs adjacent to highly susceptible fragile sites. This is highly likely to increase the chances of integration at fragile sites, as is found in many HPV-associated cancers.

**FINE-SCALE MAPPING OF NUCLEOSOME ORGANIZATION IN THE C. ELEGANS EMBRYO**

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DNA is wrapped ~1.7 times around a histone octamer to form the nucleosome. We are interested in the physical properties by which nucleosomes and higher-order chromatin structures restrict access to the underlying DNA sequence. We interrogated nucleosome positioning and dynamics by performing a micrococcal nuclease digestion timecourse to differentially liberate mononucleosomes from *C. elegans* embryo chromatin, followed by paired-end Illumina sequencing. This approach captures known features such as the 10-bp periodicity in nucleosome digestion, along with interesting phenomena such as correlations between nucleosome position and the propensity for digestion, and between transcriptional activity and the ease with which individual nucleosomes are released upon incubation with MNase. We will discuss how these data may be used to increase our understanding of nucleosome dynamics, nucleosome ‘breathing’, and the kinetics of DNA sequence accessibility.

**INTRINSIC TRANSLOCATION BARRIER AS AN INITIAL STEP IN PAUSING BY RNA POLYMERASE II**

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Pausing of RNA polymerase II (RNAP II) by backtracking on DNA is a major regulatory mechanism in control of eukaryotic transcription. Backtracking occurs by extrusion of the 3' end of the RNA from the active center after bond formation and before translocation of RNAP II on DNA. In several documented cases, backtracking requires a special signal such as A/T-rich sequences forming an unstable RNA-DNA hybrid in the elongation complex. However, other sequence-dependent backtracking signals and conformations of RNAP II leading to backtracking remain unknown. Here, we demonstrate with *S. cerevisiae* RNAP II that a cleavage-deficient elongation factor TFIIS (TFIIS(AA)) enhances backtracked pauses during regular transcription. This is due to increased efficiency of formation of an intermediate that leads to backtracking. This intermediate may involve misalignment at the 3' end of the nascent RNA in the active center of the yeast RNAP II, and TFIIS(AA) promotes formation of this intermediate at the DNA sequences, presenting a high-energy barrier to translocation. We proposed a three-step mechanism for RNAP II pausing in which a prolonged dwell time in the pre-translocated state increases the likelihood of the 3' RNA end misalignment facilitating a backtrack pausing. These results demonstrate an important role of the intrinsic blocks to forward translocation in pausing by RNAP II.

**P-26**

**HDAC INHIBITION DISRUPTS CELLULAR DIFFERENTIATION AND PATTERNING IN THE MAMMALIAN ORGAN OF CORTI**

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The mammalian auditory sensory end organ, known as the organ of Corti, is made up of two general cell types, hair cells and supporting cells, which are specified and differentiate during embryonic development. Each of these general categories contains multiple individual cell types with distinct cellular morphologies that are important for function. Moreover, hair cells and supporting cells are patterned in a stereotyped manner along the length of the cochlea duct. While considerable work has been done to define the differentiation program of some of these cell types, little is known about how epigenetic mechanisms, such as histone modifications, influence important developmental events including lineage commitment, cell fate decisions, and changes in cellular plasticity. Using an in vitro organ culture of mouse cochleae, we applied broad HDAC inhibitors to attempt to manipulate histone acetylation levels and determine the potential role that this modification may play during cochlear development. Treatment with HDAC inhibitors at early developmental stages when cells are already post-mitotic, but have not necessarily made terminal cell fate decisions, leads to a stalling of the gradient of differentiation that normally occurs along the length of the duct. Treatment at later developmental stages, after cell fate decisions have been made and individual cells are already taking on their characteristic morphologies, results in a loss of characteristic mechanosensory stereociliary bundles on the apical surfaces of hair cells as well as changes in cell-cell contacts, leading to patterning defects within the organ of Corti. These preliminary results suggest that regulation of histone acetylation is necessary for the proper differentiation and maintenance of distinct cell type morphologies and cellular patterning within the organ of Corti. Future work aims to determine which specific HDACs mediate these effects, how their inhibition may be affecting global and gene-specific expression, and if other regulatory co-factors may be directing these changes in a locus-specific manner.

**DNA BREAK-INDUCED CHROMATIN CONDENSATION PROMOTES BRCA1 REPAIR FACTOR CHOICE THROUGH A NOVEL REPRESSIVE CHROMATIN MODULE**

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DNA double-strand break (DSB) repair ensures the elimination of highly cytotoxic DNA lesions and is orchestrated by a diverse set of DNA damage response (DDR) factors. Appropriate repair factor choice is a critical step in promoting efficient DSB repair. However, the basis for selective repair factor recruitment to DSBs is poorly understood. DDR mediators often occupy extensive DSB-surrounding chromatin domains, suggesting a role for chromatin structure in this process. Here, we show that the DSB-induced formation of a repressive chromatin environment differentially affects recruitment of BRCA1 and 53BP1, two repair factors central to homologous recombination (HR) and nonhomologous end-joining (NHEJ), respectively. Using RNA interference-based screening, we identified a novel repair module consisting of macro-histone H2A variants and the tumor suppressor RIZ1/PRDM2 (a histone H3-K9 methyltransferase), which is required for efficient HR and resistance to genotoxic stress. DNA damage results in persistent enrichment of macroH2A at DSBs, which in turn promotes the recruitment of PRDM2 along with PRDM2-mediated dimethylation of H3-K9. As a result, the macroH2A/PRDM2 module causes the condensation of DSB-proximal chromatin following an initial phase of damage-induced expansion. This process extends over several 100 kb from the DSB site and appears to be essential for efficient recruitment of BRCA1, but not 53BP1, consistent with preferential binding of BRCA1 to repressive versus active histone marks. Finally, experimentally induced chromatin condensation promotes the rapid loss of 53BP1 but not BRCA1 from sites of DNA damage. Together, these findings place DSB-induced chromatin reorganization at a central position in the regulation of repair factor choice with direct implications for repair outcome and, ultimately, genome integrity.

**DEFICIENCY FOR KREBS CYCLE SUCCINATE DEHYDROGENASE COMPLEX UNDERLIES GLOBAL EPIGENOMIC DIVERGENCE IN GASTROINTESTINAL STROMAL TUMOR**

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Gastrointestinal stromal tumors (GIST) harbor driver mutations of signal transduction kinases such as KIT, or alternatively, manifest loss-of-function defects in the mitochondrial succinate dehydrogenase (SDH) complex, a component of the Krebs cycle and electron transport chain. We have uncovered a striking divergence between the DNA methylation profiles of SDH-deficient GIST (N=24) versus KIT tyrosine kinase pathway mutated GIST (N=39). Infinium 450K methylation array analysis of fixed (FFPE) tissues disclosed an order of magnitude greater genomic hypermethylation from gastric smooth muscle reference in SDH-deficient GIST versus the KIT mutant group (84.9K vs. 8.4K targets). Epigenomic divergence was further found among SDH-mutant paraganglioma/pheochromocytoma (N=29), a developmentally distinct SDH-deficient tumor system. Comparison of SDH-mutant GIST with isocitrate dehydrogenase (IDH)-mutant glioma -- another Krebs-cycle defective tumor type -- revealed comparable measures of global hypo- and hypermethylation. These data expose a vital connection between succinate metabolism and genomic DNA methylation during tumorigenesis, and generally implicate the mitochondrial Krebs cycle in nuclear epigenomic maintenance. This study demonstrates that SDH-deficiency underlies pervasive DNA hypermethylation in multiple tumor lineages, generally defining the Krebs cycle as mitochondrial custodian of the methylome. We propose that this phenomenon may result from a failure of maintenance CpG demethylation, secondary to inhibition of the TET2 5-methylcytosine dioxygenase demethylation pathway by inhibitory metabolites that accumulate in tumors with Krebs-cycle dysfunction.

**TRANSCRIPTIONAL CHANGES LEADING TO THYROID TUMORIGENESIS  
INDUCED BY ARCHITECTURAL CHROMATIN PROTEIN HMGN4**

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Thyroid cancer is the most common type of endocrine malignancy; however the factors underlying its etiology are not fully understood. Published microarray studies have suggested that HMGN4, a variant of the HMGN family, is highly expressed in both normal and cancerous thyroid tissue. HMGNs are chromatin architectural proteins that bind specifically to nucleosome core particles and impact epigenetic regulatory processes by altering the structure of chromatin and the levels of histone modifications. HMGN4 is unique because it is encoded by an intronless gene which originated from a retropseudogene. Quantitative PCR and western blot analyses verified that HMGN4 is indeed up-regulated in human thyroid cancer cells. Up-regulation of HMGN4 leads to increased soft-agar colony formation in both human thyroid cancer cells and mouse embryonic fibroblasts (MEFs); in addition, MEFs over-expressing HMGN4 formed significantly larger tumors than control MEFs when injected into nude mice. Comparative microarray analysis reveals that HMGN4 over-expression down-regulates the expression of the tumor suppressors BRCA2, ATM, and ATRX in both MEFs and human thyroid cancer cells. DnaseI digestion assays of the BRCA2, ATM, and ATRX promoter regions indicate that up-regulation of HMGN4 alters the chromatin structure at the promoters of these genes. We suggest that the up-regulated expression of HMGN4 leads to cancer by inducing changes in chromatin structure that lead to down-regulation of tumor suppressor expression. Thus, the random expression of a retropseudogene may lead to increased tumorigenicity.

**DNA METHYLATION PROFILING IN HUMAN B CELLS REVEALS IMMUNE REGULATORY ELEMENTS AND EPIGENETIC PLASTICITY AT ALU ELEMENTS DURING B CELL**

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Memory is a hallmark of adaptive immunity, wherein lymphocytes mount a superior response to a previously encountered antigen. It has been speculated that epigenetic alterations in memory lymphocytes contribute to their functional distinction from their naive counterparts. However, the nature and extent of epigenetic alterations in memory compartments remain poorly characterized. Here we profile the DNA methylome and the transcriptome of B lymphocyte subsets representing stages of the humoral immune response before and after antigen exposure in vivo from multiple humans. A significant percentage of activation-induced losses of DNA methylation mapped to transcription factor binding sites. An additional class of demethylated loci mapped to Alu elements across the genome and accompanied repression of DNA methyltransferase 3A. The activation-dependent DNA methylation changes were largely retained in the progeny of activated B cells, generating a similar epigenetic signature in downstream memory B cells and plasma cells with distinct transcriptional programs. These findings provide insights into the methylation dynamics of the genome during cellular differentiation in an immune response.

**THE CLAMP PROTEIN LINKS MSL COMPLEX TO THE X-CHROMOSOME DURING DROSOPHILA DOSAGE COMPENSATION**

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In heterogametic species, the process of dosage compensation is required to equalize transcript levels between the sex chromosomes in males and females. The *Drosophila* Male-Specific Lethal (MSL) complex increases transcript levels on the single male X-chromosome to equal the transcript levels in XX females. However, it is not known how the MSL complex is linked to its DNA recognition elements, the critical first step in dosage compensation. Here, we demonstrate that a previously uncharacterized zinc-finger protein, CLAMP (Chromatin-Linked Adaptor for MSL Proteins) functions as the key link between MSL complex and the X-chromosome. CLAMP directly binds to the MSL complex DNA recognition elements and is required for the recruitment of MSL complex. CLAMP is a non-sex specific protein that is enriched on the X-chromosome, even in the absence of MSL complex. Synergistic interactions between CLAMP and MSL complex increase X-chromosome enrichment of both factors. The discovery of CLAMP identifies a critical factor required for the chromosome-specific targeting of dosage compensation, providing new insights into how sub-nuclear domains of coordinate gene regulation are formed within metazoan genomes.

## MECHANISMS CONTROLLING TRANSCRIPTION OF THE MYC ONCOGENE AND CELL OVERGROWTH IN DROSOPHILA VIA PSI

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Myc proteins are critical regulators of growth and cell cycle progression during animal development. Dysregulation of Myc can result in over proliferation and malignant transformation. In addition, Myc transcription must rapidly respond to environmental cues, which feed into developmental signaling pathways. In vitro studies in mammals have demonstrated activated expression of the c-Myc oncogene in response to growth factors in serum is mediated by release of paused, but transcriptionally engaged, RNA Polymerase II (Pol II). This increase in release of MYC transcription correlates with recruitment of the single-stranded DNA binding protein FUBP1 to the Myc promoter (1). However, the signals promoting recruitment of FUBP to activated c-Myc are currently unknown. In an effort to better understand activation of Myc transcription in an in vivo signaling environment, we have developed models to study the Drosophila ortholog of FUBP1, Psi. A recent coaffinity purification (Co-IP) coupled to mass spectrometry for FLAG-HA epitope-tagged proteins, has led to a comprehensive Drosophila protein interaction map (DPiM) (3). Data mining of the DPiM revealed novel interactions between Psi and both core and gene-specific transcriptional machinery with ~80% of the 30 strongest interactors directly implicated in RNA Pol II activity and/or chromatin remodeling. In particular, Psi was found in a complex with most subunits of the mediator (MED) complex and we are currently exploring whether these interactions are required for regulation of Drosophila *myc*, *dmypc*, transcription. We will present evidence that Psi is not only required for activated *dmypc* transcription, but that the ability of the Ras pathway to activate *dmypc* transcription requires Psi. Furthermore, as Mass spectrometry showed Psi in complex with an interconnected Pi3K/Insulin pathway, we are currently exploring whether the effects of Ras are dependent on interactions with the Pi3K pathway. Together the data we will present demonstrate that Psi may provide an important link between the Ras/Pi3K signalling pathway, *dmypc* transcription and cell growth.

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**COMPARISON BETWEEN CHROMATIN INSULATORS, CHS4, GAMMA AND tDNA IN THEIR ABILITY TO SUSTAIN TRANSGENE EXPRESSION WITHIN A HUMAN ARTIFICIAL CHROMOSOME (HAC)**

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Human artificial chromosomes (HACs) are vectors that offer the advantages of capacity and stability in gene delivery and expression. Several studies have even demonstrated their use for gene complementation in gene-deficient recipient cell lines and animal transgenesis. Recently, we constructed an advance HAC-based vector, alphoidtetO-HAC with a conditional centromer. In this HAC, a gene-loading site was inserted into a centrochromatin domain critical for kinetochore assembly and maintenance. While by definition this domain is permissive for transcription, there have been no long-term studies on transgene expression within centrochromatin. In this study, we compared the effects of three chromatin insulators, cHS4, gamma-satellite DNA and tDNA, on the expression of an EGFP transgene inserted into the alphoidtetO-HAC vector. We analyzed two host cell lines, human HeLa and hamster CHO for 12 weeks and found that insulators were essential for stable and strong transgene expression. The tDNA insulator composed of two functional copies of tRNA genes consistently gave high transgene expression in both cell lines. In CHO, the EGFP intensity of cell lines with insulators cHS4, gamma-satellite DNA and tDNA was 3-fold, 11-fold and 11-fold higher compared to the control without flanking insulators. However in cHS4 and the control, EGFP intensity fell significantly during the course of the 12 week experiment. In contrast no significant change in EGFP intensity was observed with either gamma-satellite DNA or tDNA insulators. In HeLa, the EGFP intensity of cell lines with insulators cHS4, gamma-satellite DNA and tDNA was 11-fold, 24-fold and 35-fold higher than the control without flanking insulators. No significant change in EGFP intensity was observed in cell line with all three insulators. In contrast, in the absence of protective insulators, EGFP intensity of the control fell significantly, nearing complete loss of EGFP florescence after 12 weeks of culturing. We extended the experiment for the HeLa insulators cell lines up to 20 week and found no significant change in EGFP intensity. We infer that while proximity to centrochromatin does allow transgene expression, it does not protect genes lacking chromatin insulators from epigenetic silencing. Barrier elements that prevent gene silencing in centrochromatin would thus help to optimize transgenesis using HAC vectors.

**LONG1, A NOVEL NON-CODING RNA IN CHROMOSOMAL INSTABILITY AND COLORECTAL CANCER PATHOGENESIS**

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The functional roles of SNPs within the 8q24 gene desert in cancer phenotype are not yet well understood. Here, we report that LONG1, a novel long non-coding RNA transcript (lncRNA) encompassing a SNP, is highly overexpressed in microsatellite-stable colorectal cancer and promotes tumor growth, metastasis and chromosomal instability. We demonstrate that MYC, miR-17-5p, and miR-20a are up-regulated by LONG1. We further identify the physical interaction between LONG1 and TCF4 resulting in an enhancement of Wnt signaling activity. We show that LONG1 is itself a Wnt downstream target suggesting the existence of a feedback loop. Our results support a new mechanism of MYC and Wnt regulation by a novel lncRNA in colorectal cancer pathogenesis.

**CG METHYLATED MICROARRAYS IDENTIFY NOVEL METHYLATED SEQUENCE BOUND BY THE CEBPB|ATF4 HETERODIMER THAT ARE ACTIVE IN VIVO**

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To evaluate the effect of CG methylation on DNA binding of sequence-specific B-ZIP transcription factors (TFs) in a high-throughput manner, we enzymatically methylated the cytosine in the CG dinucleotide on protein binding microarrays. Two Agilent DNA array designs were used. One contained 40,000 features using de Bruijn sequences where each 8-mer occurs 32 times in various positions in the DNA sequence. The second contained 180,000 features with each CG containing 8-mer present three times. The first design was better for identification of binding motifs, while the second was better for quantification. Using this novel technology, we show that CG methylation enhanced binding for CEBPA and CEBPB and inhibited binding for CREB, ATF4, JUN, JUND, CEBPD and CEBPG. The CEBPB|ATF4 heterodimer bound a novel motif CGAT|GCAA 10-fold better when methylated. EMSA confirmed these results. CEBPB ChIP-seq data using primary female mouse dermal fibroblasts with 50X methylome coverage for each strand indicate that the methylated sequences well-bound on the arrays are also bound in vivo. CEBPB bound 39% of the methylated canonical 10-mers ATTGC|GCAAT in the mouse genome. After ATF4 protein induction by thapsigargin which results in ER stress, CEBPB binds methylated CGAT|GCAA in vivo, recapitulating what was observed on the arrays. This methodology can be used to identify new methylated DNA sequences preferentially bound by TF, which may be functional in vivo.

## P-36

### **RELIABLE CHIP-SEQ RESULTS WITH DIAGENODE TRUE MICROCHIP KIT AND MICROPLEX LIBRARY PREPARATION KIT ON 10.000 CELLS**

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Chromatin immunoprecipitation coupled with high-throughput sequencing (ChIP-seq) has become the gold standard for whole-genome mapping of protein-DNA interactions. However, conventional ChIP and library preparation protocols associated with current high-throughput sequencing require abundant amounts of starting material (at least hundreds of thousands of cells per immunoprecipitation) making it difficult to apply this technology when working with low starting amounts of cells.

Diagenode has developed small-scale ChIP-seq protocols to work with limited samples.

Diagenode provides the True MicroChIP Kit that enables successful ChIP on 10,000 cells. The kit's protocol has been thoroughly optimized for chromatin shearing on low cell amounts and ChIP followed by high-throughput sequencing on an Illumina sequencer. To enable sequencing on the low amounts of DNA recovered after ChIP on 10,000 cells, we developed a library preparation protocol for limited quantities of DNA. The MicroPlex Library Preparation Kit requires only picogram amounts of immunoprecipitated DNA inputs for library preparation and it is compatible with the Illumina platforms.

We present here new ChIP-seq tools for genome-wide analysis optimized for use with low starting cell numbers. The performance of the optimized method was evaluated for read mapping, sensitivity and specificity at a range of starting cell numbers covering three orders of magnitude, starting with the published amount of  $1 \times 10^6$  cells / IP and reduced to amounts of 10.000 cells/IP.

**REPROGRAMMING OF THE CHROMATIN LANDSCAPE: STEROID RECEPTOR CROSSTALK AT THE GENOMIC LEVEL**

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Crosstalk between the estrogen receptor (ER) and the glucocorticoid receptor (GR) plays an important role in controlling many cellular processes. Physiological interactions between ER and GR are not only important for the development of certain tissues, such as the uterus and bone, but also may play an important role in breast cancer. Recent studies have shown that the GR and ER status in breast cancer is a significant factor for determining the outcome of the disease. However, the mechanistic details defining the cellular interactions between ER and GR are poorly understood. Since the regulation of receptor binding to response elements controls the transcriptional output in response to hormones, it is logical to suspect that co-treatment of cells with corticosteroids and estradiol would have an effect on the genome-wide binding landscapes for GR and ER. We investigated genome-wide binding profiles for ER and GR upon co-activation, and characterized status of the chromatin landscape. We describe a novel mechanism dictating the molecular interplay between ER and GR. Upon induction, GR modulates access of ER to specific sites in the genome by reorganization of the chromatin configuration for these elements. Binding to these newly accessible sites occurs either by direct recognition of ER response elements, or indirectly through interactions with other factors. The unveiling of this mechanism is important for understanding cellular interactions between ER and GR, and may represent a general mechanism for crosstalk between nuclear receptors.

**P-38**

**EFFECTS OF UPSTREAM BINDING FACTOR (UBF) LOSS ON RIBOSOMAL RNA GENE CHROMATIN AND SUB-NUCLEAR STRUCTURE**

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UBF is a multi-HMGB-box transcription factor that is believed to induce an epigenetic reorganization of the Ribosomal RNA (rRNA) genes, and to mediate the growth factor regulation of Ribosome Biogenesis. Here we show that inactivation of the Ubf gene in cells derived from mice carrying a homozygous conditional Ubf deletion leads to a rapid depletion of UBF protein, a shut-down of rRNA gene transcription and the arrest of cell proliferation. Concomitantly, we observe major changes in rRNA gene chromatin, nucleolar fusion and genome-wide changes in gene expression. Subsequently, oncogenically transformed UBF-null cells uniformly enter caspase-dependent apoptosis. P53 levels are, however, not elevated preceding entry into apoptosis. The effects of UBF inactivation closely resemble those induced by cisplatin, a commonly used anti-cancer drug known to displace UBF from the nucleolus. Thus, the displacement of UBF explains the major cytotoxic effects of this drug and its anticancer activity. Homozygous inactivation of the Ubf gene in mouse arrests development at the 8-16 cell morula stage, that is immediately prior to embryo compaction. It is possible that this loss of zygotic UBF expression simply blocks development as the complement of maternal ribosomes becomes limiting. However, by 8 to 16 cells ribosomal RNA levels have not yet doubled. Alternatively, the establishment of nucleoli in the early embryo through activation of the ribosomal genes could play an important non-synthetic role in ensuring chromosome stability, correct chromosome segregation or by preventing p53 accumulation. In this respect, the data for the Ubf gene inactivation contrasts starkly with that for the gene encoding the RPI initiation factor TIF-IA/Rrn3. Tif1a-null mice develop as far as an equivalent of E7.5-8, by which stage total embryonic RNA, about 80% of which is ribosomal RNA, has increased some 103 times from that present in the oocyte. This work was supported by the Canadian Institutes of Health Research.

**TET2 INTEGRATES METABOLIC, MITOCHONDRIAL, AND HORMONAL SIGNALS AND ALTERATION IN METASTATIC PROSTATE CANCER MAY FACILITATE PROGRESSION TO CASTRATE-RESISTANT DISEASE**

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Metastatic prostate cancer (mPCa) is a highly lethal disease and molecular markers identifying disease subtypes and/or therapeutic targets are needed. We sequenced the exomes of five metastatic tumors and healthy kidney tissue from an index patient with castration resistant mPCa to identify lesions associated with disease progression and metastasis. A somatic missense TET2 alteration predicted to alter protein structure was observed in all metastatic tumors but not the primary adenocarcinoma, indicating TET2 alteration occurred at the metastatic stage of disease. We sequenced TET2 in additional PCa tumors and cell lines and detected somatic missense alterations, LOH, and a frameshift truncation in a cell line derived from a metastatic prostate tumor, DU-145. We observed complex combinations of frequent and rare germline missense SNPs, which may contribute to a previously described association of TET2 with PCa risk by GWAS. Examination of TET2 binding partners by affinity purification, mass spectrometry, and forward and reverse immunoprecipitation, reveals a TET2 complex involved in androgen-mediated gene expression. In summary, TET2 loss in mPCa deserves additional scrutiny and may define a subset of metastatic disease. TET2 function integrates metabolic (glucose), oxidative (iron), mitochondrial ( $\alpha$ -ketoglutarate), and hormonal (androgen) signals. This is achieved, in part, through coincident epigenetic modification of histones and DNA by glucose and 5-hmC, respectively. TET2 loss in metastatic disease is predicted to alter androgen mediated gene expression which may be a response to androgen-ablation therapy and facilitate progression to castration resistant mPCa.

**P-40**

**PROTEIN KINASE A-MEDIATED EZH2 INACTIVATION PROMOTES CELLS DIFFERENTIATION**

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How the epigenome interprets signals from the extracellular milieu to regulate gene expression is still ill-defined. In studies aimed at understanding how the epigenome is regulated during differentiation of neuroblastoma (NB) cells, we identified that retinoids (RA), via activation of protein kinase A (PKA), phosphorylate, and inactivate EZH2 (EZH2-S21). ChIP analysis indicates that within 6 hrs of RA treatment there is a decrease in the levels of EZH2 and H3K27me3 binding to the RAR $\beta$ 2 promoter although global levels of EZH2 had not declined. RA stimulates an increase in phosphorylation of EZH2 at serine 21, which disrupts EZH2 binding to histone H3 and increases the number of cells expressing cytosolic EZH2. Pharmacologic or genetic inhibition of PKA and not AKT, as previously reported, inhibits P-EZH2-S21 levels causing increases in H3K27me3 levels and decreases in steady-state and RA-induced RAR $\beta$ 2 mRNA levels. Moreover, transfection of NB with mutant EZH2-S21A attenuates the ability of RA to induce a cytosolic localization of EZH2 and blocks EZH2 target gene expression and differentiation. In contrast transfection of a phospho-mimic EZH2-S21D, resides primarily in the cytosol, increases RAR $\beta$  mRNA levels and increases cellular differentiation. These findings are not restricted to NB, as we detect RA induced PKA-mediated increases in P-EZH2-S21 and its cytosolic localization in normal mouse embryo fibroblasts and embryonic stem cells. Our studies identify a novel mechanism by which cAMP and PKA, key mediators of extracellular signaling pathways, function to regulate EZH2 activity.

**COMPLETE TCR $\alpha$  GENE LOCUS CONTROL REGION ACTIVITY IN T CELLS DERIVED IN VITRO FROM EMBRYONIC STEM CELLS**

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Locus Control Regions (LCR) are cis-acting gene regulatory elements with the unique, integration site-independent ability to transfer the characteristics of their locus-of-origin's gene expression pattern to a linked transgene in mice. LCR activities have been discovered in numerous T cell lineage expressed gene loci. These elements can be adapted to the design of stem cell gene therapy vectors that direct robust therapeutic gene expression to the T cell progeny of engineered stem cells. Currently, transgenic mice provide the only experimental approach that wholly supports all the critical aspects of LCR activity. Herein we report manifestation of all key features of mouse T cell receptor (TCR)- $\alpha$  gene LCR function in T cells derived *in vitro* from mouse embryonic stem cells (ESC). High level, copy number-related TCR $\alpha$  LCR-linked reporter gene expression levels are cell type-restricted in this system, and upregulated during the expected stage transition of T cell development. We further report that *de novo* introduction of TCR $\alpha$  LCR linked transgenes into existing T cell lines yields incomplete LCR activity. Together, these data indicate that establishing full TCR $\alpha$  LCR activity requires critical molecular events occurring prior to final T-lineage determination. This study additionally validates a novel, tractable and more rapid approach for the study of LCR activity in T cells, and its translation to therapeutic genetic engineering.

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**GAMMA-H2AX AS A PREDICTIVE MARKER TO STUDY THE EFFICACY OF GENOTOXIC DRUGS AGAINST MELANOMA USING RECONSTITUTED 3D HUMAN SKIN MODEL**

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Due to the lack of effective treatments, patients with advanced melanoma show poor survival rates. However, melanoma's dermal location enables the application of human skin models. These models mimic many important characteristics of skin in vivo. Monitoring gamma-H2AX foci to assess DNA double-strand break (DSB) levels in reconstituted human 3D skin tissue harboring melanoma tumor cells may provide insights for drug development against cancer. In addition to the differential toxicities of genotoxic agents on the various cell types, we examine how the early drug response of normal tissues might serve as surrogates for predicting treatment efficacy on the tumors. Commercially available reconstituted 3D human skin tissue consists of dermal fibroblast and epidermal keratinocyte layers with malignant melanoma A375 cells which have been cultured to form a multilayer, highly differentiated epidermis containing various stages of cutaneous melanoma malignancy. Genotoxic drugs representative of several different categories were studied, including bleomycin (a radiomimetic), camptothecin (a topoisomerase I inhibitor), temozolomide (a DNA alkylating agent), cisplatin (a DNA intercalating agent) and gemcitabine (a nucleoside analog). DNA DSB levels were monitored by gamma-H2AX foci formation by immunohistochemistry and apoptosis by TUNEL assay. Tumor growth was monitored by H&E staining. In addition to study drug responses in tumor cells, we also examined the impacts of cancer drugs in normal cells (i.e., basal layer, keratinocytes and fibroblasts). Determining drug response in normal cells vs. cancer cells in the same 3D tissue may tell us if normal tissues may serve as surrogates to predict drug efficacy in tumors. A wide range of responses in both tumor and normal tissue by genotoxic drugs were observed, including increased genotoxicity, tumor regression and normal tissue toxicity. The relative amounts of DSB formed in both melanoma and normal tissues were drug dependent. Thus, this study provides new insights for the use of both gamma H2AX and reconstituted 3D human skin tissues to predict drug efficacies. If validated, such models would help reduce the use of animals for preclinical studies.

**BRD4 COORDINATES RECRUITMENT OF PAUSE-RELEASE FACTOR P-TEFb AND THE PAUSING COMPLEX NELF/DSIF TO REGULATE TRANSCRIPTION ELONGATION OF INTERFERON STIMULATED GENES**

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Transcription is a highly ordered, regulated multi-step process in eukaryotic gene expression. Negative elongation factor (NELF) and DRB sensitivity inducing factor (DSIF) cooperatively bind to elongating RNA polymerase II (Pol II) and induce transcriptional pausing, which is alleviated by pause-release factor, P-TEFb mediated phosphorylation of Pol II C-terminal domain at serine 2. However, the mechanism of P-TEFb recruitment and regulation of NELF/DSIF during inducible gene expression where recruitment of Pol II is rate limiting step remains poorly understood. We addressed this question in interferon (IFN) stimulated transcription, focusing on BRD4, a BET family protein that interacts with P-TEFb. Besides P-TEFb, BRD4 binds to acetylated histones through the bromodomain. We examined the assembly of transcription pausing and elongation machinery for several IFN stimulated genes (ISGs) in NIH3T3 fibroblasts with detailed time kinetic and chromatin immuno precipitation studies. Specifically, we found that IFN stimulation triggered inducible BRD4 dependent P-TEFb recruitment at the transcription start sites of multiple ISGs, which positively regulates transcription elongation. Likewise, NELF and DSIF were hardly detectable on ISGs prior to stimulation, but recruited robustly after IFN treatment. A shRNA-based knockdown of NELF revealed that it negatively regulates the passage of Pol II and DSIF across the ISGs during elongation, which reduces total ISG transcript output. Analyses with a BRD4 small molecule inhibitor showed that IFN-induced recruitment of P-TEFb and NELF/DSIF was under control of BRD4. Together, our data suggests a model where BRD4 coordinates both positive and negative regulation of ISG transcription elongation.

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**PROMOTER CROSS-TALK VIA A SHARE ENHANCER EXPLAINS PATERNALLY BIASED EXPRESSION OF NCTC1 AT THE IGF2/H19/NCTC1 IMPRINTED LOCUS**

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Developmentally regulated transcription often depends upon physical interactions between distal enhancers and their cognate promoters. Recent genomic analyses suggest that promoter-promoter interactions might play a similarly critical role in organizing the genome and establishing cell-type specific gene expression. The Igf2/H19 locus has been a valuable model for clarifying the role of long-range interactions between cis regulatory elements. Imprinted expression of the linked, reciprocally imprinted genes is explained by parent-of-origin specific chromosomal loop structures between the paternal Igf2 or maternal H19 promoters and their shared tissue-specific enhancer elements. Here we further analyze these loop structures for their composition and for their impact on expression of the linked long non-coding RNA, Nctc1. We show that Nctc1 is co-regulated with Igf2 and H19 and physically interacts with the shared muscle enhancer. In fact, all three co-regulated genes have the potential to interact not only with the shared enhancer but also with each other via their enhancer interactions. Furthermore, developmental and genetic analyses indicate functional significance for these promoter-promoter interactions. Altogether, we present a novel mechanism to explain developmental specific imprinting of Nctc1 and provide new information about enhancer mechanisms and about the role of chromatin domains in establishing gene expression patterns.

**A TRULY GLUCOCORTICOID RECEPTOR MUTANT: ROLE OF MULTIPLE DOMAINS FOR IN VIVO DIMERIZATION**

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Glucocorticoids (GCs) influence the activity of almost every cell in mammalian organisms, mainly through binding to the Glucocorticoid Receptor (GR). Although GCs are essential for life they are also implicated in the pathogenesis of disease, and produce many unwanted effects when given therapeutically. In the absence of ligand GR is associated to the hsp90 chaperone heterocomplex and primarily localizes in the cytoplasm, while the GR-ligand complex is mainly nuclear. Once in the nucleus, the activated GR regulates gene expression either by direct binding to specific sequences in the DNA, or by protein-protein interactions with other transcription factors. These two mechanisms of action were historically designated GR transactivation and GR transrepression, respectively. Although GR homodimerization is considered an essential step in the GR-transactivation pathway, it is still not clear whether GR dimerizes before or after DNA binding; or which regions of the protein are functionally involved in the homodimerization process. A point mutation (A465T) within GR's DNA-binding domain (DBD) [also known as the GRdim mutant] has been suggested to be crucial for dimerization and DNA binding. However, this mechanism has recently been challenged. Here, we analyzed GR oligomerization state in vivo by using the Number and Brightness (N&B) assay. This novel technique, based on moment-analysis, provides the average number of moving, fluorescent molecules and their brightness at every pixel of images. Therefore, N&B can be used to obtain the oligomerization state of proteins in living cells with high spatial resolution. Our results suggest a complete, reversible, and DNA-independent, ligand-induced model for GR dimerization within the nuclear compartment. We demonstrate that GRdim is able to form dimers and that an additional mutation (I634A) in the Ligand-binding domain (LBD) severely compromises homodimer formation. Thus, GR form dimers in vivo through a combined action of the LBD and the DBD regions. Transactivation and transrepression assays indicate no correlation between the monomeric/dimeric state of the receptor and its transcriptional activity. Finally, chromatin immunoprecipitation experiments suggest that dimerization status affects DNA binding only to a subset of GR binding sites. These results will have major implications on the future search for dissociated glucocorticoid ligands for human therapy.

**NURF REGULATES CELL-TYPE GENE EXPRESSION IN PART THROUGH THE UNIQUITOUS MULTIVALENT FACTOR CTCF**

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Organism development and tissue formation depend on distinct gene expression in the different cells and its aberrant expression leads to diseases or cancers. The gene expression is not only controlled by transcription factors, but also influenced by epigenetic regulators such as Nucleosome Remodeling Factor (NURF). NURF, an ATP-dependent chromatin remodeling complex consisting of three subunits including BPTF, SNF2L and pRBAP46/48, positively or negatively regulates transcription of several hundred *Drosophila* genes *in vivo*. However, how it regulates the expression of genes remains elusive in many aspects. In this investigation, we observed that some NURF-dependent genes were cell-type dependent in regulation in the double positive cells, ES cells and MEF cells of mice, but interestingly they became cell-type independent changes in nucleosome occupancy in those cells. To address this question, BPTF knock-out and knock-down ES cells and fibroblast cells were used to perform experiments. The results showed that there were physical interactions between BPTF, CTCF and SA2. ChIP data indicated that NURF, CTCF and Cohesin were enriched on the same DNA site of some genes chosen randomly and the PNI assay demonstrated that CTCF binding site possessed enhancer, insulator or silencer activity, which was BPTF dependent. All these results were verified by an analysis of different DNA fragment in the oncogene cyclin D1, a gene which is NURF dependent in multiple cell types. Further research exhibited that some CTCF binding sites were near the nucleosome which contains H2AZ instead of H2A. Our *in vitro* chromatin remodeling assay revealed that in comparison with H2A nucleosome, NURF made H2AZ nucleosome shift faster and form more distinct nucleosomes. Taken together, NURF plays a critical role in conversion of cell-independent genes in regulation to cell-dependent genes in expression through the ubiquitous multivalent factor CTCF. Our future study will focus on analysis of functional domains of BPTF which cause chromatin remodeling to completely understand the mechanism of NURF and apply it to medical treatment.

**THE EPIGENETICS OF EPITHELIAL SELF-RENEWAL IN THE INTESTINE**

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Self-renewing tissues must control both proliferation and differentiation in space and time, and maintain this control over thousands of cell divisions throughout life. The murine intestinal epithelium is an excellent model system in which to study self-renewal. The intestinal epithelium has a unique organization in which stem cells are harbored in crypts, which produce progenitors and finally clonal populations of differentiated cells that migrate in ordered cohorts up the villus axis. Disrupted maintenance of the intricate balance of proliferation and differentiation can lead to loss of epithelial integrity and barrier function or to cancer. We have learned a great deal about the genetic control of intestinal differentiation and proliferation from modern mouse genetics. However, the characterization of the control of gene expression during self-renewal of the intestinal epithelium at the whole genome level has lagged behind, due in part to the mixture of numerous cell types in various stages of differentiation and the difficulty in sorting pure cell populations, as well as the lack of genome-wide tools. Homeostasis of self-renewal tissues requires the tight control of gene expression throughout multiple stages of differentiation. Gene expression is tightly correlated with the epigenetic state through DNA methylation and histone modifications. Furthermore, maintenance of DNA methylation has been shown to be important for self-renewal and appropriate differentiation. Loss of the key enzyme required for maintenance of DNA methylation, DNA Methyltransferase 1 (Dnmt1), in the human epidermis has been shown to cause inappropriate differentiation and loss of the progenitor pool, resulting in total loss of regenerative capacity. There is a tight correlation between epigenetic status of genes and their expression in self-renewing, progenitor and fully differentiated cell types; however, the mechanism of how changes in epigenetic status direct gene expression and the progression from stem cell to its differentiated descendants is unclear. Our work addresses the importance of epigenetic modifications in the maintenance of self-renewal in the intestine. We have employed cell-sorting techniques to separate stem cells and differentiated cells from the mouse small intestinal epithelium and used mRNA sequencing and whole-genome shotgun bisulfite sequencing (WGSBS) to determine how gene expression is correlated with DNA methylation during self-renewal of the intestine. We show that genes important for stem cell maintenance and proliferation are methylated during differentiation and loss of methylation by deletion of Dnmt1 in the adult causes crypt expansion in vivo. Our research shows that DNA methylation plays an important role in the maintenance of the intestinal epithelium.

**MBD3 ACCUMULATES AT PROMOTERS AND ENHANCERS OF ACTIVE GENES**

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The Mi-2/nucleosome remodeling and histone deacetylase (NuRD) complex is a multiprotein machine proposed to regulate chromatin structure by nucleosome remodeling and histone deacetylation activities. Recent reports describing localization of NuRD are not in complete agreement and provide new insights that question previous models on NuRD action. Here, we provide location analysis of endogenous MBD3, a component of NuRD complex, in two human breast cancer cell lines (MCF7 and MDA-MB-231) using two independent genomic techniques: DNA adenine methyltransferase identification (DamID) and CHIP-Seq. Contrary to existing models, MBD3 preferentially associated with CpG rich promoters marked by H3K4me3 and lacking 5-methyl C or 5-hydroxymethyl C. MBD3 also showed cell-type specific localization across gene bodies, peaking around the transcription start site (TSS). A subset of sites bound by MBD3 was enriched in H3K27Ac and was in physical proximity to promoters in three-dimensional space, suggesting function as enhancers. MBD3 enrichment was also noted at promoters modified by H3K27me3. These data suggest that MBD3, and by extension the Mi-2/NuRD complex, may have multiple roles in fine-tuning expression for both active and silent genes. These data represent an important step in defining regulatory mechanisms by which Mi-2/NuRD complex controls chromatin structure and modification status.

**EFFECTS OF DNA METHYLATION ON NUCLEOSOME STABILITY**

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Recent experimental studies suggest that the mechanical properties of a given DNA sequence dictate its nucleosome positioning propensity, and therefore may play an important role in gene regulation. In particular, the nucleosome affinity of DNA sequences is higher in sequences that have dinucleotide repeated every 10 bp. Likewise, CpG methylation of DNA is an epigenetic modification associated with the inactivation of transcription and the formation of a repressive chromatin structure. Understanding the changes in the structure of nucleosomes with various dinucleotide and upon CpG methylation and is necessary in providing insight of the mechanisms of gene repression. The Fragment-Molecular Orbital (FMO) and the Density Functional Theory-Symmetry Adapted Perturbation Theory (DFT-SAPT) methods were utilized to systematically study the stacking effects of two different (linear and bent) DNA fibers of 18 bps length as a function of both twist and rise (which is related to its bending propensity). The DFT-SAPT method provides insight into the  $\pi$ -stacking and hydrogen-bonded interactions of DNA structures on the basis of electrostatic and dispersion contributions. Results indicate sequences with CpG methylation along with specific dinucleotides have lower interaction energies-this is associated to higher nucleosome affinity. These findings suggest that changes in the physical properties of nucleosomes induced upon CpG methylation may contribute directly to the formation of a compacted chromatin structure. Research Supported by NIH-PSOC grant #U354CA143869.

**STOICHIOMETRY OF THE CARIOGENIC SWI/SNF-LIKE CHROMATIN-REMODELING COMPLEXES SPECIFIES HEART DEVELOPMENT**

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The regulatory networks of differentiation programs in mammalian embryos have been partly characterized; however, the molecular mechanisms of lineage-specific (heart) gene regulation by global chromatin changes have been unclear. Here, we show that several distinct cardiogenic SW/SNF-like complexes exist in the developing heart, and BAF-A complexes act as a repressive regulator during cardiac differentiation. Using an innovative proteomics approach, we show that various type of ATP-dependent SWI/SNF-like chromatin-remodeling complexes exist in the developing heart, and a change in stoichiometry and in subunit composition in the heart, differed from those of the head and trunk characterized at the E8.5 mouse embryo. Early heart complexes in which BAF60b, 60c, 250a, 250b and polybromo BRD7 are quantitatively associated with the SWI2/SNF2-like ATPases, Brg and Brm. During development, these subunits show distinct spatiotemporal patterns of expression between the heart, head and trunk with elevated and specific expression of BAFs 250a, 200, 180 and 60c in the heart. Preventing the BAF250a/BAF complex led to upregulation of cardiac genes thus results impaired fully functional cardiomyocyte differentiation. We found that BAF250a directly binds to the regulatory DNA sequence located in the enhancer/promoter region of many key genes in developing heart. Consistent with this, in the absence of BAF250a, active chromatin mark and chromatin accessibility is increased, and poised chromatin mark decreased on the regulatory region due to destabilization of the HDAC/NURD complexes. Hence distinct SWI/SNF-like complexes exist in the developing heart, and BAF250a-containing BAF-A complexes defines transcriptional regulation to fine tune transcription by preventing the spread of active chromatin during early heart development.

**NON-TRANSCRIPTIONAL ROLES FOR SP1 IN PREVENTING CHROMOSOMAL INSTABILITY**

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Genomic instability is a broad term used to describe the failure of a cell to pass a complete and intact copy of its genome to its daughter cells, and can occur through the gain or loss of whole chromosomes during cell division. When dynamic and continual, this phenomenon is classified as whole chromosomal instability (CIN). CIN is a common feature of many cancers, and is associated with poor patient outcome in multiple cancer types, as well as multi-drug resistance and tumor heterogeneity, underscoring its clinical importance. Once thought to be a by-product of cell transformation, CIN is now emerging as a causative factor in cancer development. Despite its prevalence and clinical importance, the exact mechanisms that lead to CIN remain to be determined. Since its discovery in cancer, many research groups have identified mechanisms that, when lost or disrupted, can lead to chromosome missegregation and CIN. Those mechanisms include cohesion defects, defects in kinetochore-microtubule attachment at the centromere, weakening or loss of the spindle assembly checkpoint (SAC), and/or centrosome amplification. The transcription factor Specificity Protein 1 (Sp1) regulates the expression of genes involved with many cellular processes, including differentiation, cell cycle progression, DNA repair, apoptosis, and senescence. Sp1 binds to specific GC-rich elements through its highly conserved carboxy terminal zinc finger DNA binding domain and functions to recruit different factors to chromatin in order to influence transcription. Our previous work shows that Sp1 is important for maintaining chromosomal stability during mitosis. We have shown that loss of Sp1 results in abnormal chromosome alignment along the metaphase plate, creation of micronuclei, and aneuploidy. More recently, we have shown that Sp1 knockdown results in lagging chromosomes and anaphase bridges, all of which are phenotypes consistent with CIN. Preliminary data indicates that Sp1 localizes to the centromere during mitosis and loss of Sp1 results in defects in chromosome pairing and sister chromatid cohesion. This phenotype can result from defects in several different processes, including the sister chromatid cohesion machinery, kinetochore-microtubule attachment, and/or the spindle assembly checkpoint. Our data shows that the Sp1 DNA binding domain is not required for localization to the centromere, suggesting a non-transcriptional role for Sp1 at the centromere-kinetochore region during mitosis. Interestingly, this data differs from previously published works that describe Sp1 as being evicted from the chromatin during mitosis. Therefore, we hypothesize that Sp1 prevents chromosomal instability through a novel non-transcriptional mechanism at the centromere-kinetochore region during mitosis. Results of experiments testing this hypothesis will be presented.

**CHARACTERING B CELL LYMPHOMA SELF-RENEWING CELLS AND THEIR  
ROLE IN LYMPHOMA RELAPSE**

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Diffuse Large B-cell Lymphoma (DLBCL) is an aggressive form of non-Hodgkin's Lymphoma (NHL) with one-third of patients either do not respond to initial therapy or relapse after standard therapy, such as CHOP or R-CHOP. Patients who relapse see initial tumor regression but the tumor comes back and is usually chemoresistant. Although relapses normally occur early, mainly within the first 2-3 years after initial treatment, some do occur after 5 years. Treatment options for relapse and refractory DLBCLs are limited, including salvage chemotherapy followed by autologous stem cell transplantation. However only 10% of the relapsed patients can achieve 3-year progression-free survival with these treatments, underlying the urgent need of novel approaches to treat DLBCL relapse. Unfortunately, our current understanding of the molecular mechanisms associated with DLBCL relapse is limited.

**ChAP-MS: A METHOD FOR IDENTIFICATION OF PROTEINS AND HISTONE POSTTRANSLATIONAL MODIFICATIONS AT A SINGLE, NATIVE GENOMIC LOCUS**

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The field of epigenomics has been transformed by chromatin immunoprecipitation approaches that provide for the localization of a defined protein or posttranslationally-modified protein to specific chromosomal sites. While these approaches have helped us conceptualize epigenetic mechanisms, the field has been limited by the inability to define features like the proteome and histone modifications at a specific, native genomic locus in an unbiased manner. We developed an unbiased approach whereby a unique native genomic locus was isolated, which was followed by high resolution proteomic identification of specifically associated proteins and histone posttranslational modifications. This Chromatin Affinity Purification with Mass Spectrometry (ChAP-MS) technique was used to specifically enrich a 1,000 base-pair section of GAL1 chromatin under transcriptionally active and repressive conditions, and to identify the specifically bound proteins and histone posttranslational modifications. ChAP-MS should yield unprecedented insight into the regulatory mechanisms of transcription and help identify factors that epigenetically control chromatin function.

**GENOME WIDE PROFILING OF CHROMATIN TRANSITIONS DURING OSTEOBLAST DIFFERENTIATION IN hFOB CELLS**

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Bone regeneration from mesenchymal stem cells (MSC) is a natural repair mechanism that resembles the embryonic process of bone formation. Although bone undergoes life-long remodeling, there is a substantial lack of information on MSC differentiation and key regulators in this process. The purpose of this study was to investigate global chromatin changes during MSC differentiation into osteoblast and how these changes affect transcriptional regulation. **METHODS:** We used a human fetal osteoblastic cell line, hFOB, has been immortalized by SV40 pUCSVtsA58, a temperature sensitive vector. hFOB cells resemble mesenchymal stem cells when grown at 34C, and are capable of undergoing multilineage differentiation to adipocyte, osteoblast, or chondrocyte when shifted to 39C in the presence of specific growth factors. At 39C they can spontaneously differentiate into osteoblast, a process that takes several weeks. To characterize phenotypic and genotypic changes during differentiation to osteoblast and adipocyte lineages, we performed cell staining by Alizarin Red S dye and Oil Red-O, as well as qPCR for several key osteoblast and adipocyte-specific genes, such as ALP, COL1A1, RUNX2, PPAR $\gamma$ , and others.

Detection of sites in mammalian chromatin that are hypersensitive to DNase I (DHS) has been recently adapted to allow examination of global chromatin landscape and its changes in response to hormones and differentiation. To investigate global chromatin transitions during osteoblast differentiation, we performed DNase-seq. To select an optimal sample for sequencing, we developed a quality control method using negative primers matched to closed chromatin and positive primers matched to hypersensitive sites common among multiple cell lines (retrieved from ENCODE datasets). **RESULTS:** When hFOB cells are shifted to 39C and exposed to osteogenic induction media (OIM) that contains dexamethasone, the osteoblast differentiation process is significantly accelerated, with an increase in osteoblast gene expression by day 8 [Example: an 11-fold increase in Alkaline Phosphatase (ALP)]. The accumulated mineralized matrix deposit in cells exposed to OIM was also detected at day 8. The number of DHS elements increased significantly during the differentiation process. Examination of these elements will allow the characterization of regulatory circuits important in the osteoblast differentiation process.

**CONCLUSIONS:** We established a QC method that enables us to select samples with appropriate levels of DNase I digestion prior to fragment isolation and sequencing. We can apply this method to other cells and tissues following DNase I, Benzonase and similar methods of enzymatic digestion of DNA.

**FUTURE WORK:** We will extend the analysis of DHS-seq on hFOB cells during osteoblast differentiation, perform bioinformatics to investigate transitions in the chromatin landscape during osteoblast differentiation, determine and validate key regulators involved in this process.

**STAT PROTEINS: ORCHESTRATORS OF GENOMIC ENHANCERS**

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Genomic enhancers are recognized as primary elements that regulate selective gene expression. However, the interplay between signaling pathways and actively used enhancer elements is not known. We use CD4<sup>+</sup> T cells as a model of differentiation, mapping the activity of cell-type-specific enhancer elements in T helper 1 (Th1) and Th2 cells. Mapping the chromatin signature of active enhancers, we establish that signal transducers and activators of transcription (STAT) proteins have a major impact on the activation of lineage-specific enhancers. In addition, STAT proteins are responsible for the suppression of enhancers associated with alternative cell fates. Transcriptome analysis further supports a functional role for enhancers regulated by STATs. Importantly, expression of lineage-defining master regulators in STAT-deficient cells fails to fully establish the chromatin signature of STAT-dependent enhancers. Thus, these findings point to a critical role of STATs as environmental sensors in dynamically shaping the specialized enhancer architecture of differentiating cells.

**IDENTIFICATION AND CHARACTERIZATION OF CARM1 SUBSTRATES**

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Arginine methylation is a common post-translational modification that occurs in eukaryotes. It is mediated by a group of enzymes called Protein ARginine MethylTransferases (PRMTs). The PRMT family has nine members, PRMT1-9. The current project focuses on PRMT4, also referred to as CARM1 (Coactivator-Associated ARginine Methyltransferase 1). CARM1 methylates histone H3 (at Arg17), the histone acetyltransferase, p300/CBP and GRIP1, a transcriptional co-activator, all of which positively influence transcription and therefore CARM1 is considered a coactivator. The methylation events on these substrates are believed to generate docking sites for effector proteins, which will further modulate their protein-protein interactions that are involved in transcription and splicing, although the exact mechanism of how this happens is unclear. In order to better understand the role of CARM1 in cellular processes, we seek to identify its substrates. For this purpose, we generated and characterized Pan-CARM1 substrate antibodies. Using an immunoprecipitation (IP)/mass spectrometry approach, we identified over 100 putative CARM1 substrates and selected a subset of these proteins (26) for further evaluation. This screen identified a few novel CARM1 substrates, one of which is Med12, a component of the Mediator complex. Besides its negative regulatory role in general transcription, Med12 possesses multiple other functions in the Wnt signaling pathway, NANOG pathway and REST-mediated gene silencing. Med12 was shown to aid in REST-mediated silencing by recruiting G9a. Particularly, G9a interacts with Med12 at its PQL domain, where it is also methylated. Based on these observations, we propose that methylation on Med12 potentially affects its interaction with G9a, thereby influencing the repressive functions of REST. We will test this hypothesis by performing Co-IP assays between Med12 and G9a in WT and CARM1 KO cells. Additionally, ChIP-seq analysis will be done with Pan-CARM1 substrate antibodies, which will help determine the genomic distribution of CARM1 activity. ChIP-seq experiments will also be done with methyl-specific Med12 antibody and we expect to see an overlap between Med12 and CARM1 activity profiles. We envision that a major mechanism of transducing CARM1's co-activator activity is by blocking the repressive function mediated by the Med12 and G9a interaction.

**SIMULTANEOUS MAPPING OF TRANSCRIPTION FACTOR AND NUCLEOSOME OCCUPANCY**

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The binding of sequence specific transcription factors leading to the eviction nucleosomes is a fundamental process of eukaryotic gene regulation. Despite the wealth of available data concerning the occupancy of transcription factors and nucleosomes in the human genome, we currently lack methods to measure their co-localization on the same chromatin template. Here, we describe a novel method leveraging DNase I that detects transcription factors and nucleosomes with nucleotide precision within the same experiment. We find that small fragments released during DNase I digestion of intact nuclei delineate the precise boundaries of transcription factor binding sites and then these fragments are in a quantitative relationship with transcription factor occupancy. We identified >500,000 nucleosomes that flank canonical DNase I hypersensitive sites in both proximal and distal configurations. We observed that transcription factor binding sites are depleted within sequences overlapping the nucleosome core particle, while enriched at the boundaries, demonstrating nucleosome positioning by bound sequence specific transcription factors. Finally, we resolve high-resolution structures of both transcription factor and nucleosome occupancy at active human promoters.

**SETBP1 IS AN ONCOGENE CAPABLE OF INDUCING MYELOID LEUKEMIA DEVELOPMENT**

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We have found previously that overexpression of Setbp1, an AT-hook transcription factor, can immortalize mouse myeloid progenitors in culture, suggesting Setbp1 activation could be a driver mutation capable of inducing myeloid leukemia development. To test this idea, we transduced 5-fluorouracil treated murine bone marrow progenitors with a retrovirus expressing Setbp1 and marker GFP, and subsequently transplanted them into lethally-irradiated congenic mice. Engraftment analysis of transduced cells in recipient mice showed that their representation in donor cells increased with time suggesting that Setbp1 expression promotes proliferation of hematopoietic progenitor cells. Setbp1 overexpression in hematopoietic stem cells (HSCs) may also promote their commitment to the myeloid lineage as significantly higher percentage of cells expressed myeloid marker Gr-1. More interestingly, 45% of transplanted mice developed myeloid leukemia within a year starting as early as 55 days post transplantation. Leukemic mice displayed enlarged spleens and leukemic infiltration into non-hematopoietic tissues including liver and lung. Secondary recipients of spleen cells from leukemic mice developed the same disease with much shorter latency, suggesting that additional mutations may be required for Setbp1-induced leukemic transformation. We have identified Mllt3 as a potential cooperating partner for Setbp1 during leukemia development by cloning retroviral insertions in these Setbp1-induced leukemias. Furthermore, enforced expression of Setbp1 in lin<sup>-</sup> sca1<sup>+</sup>kit<sup>+</sup> (LSK) cells increased their in vivo repopulating capabilities after secondary bone marrow transplant, suggesting that Setbp1 may promote self-renewal of HSCs. Thus, these data suggests that Setbp1 is an oncogene in the hematopoietic system capable of inducing myeloid leukemia development by disrupting normal development of hematopoietic stem and progenitors.

**BIOCHEMICAL INSIGHTS INTO THE MECHANISM OF MOT1**

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Mot1, an essential protein in *S. cerevisiae*, regulates the dynamics of TATA-binding protein (TBP) by displacing it from promoters in an ATP-dependent manner. Mot1 also belongs to the Swi2/Snf2 enzyme family, whose members broadly regulate various processes like transcription, replication and repair. While many of the Swi2/Snf2 ATPases are subunits of large complexes, Mot1 and its human homolog, BTAF1, act as single polypeptides on TBP. In order to gain insight into the mechanism by which these enzymes disrupt protein-DNA interactions, we used a FeBABE-mediated hydroxyl radical cleavage assay to map the domains of Mot1 that interact with promoter DNA. Using this assay, we found first that the Mot1 ATPase domain interacts with DNA upstream of the TATA box. Using the ATP analog, ADP-AIF4, we trapped a putative transition-state complex in which the ATPase domain has undergone a conformational change in which an additional domain is engaged with DNA and the complex is primed for TBP-DNA dissociation. Gaps primarily on one strand of DNA upstream of the TATA box inhibit the catalytic activity of Mot1 suggesting that Mot1 displaces TBP by tracking on a single strand of DNA. The C-terminal Mot1 ATPase domain is tethered to TBP by a spring-like array of HEAT repeats. We propose that the HEAT array might not function as a passive tether and instead may store conformational energy in the spring so that it can be used to facilitate the dissociation of TBP from DNA.

**MICRORNA-MEDIATED REGULATION OF THE BRG1 CHROMATIN REMODELING COMPLEX IN HUMAN EMBRYONIC STEM CELLS**

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Embryonic stem (ES) cells hold great promise for regenerative medicine because of their unique characteristics of self-renewal and pluripotency. Balance between ES cell pluripotency and lineage commitment is maintained in part by epigenetic regulators such as the developmentally-regulated Brg1 chromatin remodeling complex. Subunits of the Brg1 complex, termed BAFs, are assembled in a combinatorial fashion to dictate context-dependent functional specificity. Although BAF expression has been studied in mouse ES cells, the mechanisms of regulation remain unknown. Here we provide, to our knowledge, the first mechanistic insight into BAF regulation in ES cells. We utilized *in vitro* culture and differentiation of hES cell lines to explore the regulation of BAFs in early human development. Through gain- and loss-of-function experiments we identified a microRNA-mediated regulatory event critical for BAF regulation. The ES-cell specific miR-302 family directly represses BAF170 in hES cells. This repression is relieved upon differentiation and miR-302 inhibition. The importance of BAF170 repression for gene expression was explored through genome-wide microarray studies. 352 genes were significantly affected at least 1.5 fold by BAF170 KD with 63% also misregulated upon miR-302 inhibition. Functional analysis revealed enrichment in Nodal signaling. qRT-PCR confirmed that miR-302 and BAF170 conversely regulate endodermal differentiation markers and targets of Nodal signaling, a pathway known to regulate both ES cell pluripotency and endodermal differentiation. Our data support a role for miR-302-mediated BAF170 repression in maintaining pluripotency through suppressing endodermal differentiation and suggest that relief of this inhibition is important for human endodermal lineage specification. This places the BRG1 complex at the center of cell fate decisions during early human development and provides mechanistic insight into the essential role of this complex in balancing stem cell pluripotency and differentiation.

**MECHANISM OF TRANSLATION TRANSCRIPTION OF AN OXIDATIVE  
CYCLOPURINE DNA DAMAGE**

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The 8, 5'-Cyclopurines-2'-deoxyadenosine DNA lesion (CydA) is one of the major oxidative DNA damage that strongly blocks elongation by RNA polymerase II (Pol II) and triggers transcription-coupled DNA repair. Here, we present a functional dissection of individual steps in Pol II negotiation with a CydA that allows bypass of the lesion without repair. A major barrier to Pol II emerges subsequently to CydA loading to the active site and after proper UMP incorporation opposite the CydA (step 1). The barrier involves Pol II failure in loading the adjacent base 5' to the CydA into the active site resulting in slow non-templated AMP insertion according to an A-rule (step 2) known for DNA polymerases and recently demonstrated for UV-induced CPD lesion bypass by Pol II. The last rate-limiting step in the bypass involves extension of the 5' A with the next cognate nucleotide (step 3), which includes an impaired translocation of the CydA lesion beyond the active center of Pol II (i-1 site). Alternatively, Pol II can also catalyze slow templated UMP incorporation opposite the CydA adjacent 5' base leading to an error-free bypass (step 2). Our findings corroborate previous observations on the CydA bypass in human cells that showed both error-free bypass, as well as a preferred misincorporation of adenine opposite the adjacent base 5' to the lesion. Notably, the CydA bypass in vitro is substantially slower than in living cells indicating involvement of special factors assisting the process in living cells. These findings reveal a biologically important mechanism employed by Pol II to limit the burden of lesion-arrested transcription and transcriptional mutagenesis resulting from endogenous oxidative DNA damage.

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**STEROID RECEPTOR RNA ACTIVATOR (SRA) AS A 'BIVALENT' LONG NON-CODING RNA: INTERACTION WITH MLL AND PRC2 COMPLEXES**

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Steroid Receptor RNA Activator (SRA) is the first identified long non-coding (lnc) RNA which is involved in transcriptional activation of steroid hormone responsive genes via a direct and specific interaction with the RNA helicases p68/p72 together with other coactivator complexes. Recently, SRA has been shown to directly associate with two nuclear receptors, i.e. estrogen and androgen receptors. However, little is known about how SRA controls gene expression and how the lncRNA might integrate its co-activation activity with epigenetic mechanisms. We have previously shown that SRA is present in the complex with the chromatin insulator CTCF, and that CTCF and the RNA helicase p68 form a complex *in vivo*. Here, we show that the lncRNA SRA can pull down CTCF and the cohesin component SMC1A from nuclear extract. However, the association between CTCF and SRA is indirect, which might be mediated by p68. Using chromatin isolation by RNA purification technique, we find that the lncRNA SRA is present at promoter regions of previously characterized SRA activated or repressed genes. Many of these sites are also occupied by p68, suggesting that SRA is a functional partner of p68 at these target genes. The RNA pulldown assay also reveals not only a direct interaction between SRA and p68 as previously described, but also a direct binding between the lncRNA and the trithorax and polycomb complexes MLL and PRC2, respectively. Further, co-immunoprecipitation experiments indicate that a single SRA molecule can recruit both the MLL and PRC2 complexes. In addition, a genome wide analysis shows that the majority of genomic regions which are occupied by p68, are co-bound by histone H3 lysine 4 trimethylation (H3K4me3) in HeLa cells. Moreover, p68 binding is also observed at many loci having both H3K4me3 and H3K27me3 marks which, when present together, are indicative of bivalent domains. Taken together, this study suggests that SRA might act as a 'bivalent' lncRNA by directly scaffolding two distinct epigenetic machineries involving methylation of histone H3 lysine 4 and lysine 27.

**IDENTIFICATION OF THE HISTONE H2A REPRESSION DOMAIN AS A REGULATOR OF H3K79 METHYLATION AND TRANSCRIPTION ELONGATION IN YEAST**

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Histone 'cross-talk' represents a fundamental way by which histone post-translational modifications (PTMs) regulate the structure and function of chromatin. Here we show in the budding yeast *Saccharomyces cerevisiae* that a H2A N-terminal region referred to as the H2A repression (HAR) domain is important for trimethylation of H3K79 (H3K79me<sub>3</sub>). Consistent with a recently published report, we also find that the HAR domain regulates mono-ubiquitylation of H2BK123 (H2BK123ub<sub>1</sub>) which, as we show for H3K79me<sub>3</sub>, is a regulatory pathway observed across multiple genetic backgrounds. This was in contrast to the previously reported regulation of H3K4 trimethylation by the HAR domain, which we find restricted to a particular genetic background. We further show that the HAR domain promotes H3K79me<sub>3</sub> by maintaining wild-type levels of H2BK123ub<sub>1</sub>, but this mechanism is independent of recruitment of the H2B ubiquitylation machinery to chromatin. Finally, we provide genetic evidence that the HAR domain contributes to telomeric silencing and the process of transcription elongation consistent with the established role of H2BK123ub<sub>1</sub> in these processes. In sum, these data highlight a 'cross-talk' pathway involving the H2A tail that governs H2B ubiquitylation and H3 methylation in the process of transcriptional regulation.

**PHOSPHORYLATION STATUS OF ASCL1 REGULATES NEUROBLASTOMA SELF-RENEWAL AND DIFFERENTIATION**Wylie, L.A.<sup>1,2</sup>, Cheng, K.<sup>1</sup>, Thiele, C.J.<sup>2</sup> and Philpott, A.<sup>1</sup><sup>1</sup>Department of Oncology, University of Cambridge, Cambridge, UK <sup>2</sup>Pediatric Oncology Branch, CCR, NCI, Bethesda, MD

Neuroblastoma (NB) is a tumor of infancy that accounts for 15% of all pediatric cancer mortality. NB bears striking similarity to undifferentiated neuroblasts of the sympathetic nervous system in gene expression profiles and histological appearance. In most neuroblastoma tumors, the cell cycle is hyperactive due to overexpression of cyclin D1 and amplification of MYCN. However, retinoic acid (RA) induces differentiation and cell cycle exit of some NB cell lines in vitro and provides significant benefit to patients in the clinic. As neuroblastoma appears to result from sustained proliferation and inhibition of differentiation, we sought to understand how cell cycle and differentiation are linked within NB. We focused our efforts on Ascl1, a proneural transcription factor that is both necessary and sufficient for neural differentiation of noradrenergic neurons and has been shown to be regulated by the cell cycle. Paradoxically, Ascl1 is both highly expressed and associated with poor prognosis in NB. However, we hypothesized that Ascl1 phosphostatus critically regulates its ability to induce differentiation. We show that Ascl1 is highly expressed and phosphorylated across multiple NB cell lines and is largely phosphorylated by CDKs. Phosphorylated Ascl1 actively promotes the G1-S transition by upregulating E2f, Skp2, and Cdk2. These targets all promote CDK activity, further maintaining Ascl1 phosphorylation. However, a phosphomutant form of Ascl1, where putative CDK sites are mutated, arrests cells in the G1 phase by preferentially upregulating Ebf3 and p27. These targets inhibit CDK activity promoting Ascl1 dephosphorylation and further upregulation of Ebf3 and p27. We propose a model whereby Ascl1 sits between two positive feedback loops, both forming a critical link between cell cycle and differentiation, but also as an agent to commit cells to either a self-renewing undifferentiated state or a postmitotic differentiated state. Given this model, we hypothesized that pharmacologic inhibition of CDK activity when combined with a pro-differentiation agent such as RA would synergistically cause differentiation in neuroblastoma. Indeed, CDK inhibitor and RA combination treatment causes increased differentiation in RA-susceptible cell lines and induces differentiation in RA-resistant cell lines. Given that most neuroblastoma tumors have an overactive cell cycle and also highly express Ascl1, combination therapy of CDK inhibitor and RA may provide generalized benefit to NB patients.

**LINKING EPIGENETIC REGULATORS EZH1 AND EZH2 TO NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD)**

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Epigenetic mechanisms of nuclear chromatin remodeling are increasingly recognized as crucial factors in the pathophysiology of NAFLD. Polycomb group members Ezh1 and Ezh2 are important key epigenetic regulator of embryonic stem cell identity, but their role in NAFLD is poorly understood. We studied the function of the histone methyltransferases Ezh1 and Ezh2 in NAFLD development using mice with germ-line and hepatocyte-specific gene deletions, respectively. Germline Ezh1-null (-/-) and hepatocyte-specific Ezh2-null (f/f;AC) male mice were examined at 3 and 8 months of age using histology, liver enzyme tests, RNA-seq and Chip-Seq analyses for H3K4me3 and H3K27me3. Ezh1 ablation induces steatosis. Expression of genes associated with lipogenesis was higher in Ezh1-null mice compared to wild-type mice. In Ezh1&2 double knockout (DKO) mice steatohepatitis was accompanied by the development of fibrosis. This was correlated by pronounced loss of H3K27 trimethylation, and accompanied by increased levels of oxidative stress enzymes and collagen-related genes in livers of Ezh1&2 DKO mice. These results reveal a previously unknown function of EZH1 and EZH2 in regulating NAFLD and strongly suggest that epigenetic regulation may be a determining factor to susceptibilities to NAFLD.

**CHROMATIN DYNAMICS IN HEMATOPOIESIS REVEAL CONCOMITANT ACTIVATION OF TISSUE-SELECTIVE ENHANCERS WITH LIMITED LINEAGE SPECIFICITY**

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Multilineage progenitors establish lineage-specific transcriptional programs to engender cell diversity. Transcription factors such as GATA1 and PU.1 specify blood lineages, but it is unclear when chromatin at lineage-restricted cis-elements becomes fully permissive for transcription. Using ChIP-seq to profile histone marks, we studied enhancer dynamics during differentiation of primary mouse blood stem, progenitor, and specified cells. Enhancers functional in single cell types showed stably positioned H3K4me2- and H3K27ac-marked nucleosomes and DNase hypersensitivity in sibling lineages. Thus, epigenetic states near binding sites for lineage-restricted transcription factors show surprising overlap in distinct cells. Multipotent progenitors showed scant H3K4me2 at enhancers that function in their committed progeny, revealing independent consolidation of this mark after cells diverge in ontogeny. Nevertheless, primordial enhancer marks in progenitors can explain classical observations on multilineage priming. Our findings also suggest a chromatin basis for reprogramming or transdifferentiation and provide a framework to understand how TFs interact with chromatin to drive hematopoiesis.

**THE NUCLEOSOME BINDING PROTEIN HMGN5 AND THE LAMINA ASSOCIATED PROTEIN LAP2A INTERACT AND RECIPROCALLY AFFECT THEIR GENOME WIDE CHROMATIN BINDING**

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Chromatin structure and dynamics are regulated by the combined action of numerous nuclear components including that of architectural proteins such as members of the high mobility group (HMG) superfamily which bind to nucleosomes without any specificity for the underlying DNA sequence. HMGN5, the most recently discovered member of HMGN family, de-compacts chromatin by competing with linker histone H1 and modulates the fidelity of the cellular transcription profile. To gain additional insights into the mechanisms whereby HMGN5 affects chromatin structure and function, we searched for HMGN5-interacting components using the HALO-TAG pull down assay. This approach led to the identification of Lamina-associated polypeptide 2 alpha (LAP2 alpha) as a new HMGN5 interacting protein. We found that LAP2 alpha interacts with HMGN5 both in-vitro and in-vivo. Fluorescence recovery after photobleaching analysis in living cells lacking HMGN5 reveals that the protein affects chromatin binding of LAP2alpha. Chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-seq) indicates that HMGN5 and LAP2 alpha co-localized in the promoter region of numerous genes. Significantly, loss of either HMGN5 or LAP2 alpha resulted in dramatic redistribution of the other protein on chromatin without disrupting global chromatin structure. Our studies reveal a cross-talk between a nucleosome binding protein and a nuclear lamina binding protein and provide additional insights into mechanisms whereby the nuclear lamin network regulates chromatin function.

**THE REGULATORY REGIONS OF KEY DEVELOPMENTAL GENES ARE IMPROPERLY REPROGRAMMED IN HUMAN INDUCED PLURIPOTENT STEM (iPS) CELLS**

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Adult mammalian somatic cells can be reprogrammed into pluripotent stem cells (iPSCs) by manipulating the expression of defined transcription factors. Induced pluripotent cells are similar to embryonic stem cells (ESCs) in many respects, but are also reported to have altered differentiation potential, different DNA methylation patterns and a different spectrum of copy number variations (CNVs). The biological significance of those dissimilarities in iPSCs is largely unknown. Reprogramming involves conferring iPSCs with ESC-like chromatin characteristics. It is still unclear how completely the chromatin structure in iPSCs is reconfigured, and if any imperfections are functionally relevant. We mapped DNase hypersensitive (DHS) sites in nine samples: three human Embryonic Stem Cell (ESC) lines and three human iPS cell lines with their matched parental fibroblast cells. DHS identify regions of nucleosome eviction and regulatory factor binding to DNA, also called 'open chromatin'. Genome-wide, ~95% of DHS sites were shared, but there were ~5000 sites with different open chromatin profiles. Among the 50 genes with the highest density of differential DHS sites, we find a striking enrichment of loci encoding key transcription factors that function in early embryonic development. Interestingly, the RNA levels of most of these genes is indistinguishable between ES and iPS cells themselves, but many have different expression profiles in iPSs versus ES cells upon the induction of differentiation. Our results suggest that the altered chromatin architecture in iPS cells foreshadows their development potential.

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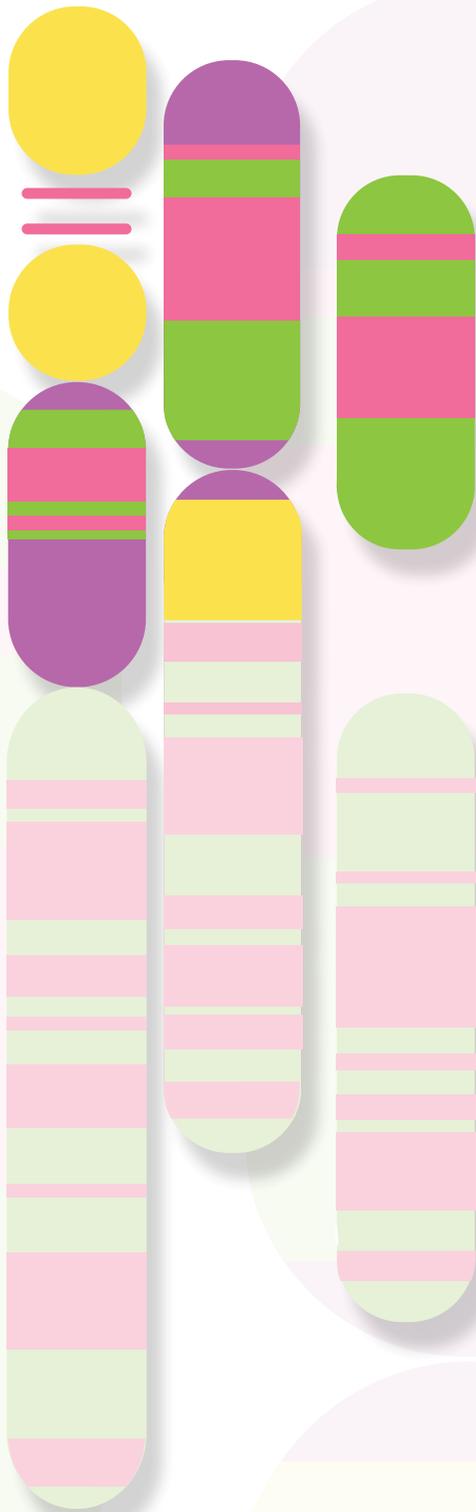
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