NCI Symposium on Chromosome Biology: Chromatin, ncRNA, Methylation & Disease

PROGRAM AND ABSTRACT BOOK

April 16-17, 2015

Natcher Auditorium, NIH Campus
Bethesda, Maryland

Hosted by the
Center of Excellence in Chromosome Biology
Center for Cancer Research
National Cancer Institute
National Institutes of Health
U. S. Department of Health and Human Services

Welcome to the National Cancer Institute (NCI) Symposium on Chromosome Biology "Chromatin, ncRNA, Methylation & Disease". 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 a focus on DNA methylation, ncRNA interactions with chromatin, and (epi)genome maintenance in development and disease.

We hope this symposium offers you an opportunity to learn more about exciting developments in chromosome biology, to share your research, and to discuss implementing these advances in clinical applications.

Sincerely,

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

Yamini Dalal, Ph.D., Symposium Co-Chair
Philipp Oberdoerffer, Ph.D., Symposium Co-Chair
Gordon Hager, Ph.D., Chair CECB
Munira Basrai, Ph.D.
Michael Bustin, Ph.D.
David Clark, Ph.D.
Susan Gottesman, Ph.D.
Shiv Grewal, Ph.D.
David Levens, M.D., Ph.D.
Michael Lichten, Ph.D.
Tom Misteli, Ph.D.
Kathrin Muegge, M.D.
Andre Nussenzweig, Ph.D.
Thomas Ried, M.D.
Carl Wu, Ph.D.

NCI Symposium on Chromatin, ncRNA, Methylation & Disease

TABLE OF CONTENTS

Agenda 1
Author Index 5
Poster Abstracts 11
Participant List 93

Agenda

NCI Symposium on
Chromatin, ncRNA, Methylation & Disease
Natcher Auditorium, NIH, Bethesda, MD
April 16-17, 2015

Thursday, April 16, 2015

8:00 a.m. Registration

9:00 a.m. **Welcome**
Gordon Hager, Ph.D., Chair of the CECB, National Cancer Institute

SESSION 1: DNA METHYLATION ACROSS GENOMES AND SPECIES
Chair: *Yamini Dalal, Ph.D., National Cancer Institute*

9:15 a.m. **"Epigenetic Gene Regulation in a *Arabidopsis*"**
Steve Jacobsen, Ph.D., University of California, Los Angeles

9:45 a.m. **"Molecular Co-evolution of Genomes and Chromatin"**
Daniel Zilberman, Ph.D., University of California, Berkeley

10:15 a.m. **"Epigenomic Signatures of Neuronal Diversity in the Mammalian Brain"**
Joseph Ecker, Ph.D., Salk Institute

10:45 a.m. Break

SESSION 2: CHROMATIN MAINTENANCE IN DEVELOPMENT
Chair: *Dinah Singer, Ph.D., National Cancer Institute*

11:00 a.m. **"H2A.Z: A Molecular Rheostat for Developmental Gene Expression Control"**
Laurie Boyer, Ph.D., Massachusetts Institute of Technology

11:30 a.m. **"Cellular Plasticity of Lsh Mutant Cells"**
Kathrin Muegge, M.D., National Cancer Institute/Leidos Biomedical Research, Inc.

12:00 p.m. **"A Sweet Story About Chromatin"**
Barbara Panning, Ph.D., University of California, San Francisco

12:30 p.m. **LUNCH BREAK AND POSTER VIEWING (Authors present from 1:00-2:30)**

SESSION 3: ncRNA INTERACTIONS WITH CHROMATIN STRUCTURE
Chair: *Shiv Grewal, Ph.D., National Cancer Institute*

2:30 p.m. **"Epigenetic Regulation of Chromatin States at Centromeres and Retrotransposons in Fission Yeast"**
Karl Ekwall, Ph.D., Karolinska Institute

3:00 p.m. **"Noncoding RNAs Drive Chromatin Assembly at Centromeres"**
Yamini Dalal, Ph.D., National Cancer Institute

3:30 p.m. **"Activation of X Inactivation"**
Joost Gribnau, Ph.D., Erasmus University Medical Center

4:00 p.m. Break

SESSION 4: TRANSCRIPTOME REGULATION
Chair: *David Levens, M.D., Ph.D., National Cancer Institute*

4:15 p.m. **"B Cell Genomics"**
Rafael Casellas, Ph.D., National Institute of Arthritis and Musculoskeletal and Skin Diseases

4:45 p.m. **"TBD"**
Mike McManus, Ph.D., University of California, San Francisco

5:15 p.m. **"Regulatory Landscape of Embryonic Stem Cells"**
Richard Young, Ph.D., Whitehead Institute/Massachusetts Institute of Technology

5:45 p.m. Adjourn

Friday, April 17, 2015

SESSION 5: NUCLEAR ORGANIZATION
Chair: *Susan Gottesman, Ph.D., National Cancer Institute*

9:00 a.m. **"Chromatin Structure, Insulators, and Long Range Interactions in the Nucleus"**
Gary Felsenfeld*, Ph.D., National Institute of Diabetes and Digestive and Kidney Diseases*

9:30 a.m. **"Stress-induced Rearrangement of Chromosome 3D Organization"**
Victor Corces, Ph.D., Emory University

10:00 a.m. **"How the Nuclear Envelope Controls Genome Function"**
Martin Hetzer, Ph.D., Salk Institute

10:30 a.m. Break

SESSION 6: DNA PACKAGING, AGING AND DISEASE
Chair*: Mirit Aladjem, Ph.D., National Cancer Institute*

10:45 a.m. **"Histone Variations in Cancer"**
Emily Bernstein, Ph.D., Mt. Sinai Hospital

11:15 a.m. **"Chromatin Remodeling by Architectural Proteins"**
Michael Bustin, Ph.D., National Cancer Institute

11:45 a.m. **"Epigenetic Alterations in Age-associated Disease"**
Shelley Berger, Ph.D., University of Pennsylvania

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

SESSION 7: CHROMATIN AND THE DNA DAMAGE RESPONSE
Chair: *Andre Nussenzweig, Ph.D., National Cancer Institute*

1:15 p.m. **"Regulation of DNA Double-strand Break Repair by the Cell Cycle"**
Daniel Durocher, Ph.D., University of Toronto

1:45 p.m. **"Stop Relaxing: Genome Maintenance Meets Chromatin Condensation"**
Philipp Oberdoerffer, Ph.D., National Cancer Institute

2:15 p.m. **"Nucleosome Dynamics During Processing of DNA Breaks"**
Brendan Price, Ph.D., Dana-Farber Cancer Institute/Harvard University

2:45 p.m. Break

SESSION 8: NUCLEAR INTEGRITY
Chair: *Philipp Oberdoerffer, Ph.D., National Cancer Institute*

3:00 p.m. **"Mechanisms Controlling the Integrity of Replicating Chromosomes"**
Marco Foiani, *Ph.D.,* FIRC Institute of Molecular Oncology and University of Milan

3:30 p.m. **"Unraveling the Molecular Basis of Immunodeficiency in ICF Syndrome"**
Haico van Attikum, Ph.D., Leiden University Medical Center

4:00 p.m. **"Control of Centrosomes by Telomeres and Centromeres - In Both Meiosis and Mitosis"**
Julie Cooper, Ph.D., National Cancer Institute

4:30 p.m. Adjourn

Poster Author Index

Poster Abstracts

Poster No.	Presenting Author	Title	Page No.
P-1	Adar, Sheera	XR-Seq: Genome-Wide Mapping Of Human Global And Transcription-Coupled Repair At Single Nucleotide Resolution	12
P-2	Aprelikova, Olga	Cooperation Of JMJD6 With C-Myc During Cellular Transformation	13
P-3	Ballahcanda, Devaiah	BRD4 Is A Histone Acetyltransferase That Evicts Nucleosomes From Chromatin	14
P-4	Bamhart-Dailey, Meghan	HJURP Interacts With Condensin II And RuvBL1/BL2 To Facilitate Centromeric Chromatin Assembly	15
P-5	Becker, Matthias	Inhibition Of H3K27-Specific Demethylase Activity During Murine ES Cell Differentiation Induces DNA Damage Response	16
P-6	Bharti, Sanjay	Structure-Function Analyses Of Patient-Derived Missense Mutations In The Fanconi-Anemia Complementation Group J (FANCJ) DNA Helicase	17
P-7	Bilke, Sven	A Chromatin Structure Based Model Accurately Predicts Replication Timing Program In Human Cells	18
P-8	Chang, Han-Wen	Mechanism Of DNA Replication Through Chromatin	19
P-9	Chereji, Razvan	Nucleosome Organization In Yeast	20
P-10	Deng, Tao	Functional Compensation Among HMGN Variants Modulates The DNase I Hypersensitive Sites At Enhancers	21
P-11	Ergen, Aysegul	BRCA1 Deficiency Delays MLL-AF9 Induced Leukemia	22
P-12	Farrell, Catherine	RefSeq And Epigenomics: Annotation And Discovery	23
P-13	Fletez-Brant, Kipper	Histone Binding Strength Is Quantitatively Associated With Gene Expression Across Individuals	24
P-14	Folco, H. Diego	The CENP-A N-Tail Confers Epigenetic Stability To Centromeres Via The CENP-T Branch Of The CCAN In Fission Yeast	25
P-15	Fortin, Jean-Philippe	Reconstructing Hi-C Data Using Long-Range Correlations In Epigenetic Data	26
P-16	Furusawa, Takashi	Chromatin Decompaction By The Nucleosomal Binding Protein HMGN5 Impairs Nuclear Sturdiness	27
P-17	Han, Yixing	The Role OF LSH In Regulating Proliferation And Differentiation Of NSCs	28
P-18	Han, Yoo-Jeong	A Novel Strategy To Inhibit CpG Island Hypermethylation And Restore BRCA1 Expression	29
P-19	He, Bing	Nucleosomal Binding Proteins HMGN1 and HMGN2 Modulate The Rate Of Ameloblast Differentiation	30

Poster No.	Presenting Author	Title	Page No.
P-20	He, Ximiao	Methylated Cytosines (5mC) Mutate To Transcription Factor Binding Sites That Drive Vertebrate Evolution	31
P-21	Hoffman, Jackson	Novel BRG1-BAF Complex-Independent Regulation Of Gene Expression By Baf60a	32
P-22	Imashimizu, Masahiko	Mechanisms Of RNA Polymerase Pausing Associated With A Translocation Block *In Vivo*	33
P-23	Jeffers, Tess	Chromosome-Scale And Local Patterns Of Nucleosome Fragility In *C. Elegans*	34
P-24	Katebi, Ataur	Nucleosome Repeat Length Relates To The Gene Expression Level In Yeast	35
P-25	Khan, Irfan	Biochemical Characterization Of The Human Mitochondrial DNA Helicase Twinkle	36
P-26	Kim, Jeongkyu	Impact of Controlled DNA Double-Strand Break Induction On Transcriptome Maintenance *In Vivo*	37
P-27	Kim, Rinho	The Role Of TET1-Mediated DNA Hydroxymethylation In Self-Renewal And Differentiation Of Intestinal Stem Cells	38
P-28	Kizhakke Mattada, Sathyan	Alpha-Amino Terminal Trimethlylation Of The Centromeric Histone CENP-A Is Required For Maintaining Bipolar Spindle And Regulated Cell Proliferation	39
P-29	Kouzine, Fedor	Unusual DNA Structures Are A Common Feature Of Mammalian Genomes	40
P-30	Krivega, Ivan	Inhibition of G9a Methyltransferase Stimulates Fetal Hemoglobin Production By Facilitating LCR/Beta-Globin Looping	41
P-31	Laiho, Marikki	RNA Polymerase I As A Cancer Therapeutic Target	42
P-32	Lee, Hungoo	Genome-Wide Functions Of Polycomb Complexes Regulate Pervasive Transcription	43
P-33	Lee, Nicholas	Highly Efficient CRISPR/Cas9-Mediated TAR Cloning Of Genes And Chromosomal Loci From Complex Genomes In Yeast	44
P-34	Li, Shan	Human Enhancers Are Fragile And Prone To Deactivating Mutations	45
P-35	Limou, Sophie	First Longitudinal Epigenome-Wide Association Study Of Pre- And Post-HIV Infected Subjects	46
P-36	Lin, Shuye	Epigenetic Upregulation Of Metallothionein 2A By Diallyl Trisulfide Enhances Chemotherapeutic Sensitivity Of Human Gastric Cancer Cells To Docetaxel Through Attenuating NF-KB Activation	47
P-37	Liu, Limin	The Chromosome Passenger Complex Is Converted From A Destroyer To A Protector Of Cohesion By Recruitment Of Sgo1	48

Poster No.	Presenting Author	Title	Page No.
P-38	Liu, Mengying (Mona)	Analysis Of Control Of Methionine Metabolism By The SIN3 Corepressor	49
P-39	Lopez-Bertoni, Hernando	Epigenetic Modulation Of A miR-296-5p:HMGA1 Axis Regulates The Glioblastoma Stem Cell Phenotype	50
P-40	Mathe, Ewy	Defining The Active Regulatory Element Landscape Upon Activation Of B Cells	51
P-41	Milliman, Eric	The Role Of The Linker Histone In Glucocorticoid Receptor Transcriptional Activation	52
P-42	Mitchell, Khadijah	A Histone Modifying Enzyme Gene Signature In Lung Tumors From African Americans	53
P-43	Mukherjee, Sanjit	Integrated Analysis Of High Resolution DNA Methylation Profiles In Differentiated Mouse Primary Dermal Keratinocytes And Fibroblasts	54
P-44	Nehru, Vishal	SPT6 Interacts With WHSC1 During Transcription Elongation In Interferon-Stimulated Genes	55
P-45	Nelson, George	Adjusting Infinium Methylation Profiles To Suppress Signals From Varying Cell Proportion	56
P-46	Nizovtseva, Ekaterina	Opposite Effects Of Histone H1 And HMGN5 Protein On Distant Communication In Chromatin	57
P-47	Norouzi, Davood	Topological Polymorphism Of Two-Start Nucleosome Fibers And The Mechanism Of Gene Looping	58
P-48	Ocampo, Josefina	Dynamic Interplay Between ISW1, ISW2, CHD1 and RSC Remodelers Determines Nucleosome Spacing And Phasing In Yeast	59
P-49	Odeh, Hana	SENP2 Association With Intracellular Membranes	60
P-50	Onodera, Atsushi	Polycomb And Trithorax Complexes Control Epigenetic Memory Of T Helper Cells	61
P-51	Padilla-Benavides, Teresita	Brg1 Controls The Expression Of *Pax7* To Promote Viability And Proliferation Of Mouse Primary Myoblasts	62
P-52	Paramasivam, Manikandan	Interaction of Fanconi Anemia (FA) Protein FANCD2 With Genomic Interstrand Crosslinks (ICLs)	63
P-53	Plank, Jennifer	Transcriptional Repressor Eto2 Regulates Erythropoiesis And Human Hemoglobin Switching	64
P-54	Rahman, Mohammad	Polo-Like Kinase 1 (PLK-1) Is Required For Nuclear Envelope Remodeling During *C. Elegans* Early Embryonic Divisions	65
P-55	Ren, Xiaojun	*In Vivo* Stoichiometry And Assembly Of Polycomb Repressive Complex 1 On Chromatin Revealed By Single-Molecule Imaging	66

Poster No.	Presenting Author	Title	Page No.
P-56	Sawyer, Iain	Cajal Bodies Shape Genome Conformation	67
P-57	Shachar, Sigal	Systematic Identification Of Genome Positioning Factors By High-Throughput Imaging Screening	68
P-58	Shaytan, Alexey	Dynamics Of Histone Tails And Linker DNA In Nucleosomes: Microsecond Molecular Dynamics Simulations	69
P-59	Sheaffer, Karyn	DNA Hypomethylation Contributes To The Neoplastic Process In Intestinal Cancer	70
P-60	Shimbo, Takashi	MBD3 Regulates Chromatin Accessibility At Active Promoters	71
P-61	Sommers, Joshua	Effect Of RPA-Binding Small Molecules On RPA-Stimulated DNA Unwinding By Human RECQ1 And FANCJ Helicases	72
P-62	Sowash, Aislinn	Sp1 Binds To Centrochromatin To Maintain The Centrochromatin Landscape And Preserve Chromosome Stability	73
P-63	Sun, Deqiang	Epigenome Method Development And Its Application To Aging Of Hematopoietic Stem Cells	74
P-64	Sung, Myong-Hee "Mia"	Profiling Protein Occupants Of The Genome: Is TF Footprinting Ready For Prime Time?	75
P-65	Swindstead, Erin	Steroid Receptors Can Facilitate The Binding Of The Pioneer Factor Foxa1 In Breast Cancer Cell Lines Through A Dynamic Assisted Loading (DynALoad) Mechanism	76
P-66	Syed, Khund Sayeed	CG Methylated Microarrays Identify Novel Methylated Sequences Bound By CEBPG, CEBPE Homodimers And Heterodimers Of ATF4 With CEBP Family Members	77
P-67	Takaku, Motoki	GATA3-Mediated Chromatin Establishment In Breast Cancer Cells	78
P-68	Veschi, Veronica	An Epigenetic Focused siRNA Screen Identifies Novel Druggable Targets That Inhibit Growth And Induce Differentiation In Neuroblastoma	79
P-69	Vishwakarma, Bandana Ajay	*Setbp1* Induces Leukemia Development Through Repression Of *Runx1*	80
P-70	Wade, Paul	Inheritance Of DNA Methylation In The Mouse	81
P-71	Weyemi, Urbain	Histone H2AX Is A Novel Regulator Of Epithelial To Mesenchymal Transition	82
P-72	Willox, Brad	Epstein-Barr Virus Oncoprotein Super-Enhancers Control B Cell Growth	83
P-73	Wolf, Gemot	KRAB Zinc Finger Proteins Are Evolutionarily Adaptive Repressors Of Parasitic DNA Elements	84
P-74	Xiong, Jianhua	Stemness Factor Sall4 Is Required For DNA Damage Response In Embryonic Stem Cells	85

Poster No.	Presenting Author	Title	Page No.
P-75	Xue, Catherine	Charting Epigenomes: Applying a Principled Approach For ChIP-seq Optimization	86
P-76	Yadav, Manish Kumar	Changes In The Methylation Pattern of p53 Gene Promoter In The Megaloblastic Bone Marrow Disease	87
P-77	Yoon, Je-Hyun	microRNA Transferase Function Of AUF1 p37	88
P-78	Zaidi, Hussain	Quantitative Analysis Of Transcription Factor Dynamics In Yeast Genome	89
P-79	Zasadzinska, Ewelina	The Role Of WDR18 Protein In CENP-A Deposition Pathway	90
P-80	Zhang, Shaofei	Functionally Redundant And Compensatory Binding Of HMGN Variants To Nucleosomes Affect DNaseI Hypersensitivity Of Chromatin And Gene Expression In Mouse B Lymphocytes	91
P-81	Zhao, Haiqing	The Binding Landscape of CENP-A/H4 Dimer Reveals Thermodynamic Distinctions from H3/H4 Dimer	92

Abstracts

P-1

XR-SEQ: GENOME-WIDE MAPPING OF HUMAN GLOBAL AND TRANSCRIPTION-COUPLED REPAIR AT SINGLE NUCLEOTIDE RESOLUTION

Adar, S.[1], Hu, J.[2], Selby, C.P.[2], Sancar, A.[2], and Lieb, J.D.[3]
[1]Integrative Program for Biological and Genome Sciences and [2]Department of Biochemistry and Biophysics, University of North Carolina School of Medicine, Chapel Hill, NC; [3]Department of Human Genetics, University of Chicago, Chicago, IL

Nucleotide excision repair is the major DNA repair pathway for removal of bulky adducts induced by chemical carcinogens and therapeutic agents, as well as the UV induced cyclobutyl pyrimidine dimers (CPDs) and 6-4 photoproducts. During human excision repair, dual incision of the damaged strand results in removal of a ~30 nucleotide-long single stranded oligomer. In eXcision Repair-seq (XR-seq) we capture the excised oligonucleotide released *in vivo,* and subject it to high-throughput sequencing. We used XR-seq to produce stranded, nucleotide-resolution maps of repair of two UV-induced DNA damages, cyclobutane pyrimidine dimers (CPDs) and (6-4) pyrimidine-pyrimidone photoproducts ((6-4)PPs). In wild-type cells, CPD repair was highly associated with transcription, specifically with the template strand. Experiments in cells defective in either transcription-coupled excision repair or general excision repair isolated the contribution of each pathway to the overall repair pattern. This showed that transcription-coupled repair of both photoproducts occurs exclusively on the template strand and is highly correlated with the expression level of the genes. XR-seq maps capture transcription-coupled repair at sites of divergent gene promoters and bi-directional eRNA production at enhancers. XR-seq and the resulting genome wide repair maps will aid in quantifying how DNA damage and repair efficiencies vary with respect to genomic position and chromatin status, information which will be valuable to incorporate into models of carcinogenesis, cancer risk, and genome stability.

P-2

COOPERATION OF JMJD6 WITH C-MYC DURING CELLULAR TRANSFORMATION

Aprelikova, O., El Touny, L.H., Chen, K., Han, J., and Green, J.E.
Laboratory of Cancer Biology and Genetics, Center for Cancer Research, NCI, NIH, Bethesda, MD

Oncogene overexpression in primary cells usually triggers the induction of a cellular safeguard response that promotes senescence or apoptosis. Therefore, oncogene induced tumorigenesis requires cooperating genetic events to overcome these biologic obstacles. In order to address this on a genomic scale, we performed array CGH on 8 genetically-engineered mouse models of mammary cancer. The MMTV-Myc model displayed a paucity of CNVs except for the amplification of the distal region of mouse chromosome 11 in 80% of the tumors. This region is syntenic with human chromosome 17q23-25 that is often amplified in human breast cancer. We hypothesized that some of the 243 genes within this amplicon would cooperate with Myc to enhance tumorigenesis, especially since Myc can exert anti-tumorigenic effects through its ability to induce apoptosis. Analysis of 7 selected candidate genes based upon their high expression in Myc-driven tumors identified JMJD6 as a gene that cooperates with Myc to enhance tumorigenesis. This gene has pleotropic functions in histone and non-histone protein modifications through catalyzing lysine hydroxylation and, therefore, is capable of regulating gene expression and protein activity. Recently JMJD6 was identified as a driver and a marker for poor prognosis in breast cancer although its mechanism of action was not elucidated. We have discovered that JMJD6 inhibits p19ARF protein and mRNA expression in normal mammary gland epithelial cells. A chromatin immunoprecipitation assay demonstrated that JMJD6 is bound to the p19ARF promoter and exerts its inhibitory function through demethylation of H4R3me2a, which is an epigenetic mark of active chromatin. As a result of p19ARF silencing, we observe reduced levels of p53 protein in JMJD6-expressing cells and diminished Myc-induced apoptosis after varying stress conditions. This mechanism might explain the cooperation of Myc with JMJD6 during tumor initiation in MMTV-Myc mouse models. We have demonstrated that in an established Myc-driven tumor model that is not metastatic, JMJD6 expression increases tumor burden, promotes tumor metastasis and induces multiple EMT markers (Twist and Snail) as well as several anti-apoptotic genes from the Bcl2 family. Given the pleotropic pro-tumorigenic activities of JMJD6, it may be useful as a prognostic factor and a therapeutic target for Myc-driven mammary tumorigenesis.

P-3

BRD4 IS A HISTONE ACETYLTRANSFERASE THAT EVICTS NUCLEOSOMES FROM CHROMATIN

Ballahcanda, D.[1], Case-Borden, C.[1], Gegonne, A.[1], Dey, A.[2], Ozato, K.[2], and Singer, D.S.[1]
[1]Experimental Immunology Branch, Center for Cancer Research, NCI; [2]Laboratory of Molecular Growth Regulation, NICHD, NIH, Bethesda, MD

Bromodomain protein 4 (BRD4) is a master transcriptional and epigenetic regulator which plays a pivotal role in cancer development and immune diseases. While its association with chromatin is known to de-compact it and activate transcription of key proto-oncogenes, the mechanism involved is currently unknown. BRD4 plays an important role in transcription through phosphorylation of the RNA polymerase II, but its role in epigenetic regulation is yet to be clearly elucidated. It is known to bind and stay associated with chromatin during mitosis as a mitotic bookmark, reactivating transcription after mitotic silencing. Inhibiting its interaction with chromatin has been recently shown to be a successful therapeutic strategy against a variety of cancers that include acute myeloid leukemia, Burkitt's lymphoma, breast, colon and lung cancer. Therefore, deciphering the precise role of BRD4 on the chromatin is of critical importance. Here, we report that BRD4 has novel and intrinsic histone acetyltransferase (HAT) activity through which it acetylates histones H3 and H4 in nucleosomes. BRD4 HAT activity was mapped to two consensus acetyl CoA binding sites and a 40 amino acid catalytic site on BRD4 through point and deletion mutants. BRD4 HAT activity is distinct from all other known HAT's with a unique lysine acetylation 'fingerprint' that includes acetylation of all histone H4 tail lysines and H3 tail lysines at K4, K9, K18 and K27 positions but not at K14. In addition, BRD4 acetylates H3K122, a key lysine residue critical for nucleosome stability located on the dyad axis of the nucleosome. Indeed, we show that BRD4 HAT activity and H3K122 acetylation is responsible for nucleosome eviction and opening of the chromatin, as evidenced by the ability of BRD4, but not BRD4 HAT mutants, to evict nucleosomes both *in vitro* and *in vivo*. These findings are consistent with previous reports that BRD4 causes chromatin de-compaction and influences higher order chromatin structure. Nucleosome clearance by BRD4 is selective and localized to the gene loci it is known to regulate such as c-Myc, c-Fos and Aurora B kinase. Consistent with its role in localized chromatin de-compaction, BRD4 HAT activity regulates transcription at these gene loci as well. These findings suggest that BRD4 HAT activity plays a key role in chromatin remodeling and transcription. Based on our findings, we propose a new model where BRD4 is recruited to specific gene loci of M/G1 genes such as c-Myc and c-Fos, where it clears nucleosomes through its HAT activity to allow access to the transcriptional machinery.

P-4

HJURP INTERACTS WITH CONDENSIN II AND RUVBL1/BL2 TO FACILITATE CENTROMERIC CHROMATIN ASSEMBLY

Barnhart-Dailey, M.C., Zasadzinska, E., and Foltz, D.R.
Department of Biochemistry and Molecular Genetics, University of Virginia, Charlottesville, VA

Centromeric chromatin is marked by the presence of nucleosomes containing the histone H3 variant, CENP-A. Contrary to canonical H3.1 nucleosome assembly, no new CENP-A nucleosomes are deposited during S-phase, essentially diluting the CENP-A nucleosome content by half each replication cycle. In order to restore CENP-A to its full complement, new CENP-A nucleosomes are assembled in early G1 immediately after the cell exits mitosis in human cells. A key protein in the assembly of new centromeric chromatin is the CENP-A specific chaperone HJURP. Depletion of HJURP from human cells results in a loss of new CENP-A at the centromere, and over time, a complete loss of centromeric CENP-A as the "old" CENP-A is diluted with each cell cycle. Previously, we showed that targeting of HJURP to non-centromeric loci is sufficient to determine the site of new CENP-A nucleosome deposition (Barnhart, et al. 2011). Additional proteins associated with HJURP and CENP-A in the pre-nucleosomal complex may also contribute to the remodeling and assembly of centromeric chromatin during G1. In this most recent work we delineate novel interactions between HJURP and the Condensin II and RuvB-like complexes, which are involved in the ability of HJURP to deposit new CENP-A nucleosomes *in vivo*. The condensin II complex is known to be required for complete axial shortening of mitotic chromosomes during prometaphase as the cell prepares to divide its chromosomes in the oncoming mitosis. It is composed of two SMC subunits (SMC2 and SMC4) that are common between the condensin I and condensin II complexes. The condensin II complex also contains three non-SMC subunits, CAP-H2, CAP-D3, and CAP-G2, which are unique from their condensin I counterparts. Previous work has implicated Condensin complexes in centromere deposition (Bernad et al. 2011, Samoshkin et al. 2009). Here we demonstrate that HJURP interacts specifically with condensin II subunits *in vivo.* In addition we show HJURP induces a chromatin decondensation that is exaggerated by condensin II depletion. We uncover condensin II localization at early G1 centromeres and identify a requirement for the complex in complete CENP-A deposition. In addition, we show an interaction between the C-terminus of HJURP and the AAA-ATPase proteins RuvBL1 and RuvBL2. The RuvBL1 and RuvBL2 proteins are part of the CENP-A pre-nucleosomal complex (Foltz et al. 2009, Shuaib et al. 2010). Depletion of these proteins demonstrates the requirement of RuvBL1 and RuvBL2 for assembly of new CENP-A nucleosomes at the human centromere. Together these experiments highlight the requirement of multiple chromatin organizing complexes for efficient deposition of new CENP-A nucleosomes *in vivo*.

P-5

INHIBITION OF H3K27-SPECIFIC DEMETHYLASE ACTIVITY DURING MURINE ES CELL DIFFERENTIATION INDUCES DNA DAMAGE RESPONSE

Hofstetter, C., Kampka, J., Müller, A.M., and Becker, M.
Institute for Medical Radiation and Cell Research (MSZ) in the Center of Experimental and Molecular Medicine (ZEMM), Würzburg University, Würzburg, Germany

Pluripotent embryonic stem (ES) cells are characterized by their capacity to self-renew indefinitely while maintaining their potential to differentiate into all cell types of an adult organism. Both the undifferentiated and differentiated states are characterized by specific gene expression programs which are determined at the chromatin level. The repressive H3K27me3 chromatin mark is stringently regulated in undifferentiated and differentiating ES cells. In this study, by employing a small molecule inhibitor (GSK-J4) and by targeted gene knockdown/knockout we analyzed the function of the H3K27me2,3-specific demethylases KDM6A and KDM6B in undifferentiated and differentiating ES cells. Surprisingly, we observed that inhibition of the H3K27 demethylase activity induced DNA damage, activation of the DNA damage response (DDR) and cell death in differentiating but not in undifferentiated ES cells. Lack of H3K27me3 attenuated the GSK-J4-induced DDR in differentiating Eed KO ES cells suggesting a critical role for H3K27me3 in DDR. Collectively our findings indicate that during ES cell differentiation KDM6A and KDM6B apart from regulating gene expression patterns have additional functions in preventing DNA damage.

P-6

STRUCTURE-FUNCTION ANALYSES OF PATIENT-DERIVED MISSENSE MUTATIONS IN THE FANCONI-ANEMIA COMPLEMENTATION GROUP J (FANCJ) DNA HELICASE

Bharti, S.K.[1], Sommers, J.A.[1], Khan, I.[1], Wu, Y.[2], and Brosh Jr., R.M.[1]
[1]Laboratory of Molecular Gerontology, NIA, NIH, Baltimore, MD; [2]Department of Biochemistry, University of Saskatchewan, Saskatoon, Canada

Fanconi Anemia (FA) is a rare genetic DNA repair disorder characterized by progressive bone marrow failure, congenital abnormalities, and cancer. Of the 16 genes currently linked to FA, the FA Group J (FANCJ) gene is unique that it encodes an ATP-dependent DNA helicase. Mutations in FANCJ are not only genetically linked to FA, but also associated with breast and ovarian cancer. Here we performed structure function studies of two FA patient-derived FANCJ mutations, R707C and H396D, resulting in substitution of arginine (R) to cysteine (C) in helicase motif IV and substitution of histidine (H) to aspartic acid (D) in Walker A (motif I) ATP binding pocket, respectively. The human recombinant FANCJ proteins, expressed in insect cells and purified to >95% homogeneity, were tested for DNA unwinding (helicase) on a forked duplex (19 bp) and an entropically favoured unimolecular G-quadruplex (G4) DNA substrate. FANCJ-R707C retained partial (~30%) helicase activity on either the forked duplex or G4 DNA substrates, whereas FANCJ-H396D failed to unwind either substrate. Single-turnover kinetic assays confirmed the differential effects of the FANCJ missense mutations on DNA helicase activity. To understand their molecular defects, the two FANCJ mutants were evaluated for DNA binding and ATP hydrolysis. FANCJ-R707C retained partial DNA binding and ATPase (~30% activity compared to FANCJ-WT), whereas FANCJ-H396D hardly bound DNA at all and retained only marginal ATPase activity (~10% compared to FANCJ-WT). Thus, the severity of the defects in both DNA binding and ATP hydrolysis correlated with the impact of the FANCJ mutations on helicase activity.
To study the biological effects of the FANCJ missense mutations, we used DT 40 FANCJ-/- cells expressing human FANCJ proteins. Consistent with its known role in homologous recombination repair, FANCJ-/- cells are sensitive to the DNA interstrand cross-linking (ICL) agent cisplatin or topoisomerase inhibitor camptothecin, which both interfere with DNA replication resulting in double-strand breaks (DSB). Expression of either the FANCJ-R707C or FANCJ-H396D mutant failed to rescue cisplatin sensitivity as measure by cell proliferation assay or DSB induction as measured by immunofluorescent detection of the DNA damage marker g-H2AX. In striking contrast, expression of FANCJ-R707C in FANCJ-/- cells restored camptothecin resistance similar to FANCJ-WT, whereas FANCJ-H396D completely failed to rescue the fancj null cell line. These results lead us to conclude that a quantitatively lower threshold of FANCJ catalytic activity is required for repair of camptothecin-induced DNA damage compared to cisplatin-induced damage. Our findings are consistent with a model in which FANCJ helicase participates in a classic pathway of ICL repair that is dependent on other FA gene products, whereas the role of FANCJ in the repair of broken replication forks caused by poisoned topoisomerase cleavage complexes occurs by a pathway distinct from the FA pathway. Moreover, the results provide new insight to the molecular phenotypes of clinically relevant FANCJ missense mutations and their roles in DNA repair that are relevant to human disease and cancer.

P-7

A CHROMATIN STRUCTURE BASED MODEL ACCURATELY PREDICTS REPLICATION TIMING PROGRAM IN HUMAN CELLS

Bilke, S., Gindin, Y., and Meltzer, P.S.
Genetics Branch, Center for Cancer Research, NCI, NIH, Bethesda, MD

Background and Hypotheses: DNA replication is a tightly regulated process that follows a strict, yet poorly understood, temporal program. This timing program is intricately linked to many aspects of cell biology, it is cell type specific and altered in cancer cells. Although on the genome scale DNA replication appears as a highly orchestrated process, at the level of individual initiation events it is found to be stochastic. The mechanisms controlling global DNA replication timing remains largely unknown. Recently, stochastic DNA replication models, where global timing emerges from the collective action of unregulated initiation events, have been proposed. Yet it is still not known which factors determine the complex timing programs observed in metazoan genomes. Contributing to the dearth of such models is the incomplete characterization of replication initiation sites in these genomes. Here we show that this issue does not prevent building a successful DNA replication timing model because we find that (a) the replication timing program is so robust that knowledge of exact firing probabilities is unnecessary and(b) high efficiency replicators are sufficiently localized by a specific chromatin mark.

Study Design and Methods: We arrive at these conclusions based on simulations of a simple mechanistic model and comparison to experimental timing data. The input to our model is an "Initiation Probability Landscape" (IPLS), a mathematical construct representing the location of high efficiency initiation sites. We find that even a simple IPLS based on the position of transcription start sites produces a remarkably precise model (r=0.75 prediction vs. experiment). In principle, any genomic dataset can be used to define an IPLS and we systematically tried all ENCODE datasets, performed simulations and ranked the resulting models according to the precision of the predictions. A number of chromatin marks dominate the top of this ranking, but only one specific chromatin mark remains fully predictive after reducing the mutual independence between the top contenders.

Results and Conclusions: Simulations based on this optimal model demonstrate that the replication timing program in human cells (and other metazoans) can be entirely understood based on a diffusion process where initiation occurs in a time-stochastic manner, i.e. without any active regulation of initiation timing. Yet, the location of initiation is not entirely random because high efficiency initiation sites are located at specific sites and their genomic distribution determines the global timing program. The model predicts the replication timing program with a precision paralleling that of independent experimental repeats (r=0.92 prediction vs experiment compared to r=0.94 for experiments performed in different labs). It recapitulates cell-specific timing patterns including abnormal timing behavior in cancer cells. These results strongly support the concept that replication timing is a stochastic process ultimately determined by chromatin structure which itself is a consequence of the topological organization of genes and functional regulatory elements on the chromosome as encoded in the DNA sequence.

P-8

MECHANISM OF DNA REPLICATION THROUGH CHROMATIN

Chang, H.-W.[1], Pandey, M.[2], Kulaeva, O.I.[1], Patel, S.S.[2], and Studitsky, V.M.[1,3]
[1]Fox Chase Cancer Center, Philadelphia, PA; [2]Department of Biochemistry and Molecular Biology, Rutgers-Robert Wood Johnson Medical School, Piscataway, NJ; [3]School of Biology, Lomonosov Moscow State University, Russia

Accurate maintenance of chromatin structure and associated epigenetic and regulatory histone marks during DNA replication is essential for normal functioning of the daughter cells. However, the mechanisms of maintaining chromatin architecture during replication are unknown. We have studied nucleosome traversal by T7 replisome *in vitro*. Nucleosome is a strong barrier for replication, with particularly strong pausing of DNA polymerase at the +(27-39) and +(41-63) regions of nucleosomal DNA. After replication ~50% of nucleosomes survive and transfer to nucleosome-distal DNA region. The exonuclease activity of T7 DNA polymerase increases the overall rate of progression of the replisome through a nucleosome, likely by resolving non-productive complexes and by facilitating nucleosome translocation, although it partially compromises the efficiency of nucleosome survival. The presence of nucleosome-free DNA upstream of the replication fork augments progression of DNA polymerase through the nucleosome. Our data suggest a novel mechanism for maintenance of nucleosomes carrying the epigenetic and regulatory codes during replication.

P-9

NUCLEOSOME ORGANIZATION IN YEAST

Chereji, R.V., Ocampo, J., Burke, T., and Clark, D.J.
Program in Genomics of Differentiation, NICHD, NIH, Bethesda, MD

During the past decade, genome-wide nucleosome mapping experiments suggested a conserved stereotypical nucleosome organization near gene promoters, consisting of regular nucleosome arrays on the gene bodies and a nucleosome-depleted region immediately upstream of the transcription start sites. We study the factors that play key roles in establishing this organization. We show that *in vitro*, the reconstituted nucleosomes have a different organization than the one that we see in live cells, so the DNA sequence has not a major contribution in nucleosome positioning *in vivo.* We identify the actors that play a key role in nucleosome positioning: non-histone proteins that bind to gene promoters, chromatin remodelers and transcription. We discuss rigorous statistical mechanics models which can explain the nucleosome phasing observed *in vivo*, and we propose a simple model for the action of chromatin remodelers.

P-10

FUNCTIONAL COMPENSATION AMONG HMGN VARIANTS MODULATES THE DNase I HYPERSENSITIVE SITES AT ENHANCERS

Deng, T.[1], Zhu, I.[2], Zhang, S.[1], Landsman, D.[2], and Bustin, M.[1]
[1]Laboratory of Metabolism, Center for Cancer Research, NCI; [2]Computational Biology Branch, National Center for Biotechnology Information, NLM, NIH, Bethesda, MD

DNase I hypersensitive sites (DHSs) are a hallmark of chromatin regions containing regulatory DNA such as enhancers and promoters; however, the factors affecting the establishment and maintenance of these sites are not fully understood. We now show that HMGN1 and HMGN2, nucleosome-binding proteins that are ubiquitously expressed in vertebrate cells, maintain the DHS landscape of mouse embryonic fibroblasts (MEFs) synergistically. Loss of one of these HMGN variants led to a compensatory increase of binding of remaining variant. Genome wide mapping of the DHSs in Hmgn1-/-, Hmgn2-/- and Hmgn1-/-n2-/- MEFs reveals that loss of both, but not a single HMGN variant, leads to significant remodeling of the DHS landscape, especially at enhancer regions marked by H3K4me1 and H3K27ac. Loss of HMGN variants affects the induced expression of stress responsive genes in MEFs, the transcription profiles of several mouse tissues, and leads to altered phenotypes that are not seen in mice lacking only one variant. We conclude that the compensatory binding of HMGN variants to chromatin maintains the DHS landscape and the transcription fidelity necessary to retain wild type phenotypes. Our studies provide insights into mechanisms that maintain regulatory sites in chromatin and into functional compensation among nucleosome binding architectural proteins.

P-11

BRCA1 DEFICIENCY DELAYS MLL-AF9 INDUCED LEUKEMIA

Ergen, A.V.[1], Zong, D.[1], Day, A.[1], Wangsa, D.[2], Santos, M.[1], and Nussenzweig, A.[1]
[1]Laboratory of Genome Integrity and [2]Genetics Branch, Center for Cancer Research, NCI, NIH, Bethesda, MD

Leukemias with Mixed Lineage Leukemia (MLL) translocations account for the majority of acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML). In our previous study, we demonstrated that in a well-studied MLL-AF9 induced transformation model of AML, Breast cancer gene 1 (BRCA1) deficiency in leukemic stem/progenitors results in reduced numbers of colony formation and reduced numbers of blasts *in vitro*. In order to determine the role of BRCA1 on AML progression *in vivo*, WT or BRCA1 deficient bone marrow cells were transformed with MLL-AF9 retroviral construct which has a GFP reporter and injected into sublethally irradiated recipient mice. In both WT and BRCA1 deficient cells, AML have developed with full penetrance and both groups of mice have similar disease latency. We then tested if BRCA1 is required for maintenance of leukemia by re-transplantation of tumor cells into secondary recipients. Disease onset was very rapid in mice which received WT AML cells. On the other hand, we observed a significant survival advantage in mice which received BRCA1 AML cells. We then performed tertiary transplantation of WT and BRCA1 null tumor cells. As in secondary transplants, there was a significant survival advantage of BRCA1 null leukemic cells. Since BRCA1 null cells gave rise AML with a later disease onset, we decided to analyze leukemic stem/progenitors in the sick mice. BRCA1 null leukemic cells had significantly lower percentage of cKit+ cells both in the bone marrow and in the spleen. These data suggest that BRCA1 is not absolutely required for leukemia maintenance but it has reduced numbers of leukemia initiating cells as they underwent serial bone marrow transplantation.MLL-AF9 transformed WT cells has gained ERK phosphorylation as they are re-transplanted into secondary and tertiary recipients whereas BRCA1 deficient cells had more genomic unstability and had significantly less levels of phosphorylated ERK.

P-12

REFSEQ AND EPIGENOMICS: ANNOTATION AND DISCOVERY

Farrell, C.M., Pruitt, K.D., Murphy, T.D., Maglott, D.R., and the RefSeq Group
National Center for Biotechnology Information, NLM, NIH, Bethesda, MD

The Reference Sequence (RefSeq) database at NCBI (www.ncbi.nlm.nih.gov/refseq/) provides an archived dataset for reference genome annotation and gene identification. Many genome-wide epigenomic studies typically use RefSeq data to determine gene, transcript and transcription start sites on the reference genome. This presentation will review the current RefSeq project in vertebrates, methods for data access, and tools to view and process genome annotation at NCBI. The advantages of obtaining RefSeq data from NCBI sources will be demonstrated, such as accessing NCBI model gene annotations. Browsers such as the UCSC Genome Browser, a common RefSeq data source for epigenomic studies, do not include those predictions. External sources may thus represent incomplete genome annotation, which could result in false association of some epigenetic marks with intergenic features. Conversely, this presentation will also review how the RefSeq project uses epigenomic data as a tool for RefSeq curation and gene determination. These uses include the verification of gene or transcript variant 5' completeness based on promoter-associated epigenetic marks and a use in defining genes when there is insufficient transcript support. This presentation will introduce plans to expand the RefSeq project to represent non-genic features, including regulatory elements (e.g., enhancers), elements involved in higher-order genome organization (e.g., insulators), and elements that are otherwise considered to be of functional importance (e.g., recombination hotspots). These planned additions would enrich current genome annotation and are expected to be valuable to biomedical research.

P-13

HISTONE BINDING STRENGTH IS QUANTITATIVELY ASSOCIATED WITH GENE EXPRESSION ACROSS INDIVIDUALS

Fletez-Brant, K.[1,2], He, Y.[3], and Hansen, K.D.[1,2]
[1]McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins School of Medicine; [2]Department of Biostatistics and [3]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

Histone modifications are known to be associated with active and inactive parts of the genome. These modifications are mainly profiled using ChIP-seq/chip, and for technical reasons most existing experiments are performed in cell lines with unlimited input material. Little is known about differences in histone marks profiled in the same cell type but in different individuals.

Using publicly available histone modification data from multiple different HapMap cell lines, we show the quantitative association that exists between a gene's expression and H3K4me3 binding at its corresponding promoter. This association shows H3K4me3 binding strength is a quantitative and functionally relevant measure, which is associated with biological variation. We establish a similar quantitative relationship between gene expression and strength binding by H3K4me1 and H3K27ac at corresponding enhancers. Finally we establish the quantitative relationship between promoter H3K4me3 binding and gene expression in rat, thereby replicating our finding in a different mammal.

P-14

THE CENP-A N-TAIL CONFERS EPIGENETIC STABILITY TO CENTROMERES VIA THE CENP-T BRANCH OF THE CCAN IN FISSION YEAST

Folco, H.D.[1, 2], Campbell, C.S.[2], Espinoza, C.A.[2], Grewal, S.I.S.[1], and Desai, A.[2]
[1]Laboratory of Biochemistry and Molecular Biology, Center for Cancer Research, NCI, NIH, Bethesda, MD; [2]Ludwig Institute for Cancer Research, University of California, San Diego, La Jolla, CA

In most eukaryotes, centromeres are defined epigenetically by presence of the histone H3 variant CENP-A. Whereas the mechanisms that load CENP-A at centromeres are being elucidated, the functions of its divergent N-terminal tail remain enigmatic. Here, we employ the well-studied fission yeast centromere to investigate the function of the $Cnp1^{CENP-A}$ N-tail. We show that alteration of the N-tail did not affect $Cnp1^{CENP-A}$ loading at centromeres and outer kinetochore formation, but displayed elevated chromosome loss. Interestingly, N-Tail mutants exhibited synthetic lethality with centromeres harboring a *tetO* array at central core. However, infrequent survivors were isolated harboring an inactive centromere depleted of $Cnp1^{CENP-A}$ and enriched in H3K9me2. Moreover, elevated centromere inactivation with concomitant presence of H3K9me2 was also observed in unaltered centromeres of N-tail mutants. Remarkably, N-tail mutants specifically reduced localization of the CCAN proteins $Cnp20^{CENP-T}$ and $Mis6^{CENP-I}$, but not $Cnp3^{CENP-C}$. Overexpression of $Cnp20^{CENP-T}$ suppressed the H3K9me2 enrichment at central core of N-tail mutants, suggesting a link between reduced CENP-T recruitment and the observed centromere inactivation phenotype. Thus, the $Cnp1^{CENP-A}$ N-tail promotes stability of centromeres in fission yeast, via recruitment of the CENP-T branch of the CCAN.

P-15

RECONSTRUCTING HI-C DATA USING LONG-RANGE CORRELATIONS IN EPIGENETIC DATA

Fortin, J.P.[1] and Hansen, K.D.[1,2]
[1]Department of Biostatistics, Johns Hopkins Bloomberg School of Public Health; [2]McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins School of Medicine, Baltimore, MD

A Hi-C experiment produces a genome-wide contact matrix whose entries estimate how often two distinct loci interact with each other. Analysis of Hi-C contact matrices have shown that at a gross scale, the genome can be divided into two compartments -- closed and open -- and that this compartmentalization is cell-type specific. Recent work has shown that 36% of these compartments change during stem cell differentiation.

Here we show that genome compartments can be reliable estimated using DNA methylation data from the Illumina 450k platform, an inexpensive and popular methylation microarray. To do so, we show that the long-range correlations of methylation levels are substantially higher for two loci that belong to the "closed" compartment ("closed-closed" interaction) than for the two other types of interactions ("open-open" and "open-closed" interactions). By applying principal component analysis to the methylation correlation matrix, we can estimate where the "closed-closed" interactions occur and therefore obtain the genome compartmentalization. At the 100kb resolution, we obtain a domain agreement between the Hi-C and methylation greater than 80%. As an important observation, we notice that most of the loci for which the prediction fails are subdomains at which either the Hi-C domain signal or the methylation signal is weak, i.e. where the first principal component is close to 0 and therefore leads to an ambiguous compartment membership in either of the data types. We show that we are able to recover differences between cell types. This work makes it possible to systematically examine genome compartments in primary samples.

P-16

CHROMATIN DECOMPACTION BY THE NUCLEOSOMAL BINDING PROTEIN HMGN5 IMPAIRS NUCLEAR STURDINESS

Furusawa, T. and Bustin, M.
Laboratory of Metabolism, Center for Cancer Research, NCI, NIH, Bethesda, MD

In most metazoan nuclei, heterochromatin is located at the nuclear periphery in contact with the nuclear lamina, which provides mechanical stability to the nucleus. We show that in cultured cells, chromatin decompaction by the nucleosome binding protein HMGN5 decreases the sturdiness, elasticity and rigidity of the nucleus. Mice overexpressing HMGN5, either globally or only in the heart, are normal at birth but develop hypertrophic heart with large cardiomyoctyes, deformed nuclei and disrupted lamina and die of cardiac malfunction. Chromatin decompaction is seen in cardiomyocytes of newborn mice but misshaped nuclei with disrupted lamina are seen only in adult cardiomyocytes, suggesting that loss of heterochromatin diminishes the ability of the nucleus to withstand the mechanical forces of the contracting heart. Thus, heterochromatin enhances the ability of the nuclear lamina to maintain the sturdiness and shape of the eukaryotic nucleus; a structural role for chromatin that is distinct from its genetic functions.

P-17

THE ROLE OF LSH IN REGULATING PROLIFERATION AND DIFFERENTIATION OF NSCs

Han, Y. and Muegge, K.
Mouse Cancer and Genetics Program, Center for Cancer Research, NCI, NIH, Frederick, MD

The cerebral cortex develops from multipotent neural stem cells (NSCs) that begin as neuroepithelial cells in the ventricular zone (VZ). After initial symmetric divisions to self-expand, NSCs divide asymmetrically to give rise to differentiated progeny and maintain copies of themselves, demonstrating self-renewal and differentiation, two defining features of stem cells. Epigenetic regulations plays pivotal role in the cell identity maintenance as well as the stepwise cell differentiation guidance. Mutations in epigenetic modulators which induce modification landscape dynamic are related to developmental deficiency and many human diseases, including cancer and mental retardation. Chromatin factors that regulate neurogenesis in the central nervous system remain to be explored. It is important to elucidate molecular mechanisms underlying NSCs self-renewal in order to understand normal CNS development, and to develop therapies for neural pathologies.

Lsh is expressed ubiquitously in rapidly dividing cells or tissues and is linked to cell proliferation. Lsh is crucial for normal development since Lsh-deficient mice show multiple developmental defects. At molecular level, Lsh deletion leads to genome-widely DNA hypomethylation in MEF, brain and the whole embryos. Also Lsh knockdown was accompanied with histone modifications change and TFs binding regulation pattern change. To clarify the function of Lsh in NSCs self-renewal and differentiation, we performed the following experiments in mouse primary embryonic NSCs and embryonic mouse brains tissue: 1) Cell-based analysis and tissue level immunofluorescence analysis; 2) RNA-seq for early stage proliferating NSCs and verification RT-PCR for proliferation and differentiation NSCs; 3) ChIP-qPCR of histone modifications for promoters and enhancers of key regulators; 4) Image-based time-lapse immunofluorescence analysis.

We found that Lsh knockdown resulted in a profound decrease in neural progenitor proliferation and an increase in cell death even in early embryonic stage cells. In our study Lsh acute knockdown resulted in rapidly increased p21 (Cdkn1a) expression, which was robust at all, stages examined. The proliferation decrease and apoptosis increase of Lsh-/- NSCs are accompanied with cell cycle alteration through p21 expression level elevation but in a p53-independent manner. During self-renewal, p21 works at downstream of Lsh to control NSCs expansion by regulating Bmp4 and Sox2 expression. Taken together, our data suggest that Lsh represses p21 and that acute reduction of Lsh relieves this repression, allowing p21 transcription and thereby promoting cell cycle exit. By using combined time-lapse imaging and immunofluorescence, we show that Lsh deletion does not affect the asymmetric division (ACD) rate as well as the cell types both *in vitro* and *in vivo*, which indicates that Lsh-/- NSCs are of the ability to differentiation into functional subtypes. However, by checking the expression level of intermediate markers and neural progenitor markers at different time points, we found that Lsh-/- NSCs differentiation was delayed than wild type litter mates, which suggests that Lsh affects the NSCs differentiation by promoting neural linage specification.

In summary, we demonstrate functional requirements for Lsh in self-renewing proliferation and differentiation of NSCs and neuronal and glial cell fate decisions. Our results provide a fresh view of molecular signaling mechanisms coordinating NSCs self-renewal and cell lineage specification.

P-18

A NOVEL STRATEGY TO INHIBIT CpG ISLAND HYPERMETHYLATION AND RESTORE BRCA1 EXPRESSION

Han, Y.J.[1], Zhang, J.[1], Prat, A.[3,4], Yoshimatsu, T.[1], Gomez-Vega, M.[1], Kwon, J.[2], Perou, C.M.[3], Kannanganattu, P.[5], and Olopade, O.I.[1]
[1]Center for Clinical Cancer Genetics and Global Health; and Section of Hematology and Oncology, and [2]Section of Gastroenterology, Department of Medicine, University of Chicago, Chicago, IL; [3]Departement of Genetics, School of Medicine, University of North Carolina, Chapel Hill, NC; [4]Translational Genomics Group, Vall d´Hebron Institute of Oncology (VHIO), Barcelona, Spain; [5]Department of Cell and Developmental Biology, University of Illinois, Urbana, IL

BRCA1 promoter methylation is observed in 20-60% of sporadic triple negative breast cancer (TNBC) and may be an important mechanism contributing to the loss of *BRCA1* function in sporadic TNBC and other cancers with low BRCA1 expression. Demethylation and consequent reactivation of tumor suppressor genes are rationale approaches being used in the treatment of cancer. However, currently available nucleoside-based DNMT inhibitors affect genome-wide DNA methylation and cannot specically target tumor suppressor genes like *BRCA1*. Here, we describe a novel approach to modulate local DNA methylation by silencing a neighboring long non-coding RNA (lncRNA) which tethers DNMT1 at the *BRCA1* genomic locus.

The human genomic region encompassing the *BRCA1* gene is complex and includes two protein coding genes (*BRCA1* and *NBR1*), a non-coding RNA gene (*NBR2*), and a pseudogene of BRCA1 (*BRCA1P1*), within a ~170kb region of chromosome 17q21. The *BRCA1* gene on the minus strand is located head-to-head with *NBR2* on the plus strand, whereas *BRCA1P1* on the minus strand is located head-to-head with *NBR1* on the plus strand. The promoter between the *BRCA1* and *NBR2* genes and that between the *BRCA1P1* and *NBR1* genes are bidirectional, expressing transcripts in opposite directions through convergent transcription. The *BRCA1P1* pseudogene was generated by a recent evolutionary event: partial duplication of *BRCA1* gene followed by insertion of a processed pseudogene of *RPLP1*. It expresses a chimeric lncRNA retained in nuclei. Interference with the nuclear expression of BRCA1P1 lncRNA using a specific anti-sense oligonucleotide (ASO) decreased the promoter methylation of *BRCA1* and increased BRCA1 expression. RNA immunoprecipitation (RIP) assays revealed DNMT1 interactions with BRCA1 mRNA and BRCA1P1 lncRNA at the locus. Chromosome conformation capture (3C) will assess whether there is a long-range interaction between the *BRCA1* and *BRCA1P1* promoters through DNMT1 and mediators. Our data support a long-range *cis*-regulation of BRCA1 expression by neighboring BRCA1P1 lncRNA through an interaction with DNMT1 at the locus. Depleting BRCA1P1 with ASO could be developed as a therapeutic method to inhibit *BRCA1* promoter methylation and restore BRCA1 expression.

P-19

NUCLEOSOMAL BINDING PROTEINS HMGN1 AND HMGN2 MODULATE THE RATE OF AMELOBLAST DIFFERENTIATION

He, B.[1], Deng, T.[1], Yamada, Y.[2], and Bustin, M.[1]
[1]Laboratory of Metabolism, Center for Cancer Research, NCI; [2]Laboratory of Cell and Developmental Biology, NIDCR, NIH, Bethesda, MD

Ameloblast secretes enamel matrix proteins which mineralize to form the dental enamel that is necessary for daily chewing. The maturation of ameloblast is a stepwise process in which the highly proliferative progenitor ameloblast transits into the terminally differentiated ameloblast. Ameloblast differentiation is regulated by multiple factors, including the zinc finger family transcription factor Epiprofin (Epfn); however, the regulatory network of ameloblast maturation is still not fully understood. We now show that the nucleosomal binding proteins HMGN1 and HMGN2 modulate the rate of ameloblast differentiation. We find that during embryogenesis *Hmgn1 and Hmgn2* are downregulated and show a reverse tendency to the expression of ameloblast differentiation markers *Amelogenin (Amel)* and *Ameloblastin (Ambn)*, which are upregulated. In primary dental epithelial cells, overexpression of HMGN1 or HMGN2 reduced the Epfn-induced expression of *Amel* and *Ambn* while siRNA-mediated knockdown of HMGN1 or HMGN2 expression increased the *Amel* and *Ambn* expression. Thus, the Epfn-mediated *Amel* activation is controlled by HMGN variants. To test the role of HMGN in ameloblast differentiation in the biological context of an organism we created *Hmgn1*$^{-/-}$, *Hmgn2*$^{-/-}$, and *Hmgn1*$^{-/-}$;*Hmgn2*$^{-/-}$ mice. Immunofluorescence, q-PCR measurements, and RNA-seq analysis of one-day-old (P1) mouse incisors revealed that loss of HMGN1 or HMGN2 downregulated the expression of *CartD,* an ameloblast progenitor marker, and upregulated the expression level of *Amel* and *Ambn,* markers of mature ameloblasts, suggesting that loss of the HMGN variants accelerated the rate of ameloblast differentiation. Indeed, all the three *Hmgn*$^{-/-}$ mutant mice showed thicker enamel than their wide type littermates at the early differentiation stages; however, all the adult mice showed normal teeth and enamel density. Our results suggest that HMGN1 and HMGN2 regulate the rate of ameloblast differentiation by modulating the interaction of specific transcription factors with chromatin. Given that HMGN proteins are ubiquitously expressed in vertebrate cells, it is likely that they similarly affect additional regulatory networks necessary for proper embryonic development.

P-20

METHYLATED CYTOSINES (5mC) MUTATE TO TRANSCRIPTION FACTOR BINDING SITES THAT DRIVE VERTEBRATE EVOLUTION

He, X.[1], Tillo, D.[1], Syed, S.[1], Vierstra, J.[2], Deng, C.[1], Ray, G.J.[1], Stamatoyannopoulos, J.[2], Fitzgerald, P.[3], and Vinson, C.[1]
[1]Laboratory of Metabolism, Center for Cancer Research, NCI, NIH, Bethesda, MD; [2]Department of Genome Science, University of Washington, Seattle, WA; [3]Genetics Branch, Center for Cancer Research, NCI, NIH, Bethesda, MD

The abundance of all 8-base-pair long DNA sequences (8-mers) in the human genome is a bimodal distribution with all rare 8-mers containing a CG dinucleotide and all abundant 8-mers not containing a CG dinucleotide. In mammals, the cytosine in CG dinucleotides is typically methylated (5mC). 5mC is chemically unstable and spontaneously deaminates to TG/CA dinucleotides, which is thought to explain the depletion of CG dinucleotides in mammalian genomes. These new TG/CA dinucleotides generate genetic diversity that may be critical for evolutionary change by creating transcription factor binding sites (TFBS) that become tissue specific regulatory regions. We tested this idea by examining regulatory regions identified by DNase Hypersensitive Sites (DHSs) in human and mouse genomes. DHSs, both shared and tissue-specific are enriched for 8-mers containing the CG dinucleotide. In both species, 8-mers containing a TG/CA and no CG dinucleotide are enriched in tissue-specific DHSs (TS-DHSs) more than 8-mer with neither a TG/CA or CG dinucleotide. The most enriched in both genomes is the AP-1 motif (GTGA$^{C}/_{G}$TCAN), a pseudo-palindrome with two TG/CA dinucleotides. When we examine the evolution of the AP-1 motif, the TG/CA dinucleotides previously were CG dinucleotides supporting the suggestion that TG/CA dinucleotides in TFBS are molecular fossils of 5mC. Additional TS-DHS enriched TFBS containing the TG/CA dinucleotide are the palindromic E-Box motif (GCAGCTGC), the NF-1 motif (GGCA---TGCC), and the GR motif (G-ACA---TGT-C). The bimodal distribution of 8-mers initially occurs in the coelacanth, the phylogenetic lineage that evolved onto the land and persists in all descendants.

P-21

NOVEL BRG1-BAF COMPLEX-INDEPENDENT REGULATION OF GENE EXPRESSION BY BAF60A

Hoffman, J.A., Ward, J.M., and Archer, T.K.
Epigenetics and Stem Cell Biology Laboratory, NIEHS, NIH, Research Triangle Park, NC

The BAF chromatin remodeling complex is composed of a variable number of subunits, many of which are expressed in tissue and cell-type specific manners. While individual subunits are required for specific cell-fate decisions and developmental events, these are typically only considered within the context of BAF complex function. We sought to determine whether individual BAF subunits might display distinct, BAF complex-independent functions. RNA-sequencing in HepG2 cells revealed that silencing expression of either the catalytic subunit Brg1 or the Baf60a subunit resulted in largely non-overlapping changes in gene expression. The majority of differentially expressed genes (DEGs) were unique for Baf60a silencing, and among common DEGs, Brg1 and Baf60a silencing frequently had opposing effects on expression. Furthermore, pathway analysis revealed that Brg1 and Baf60a regulated distinct biochemical, metabolic, and signaling pathways. To identify unique Baf60a-interacting proteins, we performed do-immunoprecipitation and mass spectrometry experiments and found that Baf60a interacts with a distinct set of nuclear RNA-binding proteins independently of Brg1. Taken together, these findings demonstrate novel, BAF complex-independent roles for Baf60a in transcriptional and post-transcriptional regulation of gene expression.

P-22

MECHANISMS OF RNA POLYMERASE PAUSING ASSOCIATED WITH A TRANSLOCATION BLOCK *IN VIVO*

Imashimizu, M.[1], Takahashi, H.[2], Oshima, T.[3], McIntosh, C.[4], Bubunenko, M.[1], Court, D.L.[1], and Kashlev, M.[1]
[1]Gene Regulation and Chromosome Biology Laboratory, Center for Cancer Research, NCI, NIH, Frederick, MD; [2]Medical Mycology Research Center, Chiba University, Japan; [3]Graduate School of Biological Sciences, Nara Institute of Science and Technology, Ikoma, Japan; [4]Office of the Director, Center for Cancer Research, NCI, NIH, Frederick, MD

Transcription elongation is frequently interrupted by pausing signals in DNA, which regulate gene expression. Pre-translocated, post-translocated and backtrack pauses of RNA polymerase (RNAP) have been well characterized *in vitro*. However, physiologically relevant mechanisms remain poorly understood. Here we investigated RNAP pausing in *E. coli* by developing a novel approach, combining nascent elongating transcript sequencing (NET-seq) with RNase footprinting of nascent elongating transcripts (RNET-seq). This technique allows assessment of translocation states of RNAP by determining the length of the 3'-proximal RNA transcript protected by RNAP in the paused complex as isolated from the nucleoid. We identified two DNA sequence elements that govern genome-wide pausing: G and CG nucleotides, respectively located respectively at the 5' and 3' ends of the RNA-DNA hybrid within RNAP. We demonstrated that the G-dC base pair in the 5' end of the hybrid interferes with RNAP translocation. The length between the 5' G and the 3' CG elements of the pause sites fluctuates over a three nucleotide width. Thus, the G-dC can induce pausing in each of three states, post-translocated, pre-translocated, and backtracked. Pausing events are significantly enriched in the 5' untranslated region (UTR) of mRNA genes. GreA and GreB proteins, which are known to rescue backtracked RNAP, reduce pausing in the 5'UTRs, but do not affect pausing in the translated parts of genes. This finding argues that translating ribosomes suppress backtracking and associated transcription pausing. We also identified multiple pausing events in regions where collisions of RNAP molecules occur during convergent transcription.

P-23

CHROMOSOME-SCALE AND LOCAL PATTERNS OF NUCLEOSOME FRAGILITY IN *C. ELEGANS*

Jeffers, T.E.[1] and Lieb, J.D.[2]
[1]Curriculum in Quantitative and Computational Biology, Princeton University, Princeton, NJ;
[2]Department of Human Genetics, University of Chicago, Chicago, IL

Regulation of nucleosome positioning and occupancy in eukaryotic chromatin is required for gene regulation, replication, and DNA repair. The stability of the histone-DNA interaction is regulated by a complex combination of histone post-translational modifications, histone variants, DNA sequence content, genomic location, and transcriptional machinery. Because these factors also influence each other, the unique contribution of each factor has been difficult to study.

We are interested in mechanisms by which nucleosomes and higher-order chromatin structures restrict access to the underlying DNA sequence. We interrogated nucleosome positioning and the stability of nucleosome-DNA interactions by performing a micrococcal nuclease digestion timecourse using chromatin from *C. elegans* embryos. At each timepoint, the mononucleosomes that had been liberated at that point in the digestion were subjected to paired-end Illumina sequencing. Previously published work has termed the earliest nucleosomes released as "fragile" and the latest nucleosomes released as "resistant". This approach captures known features such as the 10 – 11 bp periodicity in nucleosome digestion, and additional information specific to the timecourse, such as correlations between nucleosome position and fragility, and between transcriptional activity and fragilty.

At the level of gene organization, fragile nucleosomes are enriched at the 5' and 3' of genes, while resistant nucleosomes are enriched in the coding region and in non-coding intergenic regions. We were surprised to find that at the level of the chromosome, fragile and resistant nucleosomes are distributed non-uniformly. Fragile nucleosomes are enriched at the center of the autosomal chromosomes, while resistant nucleosomes are enriched at the chromosome arms. This pattern is broadly consistent with fragility being inversely proportional to chromosome-nuclear lamina interactions measured by LEM-2 ChIP-seq.

P-24

NUCLEOSOME REPEAT LENGTH RELATES TO THE GENE EXPRESSION LEVEL IN YEAST

Katebi, A.[1], Norouzi, D.[1], Cui, F.[2], and Zhurkin, V.[1]
[1]Laboratory of Cell Biology, Center for Cancer Research, NCI, NIH, Bethesda, MD; [2]Rochester Institute of Technology, Rochester, NY

We are investigating correlation between the DNA folding in 30-nm chromatin fiber and the level of gene expression. The 30-nm fiber is generally characterized by the nucleosome repeat length (NRL) – that is, the length of the core DNA, 147 bp, plus the linker DNA length, L. We found previously that there are two families of the two-start chromatin fiber structures characterized by different DNA topology and flexibility. (Depending on the NRL value, the energetically optimal fiber structure belongs to one of the two families.) Here we analyze the high resolution nucleosome positioning data to find whether there is any correlation between the NRL and the gene expression level in yeast. We calculate the NRL values for the two groups of genes – 25% highly expressed and 25% lowly expressed genes (out of ~3,500 yeast genes that are at least 1,000 bp long). Our results show that the average NRL=161-162 bp for the highly active genes (i.e., linker L=14-15 bp), whereas NRL=167-168 bp (i.e., linker L= 20-21 bp) for the lowly transcribed genes. Based on these findings, we conclude that the highly and lowly active gene sets have distinct nucleosome fiber organization with the linker $L \approx 10n+5$ and 10n, respectively. We hypothesize that organization of the most active genes in fibers with $L \approx 10n+5$ (which are more flexible than the fibers with $L \approx 10n$) facilitates formation of gene loops, thereby inducing transcription of these genes.

P-25

BIOCHEMICAL CHARACTERIZATION OF THE HUMAN MITOCHONDRIAL DNA HELICASE TWINKLE

Khan, I., Bharti, S.K., Sommers, J.A., and Brosh Jr., R.M.
Laboratory of Molecular Gerontology, NIA, NIH, Baltimore, MD

Autosomal recessive and dominant mutations in the c10orf2 gene encoding the mitochondrial replicative DNA helicase Twinkle are genetically linked to several diseases characterized by neurodegeneration or premature aging. Despite its essential role in human mitochondrial DNA replication, Twinkle helicase has not been characterized in an extensive manner in terms of its DNA substrate specificity or DNA unwinding mechanism. To gain insight on its role in mitochondrial DNA metabolism, we have characterized a homogeneous recombinant form of Twinkle protein expressed and purified from human cells. We first examined Twinkle's DNA unwinding activity on simple and sequence-related partial duplex structures that represent key intermediates of DNA replication and repair. Twinkle required a free 5' single-stranded DNA sequence to efficiently unwind a flanking duplex in the DNA substrate molecule. Twinkle was poorly active on a synthetic replication fork structure with duplex leading and lagging strand arms. The demonstrated ability of Twinkle to robustly unwind the downstream duplex of a 5' single-stranded flap DNA substrate is consistent with a proposed role of Twinkle in strand displacement synthesis and may be relevant to its potential involvement in base excision repair of oxidative lesions known to accumulate in the mitochondrial genome. Thus, we assessed the ability of Twinkle to unwind partial duplex DNA substrates harboring the endogenous oxidative lesion 8,5'-cyclopurine-2'-deoxynucleoside (cPu). Twinkle efficiently unwound DNA substrates containing a cPu within the duplex region in either the translocating or non-translocating strand, indicating that the hexameric ring-like assembly state of Twinkle can accommodate the cPu.

DNA intermediates of homologous recombination (HR) are known to exist in mitochondria; therefore, we analyzed Twinkle's ability to catalytically act upon four-stranded Holliday Junctions (HJ) and three-stranded displacement (D)-loop structures. Twinkle failed to unwind blunt-ended HJ structures under conditions that it efficiently unwound simple duplex substrates, suggesting that unlike helicases implicated in HR repair (e.g., RecQ), Twinkle does not recognize the central core of the HJ structure. However, Twinkle efficiently catalyzed ATP-dependent branch-migration of a mobile three-stranded DNA structure in the 5' to 3' direction, but poorly in the 3' to 5' direction. In contrast, Twinkle was able to efficiently unwind fixed D-loop substrates in which the invading strand possessed either a 5' or 3' single-stranded DNA tail, suggesting that Twinkle simply requires a DNA junction for optimal loading of non-mobile D-loops.

Although mitochondrial DNA is histone-free, it is bound by the duplex DNA-interacting transcription factor TFAM which is known to exist at a genome-saturating concentration. To assess if Twinkle can displace protein bound to DNA, we tested whether an enzymatically inactive BamHI-E111A restriction endonuclease bound with high affinity to a forked duplex DNA harboring a BamHI recognition sequence was able to inhibit Twinkle unwinding of the substrate. Our biochemical studies demonstrated that Twinkle was able to displace BamHI-E111A and unwind the DNA substrate in a manner that was nearly comparable to the substrate without BamHI-E111A bound to it. Based on our findings, we conclude that Twinkle performs its DNA replication functions by unwinding duplex DNA and displacing any proteins in its path.

P-26

IMPACT OF CONTROLLED DNA DOUBLE-STRAND BREAK INDUCTION ON TRANSCRIPTOME MAINTENANCE *IN VIVO*

Kim, J.[1], Sturgill, D.[1], Tran, A.[1], Sinclair, D.[2], and Oberdoerffer, P.[1]
[1]Laboratory of Receptor Biology and Gene Expression, Center for Cancer Research, NCI, NIH, Bethesda, MD; [2]Department of Genetics, Harvard Medical School, Boston, MA

DNA double-strand breaks (DSBs) and their repair cause extensive break-proximal chromatin reorganization. The latter can result in transcriptional repression near DSBs, highlighting the potential impact of DSB (repair) on the epigenetic integrity of our genomes, and ultimately cell and tissue function. However, the study of both epigenetic and physiological consequences of DSBs in model organisms has been hindered by a scarcity of tools to induce temporally and/or locally controlled DSBs. Here, we describe a mouse model that allows for tightly regulated DSB formation at approximately one hundred defined genomic loci, including the repetitive 28S rDNA. Using this model, we find that efficient DSB repair ensures surprisingly stable gene expression profiles in primary cells. No evidence for persisting, break-proximal gene deregulation was observed except at the rDNA, which, unlike single gene loci, displayed a continuous presence of DSBs due to the repetitive nature of the locus. Together, we reveal an unexpected capacity of primary cells to maintain transcriptome integrity in response to DSBs, a process that seems to fail, however, when DSBs cannot be cleared effectively. These findings have significant implications for our understanding of DNA damage-associated epigenetic dysfunction.

P-27

THE ROLE OF TET1-MEDIATED DNA HYDROXYMETHYLATION IN SELF-RENEWAL AND DIFFERENTIATION OF INTESTINAL STEM CELLS

Kim, R., Sheaffer, K.L., Aoki, R., Choi, I., Won, K.J., and Kaestner, K.H.
Department of Genetics and Institute for Diabetes, Obesity & Metabolism, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA

5-methycytosine (5mC) can be oxidized to 5-hydroxymethylcytosine (5hmC) and further to 5-formylcytosine and 5-carboxylcytosine by the Ten Eleven Translocation (TET) hydroxylases. Both 5hmC and TET1 have been s hown to be essential for embryonic stem cell function and neuronal activity, their role in adult stem cells remains to be elucidated. The intestinal epithelium is an excellent model to study adult stem cells. Our aim was to determine if 5hmC and TETs are involved in self-renewal and differentiation of intestinal stem cells.

We isolated intestinal stem cells (ISC) and differentiated cells (DIFF) from adult mice and performed immunoprecipitation for hydroxymethylated DNA followed by high-throughput sequencing to generate genome-wide 5hmC maps. We found that 5hmC distribution was dynamically changed between ISC and DIFF. Globally 5hmC level was increased in differentiated cells and 5hmC-aquiring genes during differentiation were upregulated in differentiated cells. However, 5hmC was enriched at ISC marker genes such as Lgr5 and Olfm4 in ISC.

Next, we discovered that Tet1 mRNA was highly expressed in ISC, but not in differentiated cells. To investigate regeneration capacity of Tet1-deleted ISC, we isolated ISC from $Tet1^{-/-}$ mice and grew them using the ex vivo organoid culture system. Surprisingly, we found that Tet1-deleted ISC generated hollow spheroids instead of the budding organoids, typically seen when culturing wild-type ISCs. These spheroids were remarkably similar to intestinal spheroids derived from fetal intestine not only in morphology, but also in gene expression profile, exhibiting low levels of intestinal stem cell markers and differentiation genes, and high levels of fetal spheroid markers such as Trop2 and Cnx43.

In conclusion, Tet1-mediated DNA hydroxymethylation in ISCs is required for ISC gene activation, proper self-renewal and differentiation of ISCs.

P-28

ALPHA-AMINO TERMINAL TRIMETHYLATION OF THE CENTROMERIC HISTONE CENP-A IS REQUIRED FOR MAINTAINING BIPOLAR SPINDLE AND REGULATED CELL PROLIFERATION

Kizhakke Mattada, S.[1] and Foltz, D.R.[1,2]
Departments of [1]Biochemistry and Molecular Genetics, and [2]Cell Biology, University of Virginia, Charlottesville, VA

Nucleosomes containing the histone H3 variant CENP-A mark the centromere, which is the locus required for segregation of chromosomes. It has been shown that the majority of cancers exhibit genomic instability, however, the mechanism is unclear. Overexpression of CENP-A and its chaperone HJURP occurs in many cancers and is sufficient to cause chromosome missegregation in cell culture. The posttranslational modification (PTM) of histones resulting in the recruitment of activator and repressor complexes to chromatin is a well-established and broadly used method to regulate chromatin activity by the cell. We propose that PTMs of CENP-A have a major role in regulating centromeric chromatin and preventing chromosomal instability. Recently, we reported that N-terminal residue Gly1 becomes trimethylated on the α-amino group following initial methionine removal. Here we demonstrate that the N-terminal RCC1 methyltransferase (NRMT1) methylates CENP-A both *in vitro* and *in vivo*. Methylation occurs in the prenucleosomal as well as nucleosomal forms of CENP-A, although nucleosomal CENP-A showed higher methylation. *In vivo* as well as *in vitro* data suggest that the amino terminal tail of CENP-A is sufficient for trimethylation. We also found that methylation of nucleosomal CENP-A increases through cell cycle with highest being found during mitosis. CENP-A amino-terminal methylation is required for cell survival. Previous work showed that the amino terminus of CENP-A is essential in human cells in which the CENP-A c-terminal tail is absent. We tested whether methylation of CENP-A was required for the essential function of the CENP-A amino terminus by attempting to rescue a CENP-A knockout cell line with a CENP-A mutant that could not be methylated. Cells expressing only the CENP-A methylation mutant formed fewer colonies than the wild-type control, suggesting that methylation is required for cell survival. Expression of CENP-A methylation resistant mutants causes significant defects in the microtubule spindle during mitosis. We observe increased number of multipolar spindles in CENP-A mutant expressing cells. These multipolar spindles are not a result of centriole duplication, suggesting that spindle defects result from an imbalance of motor forces within the mitotic spindle. We observed spindle pole defects only in cells that are deficient for p53 (HeLa and HCT116 p53-/- cells). A significant increase in chromosome segregation defects was observed in both p53-/- and p53+/+ HCT116 cells. Under conditions where endogenous CENP-A was knocked down, cells expressing the CENP-A methylation mutants formed larger and higher number of colonies indicating uncontrolled growth in p53-/- cells relative to p53+/+ cells and cells expressing wild-type CENP-A. Cells expressing CENP-A methylation mutants showed an increase in tumor forming potential relative to wild-type expressing cells in xenograft experiments. Our work provides a novel link between CENP-A posttranslational modification and the generation of chromosome instability.

P-29

UNUSUAL DNA STRUCTURES ARE A COMMON FEATURE OF MAMMALIAN GENOMES

Kouzine, F.[1], Wojtowicz, D.[2], Yamane, A.[3], Baranello, L.[1], Nelson, S.[3], Resch, W.[3], Casellas, R.[3], Przytycka, T.M.[2], and Levens, D.[1]
[1]Laboratory of Pathology, Center for Cancer Research, NCI, NIH, Bethesda, MD; [2]National Center for Biotechnology Information, NLM, NIH, Bethesda, MD; [3] Laboratory of Molecular Immunogenomics, NIAMS, NIH, Bethesda, MD

The predominant form of DNA in living cells is a right-handed double helix; however some DNA sequences under certain conditions have the potential to fold into non-B DNA structures such as triplex, left-handed Z-form, quadruplex, etc. These unusual DNA structures are of great interest: their stability under physiological conditions *in vitro* and the abundance of sequences with the propensity to form these structures in the genome, suggest that unusual DNA conformations could form in cells. While considered to be important in the regulation of physiological processes performing functions not achievable using conventional B-DNA, the very existence and distribution of non-B DNA conformations across the mammalian genome is still the matter of debate.

To map non-B DNA comprehensively across the whole genome, we combined potassium permanganate foot-printing with high-throughput sequencing. High-resolution mapping revealed the existence of many non-B DNA structures inside mouse and human cells. These unusual DNAs were temporally and locally associated with gene function and cell state.

We found that non-B DNA structures remodel nucleosomes positioning and have the capacity to serve as regulatory elements to the proper expression of many genes that control critical cellular processes. Formation of the non-B DNA structures within promoter of many oncogenes may play a role in the complex transcriptional regulation of these important genes, making them putatively amenable to specific drug targeting. These results demonstrate the abundance of unusual DNA structures in mammalian genomes and suggest their wide usage in a variety of DNA transactions. The presented investigation enables for the first time to elucidate in a biologically relevant context the mechanism(s) by which these structures can modulate the genome functioning.

P-30

INHIBITION OF G9A METHYLTRANSFERASE STIMULATES FETAL HEMOGLOBIN PRODUCTION BY FACILITATING LCR/β-GLOBIN LOOPING

Krivega, I.[1,3], Byrnes, C.[2,3], de Vasconcellos, J.F.[2], Lee, Y.T.[2], Kaushal, M.[2], Dean, A.[1], and Miller, J.L.[2]
[1]Laboratory of Cellular and Developmental Biology and [2]Molecular Genomics and Therapeutics Section, Molecular Medicine Branch, NIDDK, NIH, Bethesda, MD

Globin gene expression undergoes developmental switching from embryonic (ε) through fetal (γ) to adult (δ and β) genes. Inherited mutations or deletions at the β-gene cause β-thalassemia. One of the most propitious strategies of treatment for the disease is forced switching from expression of the mutated β-globin gene to the unaffected fetal γ-globin gene in adult erythroid cells. Expression of globin genes is regulated by the upstream locus control region (LCR) enhancer. The LCR loops to globin genes utilizing the LDB1/GATA-1/TAL1/LMO2 protein complex (LDB1 complex). Additionally, histone-modifying enzymes play a significant role in regulation of globin gene expression. G9a methyltransferase, responsible for establishing H3K9me2 histone modification, is involved in repressing fetal and activating adult globin gene expression in mouse erythroid cells. Moreover, inhibition of G9a methyltransferase activity by the synthetic chemical compound UNC0638 activates γ- and represses β-globin gene expression in adult human hematopoietic precursor CD34(+) cells, but underlying mechanisms are unclear.

Human peripheral blood CD34(+) progenitor cells from three healthy adult donors were differentiated for 21 days in a three phase serum-free media system. Based upon dose titration studies, cells were treated with 1μM UNC0638 during the differentiation phase of culture (days 7-14) and compared to control cells grown without UNC0638. Inhibition of G9a methyltransferase activity caused activation of γ-globin and repression of β-globin gene expression without significant changes in α-globin gene expression or strong effect on cell differentiation. At the end of the culture period, cells treated with UNC0638 showed pan-cellular distribution of fetal hemoglobin constituting up to 30% of total hemoglobin.

Chromatin immunoprecipitation and chromosome conformation capture (3) assays were utilized to determine if the increase of fetal hemoglobin along with activation of γ-globin gene expression was associated with epigenetic modification of the β-globin locus. UNC0638 treatment caused widespread loss of H3K9me2 histone modification across the locus. G9a and LDB1 complex occupancy was significantly increased at the γ-globin gene and decreased at δ- and β-globin gene promoters. Mirroring differences in LDB1 complex occupancy, LCR/globin gene looping was changed from interaction with the β- to the γ-globin gene. Our findings demonstrate that G9a establishes conditions preventing activation of γ-globin genes during differentiation of adult erythroid cells, thereby favoring LCR looping to and activating the δ- and β-globin. In this view, G9a inhibition represents a promising approach for treatment of β-hemoglobinopathies.

P-31

RNA POLYMERASE I AS A CANCER THERPEUTIC TARGET

Peltonen, K.[1], Colis, L.[2], Liu, H.[2], Sirajuddin, P.[2], Moubarek, M.S.[3], Bieberich, C.J.[3], Schneider, D.[4], and Laiho, M.[1,2]
[1]Centre for Drug Research, University of Helsinki, Finland; [2]Department of Radiation Oncology and Molecular Radiation Sciences and Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, Baltimore, MD; [3]Department of Biological Sciences, University of Maryland, Baltimore, MD; [4]Department of Biochemistry and Molecular Genetics, University of Alabama, Birmingham, AL

RNA polymerase I (Pol I) transcription is an intricately coordinated, compartmentalized transcriptional program. Ribosomal (r) DNA is transcribed by Pol I into a long 47S rRNA precursor, and processed through multiple steps to the 18S, 5.8S and 28S mature rRNAs in the nucleolus. The human genome has ~400 copies of the rDNA, of which ~50% are transcriptionally active. Of total cellular transcription, over 60% results from Pol I activity. Pol I transcription is robust, occurs at a high rate and responds to extracellular cues. Conventionally, studies on cancer pathway alterations are solely focused on RNA polymerase II (Pol II) -driven programs affected by cancer genome abnormalities. However, a prerequisite for the cancer cell is an increase in its ribosynthetic activity to support the increased needs for protein synthesis. Pol I transcription is pervasively deregulated in cancers by oncogenic signaling by ERK/Ras/Akt/PKB/mTOR/Myc and loss of repression by p53/pRB/ARF/PTEN. Yet, attempts to exploit Pol I as a clinically relevant target are almost non-existent and relevant intervention modalities are scarce. We have recently discovered a unique small molecule pyridoquinazolinecarboxamide (BMH-21) that causes Pol I transcription blocks, is broadly effective against many cancer cell types and is well tolerated in normal cells and in mouse (1). We show that the compound, BMH-21, has wide and potent anticancer activity across NCI60 cancer cell lines and represses tumor growth *in vivo* in cancer xenograft and genetically modified mouse cancer models. BMH-21 binds GC-rich sequences, present at high frequency in the rDNA gene, and potently and rapidly represses Pol I transcription. Using a fully reconstituted transcription assay for Pol I we show that BMH-21 directly causes Pol I pausing and reduces Pol I elongation kinetics. Strikingly, we find that BMH-21 causes proteasome-dependent destruction of RPA194, the large catalytic subunit of Pol I complex, and that this correlates with cancer cell killing. We have proposed that BMH-21 cancer selectivity depends on the deregulated Pol I transcription rates. Based on our data we hypothesize that Pol I is highly vulnerable to elongation stress and to polymerase pileups due to BMH-21 binding to rDNA, which activate a checkpoint leading to the degradation of RPA194. In support, we show here that blocking of transcription initiation by silencing the Pol I preinitiation complex factors rescues RPA194 expression in BMH-21-treated cancer cells. We have further addressed the stability of the Pol I complex. We show that depletion of specific Pol I complex proteins leads to loss of RPA194 indicating that Pol I complex stability is also influenced by intrinsic factors. Our results show that Pol I activity is under proteasome-mediated control, which reveals an unexpected therapeutic opportunity. These findings strongly promote the rational of Pol I targeting and specifically, BMH-21 activity-based small molecules for testing as potential cancer therapies.

P-32

GENOME-WIDE FUNCTIONS OF POLYCOMB COMPLEXES REGULATE PERVASIVE TRANSCRIPTION

Lee, H.-G.[1], Schwartz, Y.B.[2], Kahn, T.G.[2], Simcox, A.[3], and Pirrotta, V.[1]
[1]Molecular Biology and Biochemistry Department, Rutgers University, Piscataway, NJ; [2] Molecular Biology Department, Umeå University, Umeå, Sweden; [3] Molecular Genetics Department, Ohio State University, Columbus, OH

Polycomb Group (PcG) complexes PRC1 and PRC2 are well known for silencing specific developmental genes. PRC2 is a methyltransferase targeting histone H3K27 and producing H3K27me3, essential for stable silencing. Less well known but quantitatively much more important is the genome-wide role of PRC2 that dimethylates ~70% of total H3K27. We show that H3K27me2 occurs in inverse proportion to transcriptional activity in most non-PcG target genes and intergenic regions. Surprisingly, its loss results in global transcriptional derepression proportionally greatest in silent or weakly transcribed intergenic and genic regions accompanied by increase of H3K27ac and H3K4me1. H3K27me2 levels are governed by opposing roaming activities of PRC2 and the H3K27 demethylase dUTX complexes. H3K27me2 therefore sets a threshold that prevents random, unscheduled transcription all over the genome and limits the activity of even highly transcribed genes. PRC1-type complexes also have global roles. Unexpectedly, we find a pervasive distribution of histone H2A ubiquitylated at lysine 118 (H2AK118ub) outside of canonical PcG target regions, which is mostly produced by a new variant PRC1 complex involving L(3)73Ah, a homolog of mammalian PCGF3.

P-33

HIGHLY EFFICIENT CRISPR/CAS9-MEDIATED TAR CLONING OF GENES AND CHROMOSOMAL LOCI FROM COMPLEX GENOMES IN YEAST

Lee N.C., Larionov V., and Kouprina N.
Developmental Therapeutics Branch, Center for Cancer Research, NCI, NIH, Bethesda, MD

Transformation-Associated Recombination (TAR) is a yeast (*S. cerevisiae*) based cloning method, used to selectively isolate large DNA segments from complex genomes. DNA fragments, ranging from full-length genes that include distal enhancer and native promoters to very large genomic loci up to 250kb in length. In the decade since this protocol was developed, it has been use in functional, structural and comparative genomics. However wide spread use of this protocol has been impeded by the low capture efficiency (0.5-2%) of chromosomal region during yeast transformation, necessitating large, laborious screens of hundreds of colonies to obtain a single gene positive clone. In turn, to obtain sufficient number of colonies for these large screens, extremely competent yeast cells (10^{10} cfu/ μg plasmid DNA) had to be prepared. Preparation of such competent cells is both a lengthy and complicated procedure.

Cas9 is a family of bacterial, RNA-guided, double-stranded DNA endonucleases employed by type II CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) systems. DNA target specificity of Cas9 is encoded by a 20 bp guide sequence located on the 5′ terminal of the gRNA, a small synthetic chimera of mature crRNA and tracrRNA, which is bound by Cas9. Target sequence recognition is mediated by RNA–DNA base pairing between the gRNA to the DNA target and an adjacent downstream DNA motif (NGG), the protospacer adjacent motif (PAM). The 5′ terminus of the gRNA can be cheaply and quickly modified using polymerase chain reaction (PCR) with synthetic oligomers. Hence the Cas9 nuclease can be easily 'programed' to cleave any ~20 bp sequence downstream of a PAM motif within complex genomes. It is thus not surprising that Cas9 has rapidly gained prominence in the field of genome editing.

Here, we demonstrate that pre-treatment of genomic DNA with CRISPR-Cas9 nucleases, which generates double-strand breaks near the terminal ends of the targeted region, results in a dramatic increase in the fraction of gene-positive colonies (from 2% to 32%). The implication of this new development is that far smaller colony screens can now isolate a clone with the desired chromosomal region. As fewer colonies (as few as 8 colonies) are needed, less competent yeast cell, made by less stringent protocol can be used. This added leeway makes the new TAR-CRISPR protocol easier to master and faster to use.

The use of such an improve TAR-CRIPSR protocol are many. The most obvious perhaps is the creation of a library of full length genes, each represented by a genomic copy containing its native regulatory elements. This library would lead to a significant advance in functional, structural and comparative genomics, in diagnostics, gene replacement, generation of animal models for human diseases and has a potential for gene therapy.

P-34

HUMAN ENHANCERS ARE FRAGILE AND PRONE TO DEACTIVATING MUTATIONS

Li, S. and Ovcharenko, I.
Computational Biology Branch, National Center for Biotechnology Information, NLM, NIH, Bethesda, MD

Non-coding DNA sequence variation plays an important role in establishing molecular phenotypes. However, the underlying mechanisms whereby non-coding variants affect transcriptional regulation are poorly understood. We identified nucleotides capable of disrupting binding of transcription factors and deactivating enhancers if mutated (dubbed killer mutations or KMs) in HepG2 enhancers. On average, ~11% of enhancer positions are prone to KMs. A comparable number of enhancer positions are capable of creating de novo binding sites via a single-nucleotide mutation (dubbed restoration mutations or RSs). Both KM and RS positions are evolutionary conserved and tend to form clusters within an enhancer. The coordination and correlation between KMs and RSs indicate binding sites reordering in enhancer regions across individuals. Using a massively parallel reporter assay data to validate our predictions, we observed that KMs have the most deleterious effect on enhancer activity; by contrast, RSs have a smaller effect in increasing enhancer activity. By applying our framework to lymphoblastoid cell lines, we found that KMs underlie differential binding of transcription factors and differential local chromatin accessibility. The eQTLs associated with the tissue-specific genes are strongly enriched in fragile KM positions, which are likely to be the essential positions disturbing biological activity once mutated. In summary, we conclude that the fragile KMs have the greatest impact on the level of gene expression and are likely to be the causal variants of tissue-specific gene expression and disease predisposition.

P-35

FIRST LONGITUDINAL EPIGENOME-WIDE ASSOCIATION STUDY OF PRE- AND POST-HIV INFECTED SUBJECTS

Limou, S.[1], Nelson, G.W.[2], Binns-Roemer, E.[1], Li, J.[2], Elloumi, F.[2], Goedert, J.J.[3], and Winkler, C.A.[1]
[1]Basic Research Laboratory, Basic Science Program and [2]Center for Cancer Research Informatics Core, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, MD; [3]Infections and Immunoepidemiology Branch, Division of Cancer Epidemiology and Genetics, NCI, Bethesda, MD

For nearly 25 years, extensive genetic and genomic association studies have revealed essential host factors for HIV control and disease progression, which notably led to the development of a new class of antiretroviral inhibitors (*CCR5-D32* association and CCR5 antagonists). Overall, the identified associations account for ~20% of the phenotypic variance suggesting that other factors are yet to be discovered. Epigenetic mechanisms are key regulators of gene expression that are not coded by DNA primary sequence and can impact complex diseases. Here, we evaluated for the first time whether HIV-1 infection modifies the host epigenome DNA methylation patterns. For that, we recruited 19 untreated HIV-infected individuals from the DC Gay cohort with longitudinal follow-up and PBMC samples available from pre-infection, early post-infection (<12 months), post-infection during clinical latency and post-infection at or near the inflection point. Following DNA and RNA extraction from the 80 PBMC samples, DNA was bisulfite-converted and genotyped with the Illumina Infinium Human Methylation 450 arrays, covering over 485,000 methylation sites across the genome. After normalizing the data, we compared the DNA methylation profiles of pre-infection *vs.* post-infection samples adjusting for batch effect, age, stage of infection and cell composition. Our preliminary analysis revealed that host genome DNA methylation profile is impacted by HIV-1 infection and highlighted several significantly differentially methylated sites ($P<10^{-7}$, FDR<0.05). Most genes where these differentially methylated sites are located have an immune-related function or were previously shown to interact with HIV-1 proteins (*MX1*, *TNFAIP8*, *PARP9*, and *IFI44L* genes in the top 5 hits). In conclusion, we have established a unique collection of samples representing pre-infection and post-infection timepoints, which allows for detection of DNA methylation changes within an individual and between individuals following HIV infection and during the HIV-1 infection course. This first epigenome-wide association study conducted in HIV-infected subjects has identified targets of epigenetic modifications by HIV. Our report therefore indicates that the exploration of host epigenetic mechanisms opens a new promising avenue for discovery of critical host factors interacting with the virus that might be leveraged for translation to drug or vaccine development.

P-36

EPIGENTIC UPREGULATION OF METALLOTHIONEIN 2A BY DIALLYL TRISSULFIDE ENHANCES CHEMOTHERAPUTIC SENSITIVITY OF HUMAN GASTRIC CANCER CELLS TO DOCETAXEL THROUGH ATTENUATING NF-KB ACTIVATION

Lin, S.[1], Pan, Y.[2], Xing, R.[2], Cui, J.[2], Li, W.[2], Gao, J.[4], Shen, L.[4], Wang, J.M.[3], Huang, J.[1,3], and Lu, Y.[2]

[1]College of Life Sciences & Bioengineering, School of Science, Beijing Jiaotong University, Beijing, China; [2]Laboratory of Molecular Oncology, Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education), Peking University Cancer Hospital /Institute, Beijing, China; [3]Laboratory of Molecular Immunoregulation, Cancer and Inflammation Program, Center for Cancer Research, NCI, NIH, Frederick, MD; [4]Key laboratory of Carcinogenesis and Translational Research (Ministry of Education), Department of GI Oncology, Peking University School of Oncology, Peking Cancer Hospital, Beijing, China

Aims: MT2A and NF-κB are both involved in carcinogenesis and chemosensitivity. We previously showed decreased expression of metallothionein 2A (MT2A) and IκB-α associated with poor prognosis of GC patients. The present study investigated the effect of diallyl trisulfide (DATS), a garlic-derived compound, and docetaxel, on regulation and function of MT2A in relation to NF-κB in GC cells.

Results: DATS attenuated NF-κB signaling in GC cells, resulting in G2/M cell cycle arrest and apoptosis, which culminated in the inhibition of cell proliferation and tumorigenesis in nude mice. The anti-GC effect of DATS was attributable to its capacity to mediate epigenetic upregulation of MT2A, which, in turn, enhanced transcriptional activity of IκB-α to suppress NF-κB activation in GC cells. Combination of DATS with docetaxel exhibited a synergistic anti-GC activity accompanied by MT2A upregulation and NF-κB inactivation. Histopathologic analysis of GC specimens from patients showed a significant increase in MT2A expression following docetaxel treatment. GC Patients with high MT2A expression at posttreatment showed significantly improved response to chemotherapy and prolonged survival compared with those with low MT2A expression in tumor.

Innovation and Conclusion: We conclude that DATS exerts its anti-GC activity and enhances chemosensitivity of GC to docetaxel by epigenetic upregulation of MT2A to attenuate NF-κB signaling. Our findings delineate a mechanistic basis of MT2A/NF-κB signaling for DATS- and docetaxel-mediated anti-GC effect, suggesting that MT2A may be a chemosensitivity indicator in GC patients receiving docetaxel-based treatment, and a promising target for effective treatment of GC by combination of DATS and DOC.

P-37

THE CHROMOSOME PASSENGER COMPLEX IS CONVERTED FROM A DESTROYER TO A PROTECTOR OF COHESION BY RECRUITMENT OF SGO1

Liu, L.[1], Kestner, C.A.[1], Sarkeshik, A.[2], Yates III, J.R.[2], Foltz, D.R.[1], and Stukenberg, P.T.[1]
[1]Department of Biochemistry and Molecular Genetics, University of Virginia, School of Medicine, Charlottesville, VA; [2]Department of Chemical Physiology, The Scripps Research Institute, La Jolla, CA

The Chromosome Passenger Complex (CPC) removes cohesion from chromosome arms but is enriched at centromeres where cohesion is maintained through metaphase. We identified the cohesion regulator RBMX in biochemical purifications of CPC bound to mitotic chromatin. Depletion of RBMX mislocalized CPC, Bub1, Sgo1 and two histone marks (H2ApT120 and H3pT3) that are required to localize CPC from centromeres. Each of these events was rescued by targeting Aurora-B to centromeres. The premature sister chromatid separation caused by depletion of RBMX was rescued by targeting Aurora-B to centromeres. Aurora-B not only removes cohesion from the arms but we found it also protected centromeric cohesion. Targeting Sgo1 to centromere partially rescued the loss of centromeric cohesion seen in cells depleted of Aurora B activity and Aurora-B regulated the recruitment of Sgo1 to centromeres in a chromosome autonomous manner. Together these results reveal that RBMX recruits the CPC to centromeres where Aurora-B protects centromeric cohesion by regulating centromeric levels of Sgo1. We provide a model based on self-organizing principles for how the Sgo1 protein converts the CPC from a protein

P-38

ANALYSIS OF CONTROL OF METHIONINE METABOLISM BY THE SIN3 COREPRESSOR

Liu, M. and Pile, L.A.
Department of Biological Sciences, Wayne State University, Detroit, MI

SIN3 controls histone acetylation through association with the RPD3 histone deacetylase. Our laboratory has found that a histone H3K4 demethylase named LID also co-immunoprecipitates with SIN3. These results indicate that SIN3 may regulate histone methylation in addition to acetylation. To test this possibility, we analyzed global histone methylation levels in S2 cells. Loss of SIN3 led to a small increase in H3K4me3 levels. In cells with already compromised histone methylation, altering SIN3 expression led to a larger effect in regulation of the level of this mark. These data support earlier findings from our laboratory and others suggesting that SIN3 controls histone methylation. SAM synthetase (SAM-S) generates the major methyl donor SAM. To understand how SIN3 affects this modification, we performed differential gene expression analysis in *Sin3A* knockdown, *Sam-S* knockdown and *Sin3A+Sam-S* knockdown cells by RNA-seq. We found that expression of some methionine metabolic genes, but not major histone methyltransferases or demethylases, was regulated by SIN3. To explore whether SIN3 regulates methionine metabolic intermediates, we measured the amount of metabolites in RNAi-treated cells. We observed that loss of SIN3 led to increased SAM levels. Taken together, these interesting data indicate that SIN3 regulates histone methylation via influencing levels of the methionine metabolic enzymes to control SAM levels. To further investigate how SIN3 regulates the methionine pathway, we are measuring histone modification levels at methionine metabolic genes in RNAi-treated cells by ChIP-qPCR. Results from these analyses are anticipated to provide insight into the cross-talk between histone acetylation and methylation as well as the connection between metabolism and epigenetics.

P-39

EPIGENTIC MODULATION OF A miR-296-5p:HMGA1 AXIS REGULATES THE GLIOBLASTOMA STEM CELL PHENOTYPE

Lopez-Bertoni, H.[1,2], Lal, B.[1,2], Michelson, N.[1], Li, Y.[1,2], and Laterra, J.[1,2,3,4]
[1]Hugo W. Moser Research Institute at Kennedy Krieger, Baltimore, MD; Departments of [2]Neurology, [3]Neuroscience, and [4]Oncology, Johns Hopkins School of Medicine, Baltimore, MD

The long term goal of our research is to understand the cellular and molecular mechanisms that regulate brain tumor malignancy. Cell subsets isolated from glioblastoma (GBM) have the capacity to grow as spheres (*i.e*. neurospheres) and efficiently propagate tumors in xenograft models, reflecting a stem-like, self-renewing and tumor-propagating phenotype. Furthermore, GBM stem-like cells have been shown to maintain tumor growth, contribute to resistance, and there is strong evidence suggesting their role in tumor recurrence. Characterizing the basic mechanisms driving this phenotype will allow us to identify new therapeutic targets and treatment strategies. Cell fate determination and phenotype reprogramming are complex and dynamic processes that involve epigenetic mechanisms and differentially regulated coding and non-coding RNAs. We recently showed that the coordinated actions of Oct4 and Sox2 induce a tumor-propagating stem-like state in GBM cells via a mechanism involving direct DNMT promoter transactivation, DNA methylation, and methylation-dependent repression of multiple miRNAs. However, how specific epigenetic changes contribute to the acquisition and/or maintenance of the cancer stem-like phenotype remains unclear. This study focuses on understanding the cross-talk between specific DNA methylation events, miRNA expression and regulation, and the GBM-propagating stem cell phenotype. We show that miR-296-5p expression is repressed in a DNA methylation-dependent manner under conditions that promote GBM cell stemness and that forced expression of miR-296-5p inhibits self-renewal capacity of GBM neurosphere cells *in vitro* and the growth of neurosphere-derived glioma xenografts *in vivo.* Additionally, we identify the chromatin remodeling protein HMGA1 as downstream effector of miR-296-5p action in the context of GBM stem-like cells. These results show that miR-296-5p regulate chromatin architecture by targeting HMGA1 and that the miR-296-5p:HMGA1 axis as a novel regulator of the GBM stem cell phenotype and a candidate pathway for targeted therapies directed at depleting tumors of their tumor-propagating stem cell subsets.

P-40

DEFINING THE ACTIVE REGULATORY ELEMENT LANDSCAPE UPON ACTIVATION OF B CELLS

Mathé, E., Kieffer-Kwon, K., Nelson, S., Nimura, K., and Casellas R.
Laboratory of Molecular Immunogenomics, NIAMS, NIH, Bethesda, MD

Transcriptional regulation involves a complex interplay between regulatory elements (REs), such as promoters and enhancers, that carry unique combinations of histone marks, DNA methylation, and transcription factors. Recently, by comparing the RE landscape in embryonic stem cells and activated B cells, we demonstrated that the enhancer landscape varies greatly across tissues, even for broadly transcribed genes. The underlying features of REs upon activation of B lymphocytes, including histone modifications, methylation, and transcription factor binding, is yet to be characterized.

In this study, we used DNaseI-seq to define the location of REs in resting and activated B cells. To further complete the map of the active regulatory landscape in each cell type, we overlaid whole genome single-base resolution methylation, histone modifications, ChIP-seq of lymphocyte-related transcription factors, and digital footprinting onto the REs. The function of these REs was assessed by measuring levels of transcriptionally engaged PolII using GRO-seq. By focusing on REs that are gained upon B cell activation, we are able to better understand when and how the overall increase in gene expression occurs.

Of particular interest, we observe that the activity in enhancers, as measured by H3K27Ac, is increased upon activation. This observation suggests that the gain of enhancers is contributing to the transcriptome amplification observed upon B cell activation. Further characterization of these enhancers using transcription factor recruitment and methylation will be presented here.

P-41

THE ROLE OF THE LINKER HISTONE IN GLUCOCORTICOID RECEPTOR TRANSCRIPTIONAL ACTIVATION

Milliman, E.J.[1], Ward, J.[2], and Archer, T.K.[1]
[1]Chromatin Gene Expression Group and [2]Integrative Bioinformatics Group, Epigenetics and Stem Cell Biology Laboratory, NIEHS, NIH, Durham, NC

Nuclear Hormone Receptors (NHR) are a large family of ligand-dependent transcription factors. Upon activation of NHRs by ligand binding, NHRs bind to sequence-specific DNA elements, called hormone response elements (HRE). NHRs act as a nucleation point for the recruitment of a myriad of co-activators complexes. The recruitment of these complexes remodel the local chromatin environment allowing productive transcription. The linker histone (histone H1) is a small basic protein that resides at the entry/exit points of DNA wrapped around the core histone particle. Histone H1 helps to stabilize nucleosome positions by restricting their movement on the DNA. Traditionally linker histones are regarded as a repressive protein to transcription. We have found that depletion of the histone H1 variant H1.4 allows for faster activation of Glucocorticoid Receptor (GR) target genes by qPCR. Yet, there is no effect on the repression of GR-repressed target genes. We have now set out to address the specific roles of histone H1.4 shaping chromatin architecture during hormone treatment. ChIP-seq of GR +/- depletion of H1.4 shows that 25-30% of binding sites are different between the two conditions after exposure to Dexamethasone (a GR agonist) for 1 hr. Furthermore, using the Assay for Transposase-Accessible Chromatin using Sequencing (ATAC-seq), we have assayed chromatin state with and without depletion of histone H1.4. Preliminary data suggests that depletion of H1.4 reduces the number of nucleosome free regions across the genome. Overall, our data suggest that H1.4 has a role in the transcriptional response by the glucocorticoid receptor and that this may occur at the level of GR binding site selection.

P-42

A HISTONE MODIFYING ENZYME GENE SIGNATURE IN LUNG TUMORS FROM AFRICAN AMERICANS

Mitchell, K.A., Zingone, A., and Ryan, B.M.
Laboratory of Human Carcinogenesis, Center for Cancer Research, NCI, NIH, Bethesda, MD

Background and Hypothesis: Lung cancer is the second most common cancer in the U.S. and the leading cause of cancer-related death. African Americans (AA) have the highest lung cancer incidence and mortality rates when compared to any other population. When controlling for access to care the disparity in mortality is dramatically reduced. However, the disparity in incidence persists and is driven primarily by differences between AA and European American (EA) men. Smoking is the strongest risk factor for lung cancer development. However, the prevalence of both smoking and heavy smoking is lower among AA. Although most AA choose menthol cigarettes, a recent study has shown menthol cigarette smokers have a lower incidence of lung cancer compared with their non-menthol cigarette smoking counterparts. In addition, a large study of never smokers demonstrated that AA still had a higher incidence of lung cancer. Combined, these data suggest that smoking alone does not primarily account for the observations seen in AA. These findings led us to consider other biological determinants of lung cancer disparities. We hypothesize the molecular and biological phenotype of lung cancer differs in AA compared to EA.

Study Design and Methods: The transcriptome of 66 matched tissues from the NCI-MD Case-Control Study were profiled using Affymetric Human Genome U133 Plus 2.0 Arrays. Differentially expressed genes were identified and validated using TCGA RNA-Seq data. Gene Set Enrichment Analysis and Connectivity Mapping were also carried out. Population-specific cell lines were used to perform preliminary *in vitro* screens to test *in silico* drug response predictions.

Results and Conclusions: Principal component analysis of gene expression data revealed tumor and non-involved tissues segregated independently in both AA and EA populations. Unsupervised hierarchical clustering supported this finding. Approximately 1,000 genes were differentially expressed in AA only. Genes upregulated in breast, prostate, and endometrial tumors from AA more than EA, were similarly upregulated in lung tumors from AA when compared to EA (e.g. *PSPHL*). Gene Set Enrichment Analysis highlighted different enrichment profiles in AA and EA. Specifically, histone modifying enzyme gene signatures were present in lung tumors and normal tissues from AA and not EA. Connectivity mapping analysis and Entinostat treatment strongly suggest that AA and EA lung cancers have differential susceptibility to a group of anti-cancer drugs termed HDAC inhibitors.

Relevance and Importance: By understanding the biological factors that contribute to cancer health disparities, steps can be made to reduce or limit the consequences of these disparities. This study provides evidence for a distinct tumor biology in AA and EA. This may allow for more precise diagnoses, improved drug efficacy with fewer side effects, and molecular targeting for responsive patients.

P-43

INTEGRATED ANALYSIS OF HIGH RESOLUTION DNA METHYLATION PROFILES IN DIFFERENTIATED MOUSE PRIMARY DERMAL KERATINOCYTES AND FIBROBLASTS

Chatterjee, R., Mukherjee, S., He, X., Tillo, D., and Vinson, C.
Laboratory of Metabolism, Center for Cancer Research, NCI, NIH, Bethesda, MD

Methyl CGs has been previously shown to produce DNA binding sites challenging the prevailing concept of methylation of CG islands as a gene silencer. Recent studies by other groups are following up with more compelling studies that evinces methylation can be associated with both reduced and increased expression of genes. We have previously generated genome-wide cytosine methylation maps at 91X and 36X coverage of newborn female mouse primary dermal fibroblasts and keratinocytes. We now present a high resolution genome wide DNA methylation map of female differentiated dermal fibroblasts (to adipocytes) and keratinocytes. This reveals increase in hypomethylated regions (HMRs) in differentiated keratinocytes (covering 2.91% of genome in undifferentiated to 3.21% in differentiated ones) while 2.58% regions of the genome being commonly shared, the differentiation specific HMRs were 0.63% of the genome (12,925 regions). HMRs were found to be decreased in fibroblasts when differentiated to adipocytes (2.15% of the genome in undifferentiated to 1.75% in differentiated), sharing 1.57% of common genomic regions whereas about 0.18% (8,710) regions being specific to differentiation which was about 3.5 fold lower compared to the differentiated keratinocytes. The data was also compared with mRNA-seq gene expression data.

Fibroblasts and keratinocytes has been an important cellular model for understanding the epithelial-mesenchymal transition particularly in understanding cancer progression. This high-resolution methylation map of both undifferentiated and differentiated primary dermal cells may serve as reference methylomes for future studies.

P-44

SPT6 INTERACTS WITH WHSC1 DURING TRANSCRIPTION ELONGATION IN INTERFERON-STIMULATED GENES

Nehru, V.[1], Sarai, N.[1], Nimura, K.[2], Debrosse, M.[1], Patel, M.C.[1], Tamura, T.[3], Ura, K.[2], and Ozato, K.[1]
[1]Program in Genomics of Differentiation, NICHD, NIH, Bethesda, MD; [2]Division of Gene Therapy Science, Osaka University Graduate School of Medicine, Osaka, Japan; [3]Graduate School of Medicine and Faculty of Medicine, Yokohama City University, Kanagawa, Japan

One of the pivotal steps in transcription regulation in the eukaryotes is the transcriptional elongation. In the past, many different factors involved in transcription elongation have been identified via traditional genetic and biochemical studies. However, recent advances in the genome-wide analysis and single-molecule technologies have changed our in-depth understanding of the importance and roles of these factors.

In the present study, we show that the histone methyltransferase WHSC1 (Wolf-Hirshhorn syndrome candidate 1, also known as NSD2 or MMSET) interacts with SPT6, and is necessary for SPT6 recruitment to interferon (IFN) stimulated genes (ISGs). The SPT6 (Suppressor of Ty6) is a histone chaperone specific for H3 and H4 and a transcription elongation factor, involved in the elongation process by binding to the elongating form of RNA polymerase II (Pol II). We investigated SPT6 recruitment in several ISGs in the mouse embryonic fibroblasts (MEFs) from the wild type (WT) and Whsc1-/- mice. Chromatin immunoprecipitation (ChIP) analysis revealed that SPT6 is rapidly recruited to ISGs in response to IFN stimulation. Occupancy of SPT6 on ISGs highly correlated with that of Pol II-2P. Interestingly, SPT6 recruitment to IFN-stimulated genes was strongly inhibited in Whsc1-/- MEF cells. Our data indicates that, WHSC1 recruits SPT6 to facilitate transcriptional elongation of IFN stimulated genes.

P-45

ADJUSTING INFINIUM METHYLATION PROFILES TO SUPPRESS SIGNALS FROM VARYING CELL PROPORTION

Nelson, G.W.[1], Binns, E.[2], Limou, S.[2], and Winkler, C.A.[2]
[1]Basic Science Program-Center for Cancer Research Genetics Core, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, MD; [2]Basic Research Laboratory, Basic Research Program, Center for Cancer Research, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, MD

Methylation data ideally are generated from a single cell type, but often for practical reasons a mixture of cells is used. A well-studied case involves the use of mixed peripheral blood mononuclear cells (PBMCs). The R package minfi includes a function estimate CellCounts—implementing an algorithm developed by Housman et al.[1]—that takes a methylation profile from mixed blood cells and returns estimates of the frequencies of common blood cell types in the sample. These estimates are then available for use as confounding covariates in analyses of methylation changes.

However, adjustment for cell composition as a confounding factor may not always be a useful strategy. In the case of HIV infection, one of the strongest markers of disease progression is the loss of CD4+ T cells. This change of cell composition will have a strong tendency to confound the analysis due to a loss of methylation markers characteristic of these cells. But also since CD4 count variation will be a primary component of cell composition variation, it will be a dominant component of the correction for cell composition, so this correction will tend to hide methylation changes associated with disease.

We therefore propose and construct an alternate correction strategy. The minfi package analysis is based on an extensive Infinium data set from multiple blood cell types. Given the estimated cell composition for each sample, we subtract the cell profile methylation markers from each sample, proportional to the presence of that cell type in the mixed sample. This removes the cell marker variation that potentially confounds the association analysis due to varying cell frequencies. With confounding signals of cell type removed, we can omit correcting for cell composition in the association analysis. Assuming that the methylation markers of disease progression are different from the cell type markers (if they are not there is no hope of observing them, with mixed cell samples, in any case) we may observe the change of these markers with AIDS disease progression, and may use CD4 composition as a measure of this progression.

1) Houseman, Eugene A., et al. "DNA methylation arrays as surrogate measures of cell mixture distribution." BMC bioinformatics 13.1 (2012): 86.

P-46

OPPOSITE EFFECTS OF HISTONE H1 AND HMGN5 PROTEIN ON DISTANT COMMUNICATION IN CHROMATIN

Nizovtseva, E.V.[1,2,*], Polikanov, Y.S.[2,3,*], Kulaeva, O.I.[1,4], Clouvelin, N.[5], Postnikov, Y.[6], Dimitrov, S.[7], Bustin, M.[6], Olson, W.[5], and Studitsky, V.M.[1,2,8]
[1]Department of Pharmacology, Robert Wood Johnson Medical School, Rutgers, the State University of New Jersey, Piscataway, NJ; [2]Cancer Epigenetics Program, Fox Chase Cancer Center, Philadelphia, PA; [3]Present address: Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT; [4]School of Biology, Moscow State University, Russia; [5]Department of Chemistry and Chemical Biology, BioMaPS Institute for Quantitative Biology, Rutgers, the State University of New Jersey, Piscataway, NJ; [6]Laboratory of Metabolism, Center for Cancer Research, NCI, NIH, Bethesda, MD; [7]Institut National de la Santé et de la Recherche Médicale, Université Joseph Fourier, Grenoble, France; [8]Laboratory of Epigenetics, Institute of Gene Biology, Russian Academy of Sciences, Moscow, Russia. [*]These authors contributed equally to this work.

Transcriptional enhancers in the cell nuclei can interact with the target promoters *in cis* over long stretches of chromatin, but the mechanism of this communication remains unknown. Previously we have developed a defined *in vitro* system for quantitative analysis of the rate of distant enhancer-promoter communication (EPC) and have shown that the chromatin fibers maintain efficient EPC over large distances *in cis*. Here we show that all core histone "tails" contribute to efficient EPC. In contrast, the presence of linker histone H1 results in less efficient EPC; both N-terminal and C-terminal domains of H1 contribute to the inhibition. The negative effect of histone H1 on EPC is counteracted by the HMGN5 protein. Our work suggests that the chromatin fiber is a highly dynamic, efficient communication device that can maintain a high level of DNA compaction during EPC. We have uncovered the mechanisms of highly efficient EPC in chromatin and identified the likely key players (such as histone N-terminal tails and possibly their modifications, linker histones and HMGN5 protein) that strongly affect the rate of EPC in chromatin. More generally, our data suggest that EPC could constitute a novel regulated step during gene expression in eukaryotes

P-47

TOPOLOGICAL POLYMORPHISM OF TWO-START NUCLEOSOME FIBERS AND THE MECHANISM OF GENE LOOPING

Norouzi, D. and Zhurkin, V.B.
Laboratory of Cell Biology, Center for Cancer Research, NCI, NIH, Bethesda, MD

The spatial organization of nucleosomes in 30-nm fibers remains unknown in detail. To address this issue, we analyzed all stereochemically possible configurations of two-start nucleosome fibers with short DNA linkers L = 10 - 50 bp (nucleosome repeat length NRL = 157 - 197 bp). Four superhelical parameters – inclination of nucleosomes, twist, rise and diameter – uniquely describe a uniform symmetric fiber. The energy of a fiber is defined as the sum of four terms: elastic energy of the linker DNA, steric repulsion, electrostatics and a phenomenological (H4 tail - acidic patch) interaction between two stacked nucleosomes. By optimizing the fiber energy with respect to the superhelical parameters, we found two types of topological transition in fibers. The first transition is characterized by change in the DNA linking number, $\Delta Lk = 1$, and the second one by $\Delta Lk = 2$. To the best of our knowledge, this topological polymorphism of the two-start fibers was not reported in the computations published earlier. Importantly, the optimal configurations of the fibers with linkers L = 10n and 10n+5 bp are topologically different. Our results are consistent with experimental observations, such as the inclination 60-70° (the angle between the nucleosomal disks and the fiber axis), helical rise, diameter and left-handedness of the fibers. In addition, we make several testable predictions, among them existence of different degree of DNA supercoiling in the fibers with L = 10n and 10n+5 bp, different flexibility of the two types of fibers, and a correlation between the local NRL and the level of transcription in different parts of the yeast genome. We argue that our results are directly related to the mechanism of gene regulation by means of the formation of enhancer or gene loops proposed in the literature. We have developed a multi-scale computer model which enables us to simulate arbitrary arrays of nucleosomes in the presence of enhancers, topoisomerases, and other type of chromatin remodeling processes.

P-48

DYNAMIC INTERPLAY BETWEEN ISW1, ISW2, CHD1, AND RSC REMODELERS DETERMINES NUCLEOSOME SPACING AND PHASING IN YEAST

Ocampo, J., Chereji, R.V., Eriksson, P.R., and Clark, D.J.
Program in Genomics of Differentiation, NICHD, NIH, Bethesda, MD

Genome-wide nucleosome maps for yeast have revealed that nucleosomes are regularly spaced and show a global phasing relative to the transcription start site (TSS). In addition, most genes have a nucleosome-depleted region (NDR) at the promoter. We have addressed the roles of four different chromatin remodeling complexes in nucleosome organization *in vivo*: ISW1, ISW2, CHD1 and the essential RSC complex. We constructed strains with the essential *RSC8* gene under the control of the *GAL* promoter and *isw1, isw2* or *chd1* null mutations in all possible combinations in the same genetic background. In the absence of RSC we confirmed that all the nucleosomes shift towards the TSS with consequent narrowing and filling in of the NDR with no change in nucleosome spacing, which is maintained at ~165 bp. Others have shown that the combined action of ISW1 and CHD1 is required to maintain nucleosome phasing. Here, we confirm this observation and show that nucleosome spacing in the *isw1* mutant is reduced on average by 5 bp, to ~160 bp whereas the *chd1* mutant shows little change. A more detailed analysis revealed that the absence of Isw1 or Chd1 has different effects on distinct groups of genes. In contrast, the *isw2* mutant does not show any obvious changes in global chromatin structure but there are effects on a small group of genes. The chromatin organization in the multiple mutants agree with the effects observed in the single mutants, indicating that these remodeling complexes have specific functions in chromatin organization. We propose that RSC determines the position of the +1 nucleosome, which is then used as a reference nucleosome by CHD1 and ISW1 to build nucleosomal arrays on genes.

P-49

SENP2 ASSOCIATION WITH INTRACELLULAR MEMBRANES

Odeh, H.M. and Matunis, M.J
Department of Biochemistry and Molecular Biology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD

SUMOylation regulates a wide range of essential cellular functions, many of which are associated with activities in the nucleus. There is also emerging evidence for the involvement of SUMO at intracellular membranes, however, the functional significance of SUMOylation and its regulation at membranes remain largely unexplored. To study the role of SUMO regulation at intracellular membranes, our lab focuses on characterizing the specific location and functions of SUMO proteases, collectively known as SENPs. We hypothesize that the association of SUMO proteases with intracellular membranes specifically regulates the dynamic SUMOylation of membrane-associated proteins. Consistent with this hypothesis, we have discovered that SENP2, one of the six SUMO proteases present in mammalian cells, uniquely binds to intracellular membranes. Using immunofluorescence microscopy, we initially observed that SENP2 colocalizes with the nuclear lamina, suggesting an association with the inner nuclear membrane. Through primary sequence analysis and secondary structural predictions we discovered that SENP2 has an N-terminal amphipathic alpha helix with the potential to function as an in-plane membrane anchor. Mutational analyses followed by localization and subcellular fractionation studies showed that the amphipathic helix promotes SENP2-membrane association. Analysis of specific SENP2 mutants revealed that the amphipathic helix is able to target SENP2 not only to the inner nuclear membrane, but also to ER and golgi membranes. Consistent with our findings, SENP2 was found to interact with a subset of nuclear and cytoplasmic membrane-associated proteins using mass spectrometry-based approaches. Collectively, our results indicate that SENP2 interacts directly with intracellular membranes and has the potential to regulate the SUMOylation of a wide-range of membrane-associated proteins. Further characterization of the functions and regulation of SUMOylation of membrane-associated proteins will contribute to our understanding of membrane biology and the advancement of therapeutics for a variety of associated diseases.

P-50

POLYCOMB AND TRITHORAX COMPLEXES CONTROL EPIGENETIC MEMORY OF T HELPER CELLS

Onodera, A., Hirahara, K., and Nakayama, T.
Department of Immunology, Graduate School of Medicine, Chiba University, Japan

Immune system is regulated not only by immune system-specific pathways but also by general cellular memory mechanisms such as histone modifications and DNA methylation. We focused on Trithorax (TrxG) and Polycomb (PcG) complexes, which are known as active and repressive epigenetic regulators, respectively, and investigated their roles in T helper (Th) cell function. First, we found that a TrxG complex component Menin was required for the long-term maintenance of the expression of the majority of Th2-specific genes in developed Th2 cells whereas it was dispensable for Th1 and Th2 cell differentiation. Menin was indispensable for Th17 cell differentiation as it controls IL-17A expression via binding to the Il17a gene locus and recruiting the RNA polymerase II (RNAPII). In contrast to the TrxG complexes, inactivation of Ezh2, a member of PcG complexes, was found to specifically enhance Th1 and Th2 cell differentiation and plasticity. Ezh2 directly bound and facilitated correct expression of Tbx21 and Gata3 in differentiating Th1 and Th2 cells, accompanied by substantial trimethylation at H3K27me3. We also performed *in vivo* analysis using mouse asthma models and revealed that airway inflammation was positively and negatively regulated by TrxG and PcG proteins, respectively. Finally, using ChIP-seq analysis, we found that different combinations of Ezh2 and Menin occupancy were associated with expression of specific functional gene groups important for T cell development. Functional relevance of Ezh2 and Menin co-occupancy in ES cells seemed to be different from that in T cells. In summary, our study demonstrates that TrxG and PcG proteins orchestrates the cell differentiation and function by regulating both the induction and maintenance of target gene expression.

P-51

BRG1 CONTROLS THE EXPRESSION OF PAX7 TO PROMOTE VIABILITY AND PROLIFERATION OF MOUSE PRIMARY MYOBLASTS

Padilla-Benavides, T., Nasipak, B.T., and Imbalzano, A.N.
Department of Cell and Developmental Biology, University of Massachusetts Medical School, Worcester, MA

Brg1 is the catalytic component of the evolutionarily conserved SWI/SNF ATP-dependent chromatin remodeling enzymes that are required to disrupt histone-DNA contacts on the nucleosome. SWI/SNF enzymes play key roles in regulating the balance between differentiation and proliferation. As such, Brg1 has been shown to behave as a repressor or activator of cell proliferation. While the role of the SWI/SNF complex in the differentiation of different cell lineages has been extensively studied, its role in proliferation and cell survival is not as well understood. Muscle satellite cells constitute the stem cell pool that sustains and regenerates myofibers in adult skeletal muscle. In this work we showed that deletion of Brg1 in primary mouse muscle satellite cells cultured *ex vivo* leads to a cell proliferation defect and apoptosis. We postulated that Brg1 regulates the progression of cell cycle and cell survival by activating the transcription and controlling chromatin remodeling processes at the Pax7 promotor, which is expressed during somite development and is required for controlling viability of the satellite cell population. Significantly, reintroduction of catalytically active Brg1 or Pax7 into Brg1-deficient satellite cells was sufficient to reverse the apoptotic phenotype and restore proliferative capability. These data demonstrate that Brg1 functions as a positive regulator for cellular proliferation and survival of muscle satellite cells. Therefore the regulation of gene expression through Brg1-mediated chromatin remodeling is critical not just for skeletal muscle differentiation but for myoblast cell cycle progression as well.

P-52

INTERACTION OF FANCONI ANEMIA (FA) PROTIEN FANCD2 WITH GENOMIC INTERSTRAND CROSSLINKS (ICLs)

Paramasivam, M.[1], Muniandy P.A.[2], Wang, W.[3], and Seidman, M.M.[1]
[1]Laboratory of Molecular Gerontology, NIA, NIH, Baltimore, MD; [2]University of Maryland, School of Medicine, Baltimore, MD; [3]Laboratory of Genetics, NIA, NIH, Baltimore, MD

Objective: Identification of DNA Damage Response (DDR) partners for ICL proximal and distal recruitment of FANCD2.

Methods: Laser/confocal microscopy to localize antigen tagged psoralen ICLs in the genome of living cells; the proximity ligation assay (PLA) to identify interactions between FANCD2, DDR proteins, and ICLs.

DNA adducts can trigger the DDR, in which many proteins are recruited to genomic chromatin in the vicinity of the damage and the same protein may have multiple partners and functions. The proteins include those engaged in repair, which are proximal to the lesion, while those involved in other pathways may be some distance from the damage. Currently it is not possible to distinguish lesion proximal from distal proteins. FA cells are hypersensitive to agents that form ICLs, and FA proteins are involved in the DDR following replication stress. The monoubiquitinated FA protein FANCD2 plays an important role in ICL repair during replication. We synthesized psoralens linked to the immunotag digoxigenin (Dig), and detected, by immunostaining of the tag, laser localized psoralen ICLs. We monitored the recruitment of DDR proteins to the ICLs and unexpectedly found that FANCD2 was a component of the DDR, and was important for ICL repair, in all phases of the cell cycle. By co immunoprecipitation and PLA we found novel interactions between FANCD2 and DDR proteins. We identified those protein partners important for ICL repair, used PLA to denote the partners required for recruitment of ICL proximal and distal FANCD2, and thus identified the fraction of FANCD2 involved in ICL repair.

P-53

TRANSCRIPTIONAL REPRESSOR ETO2 REGULATES ERYTHROPOIESIS AND HUMAN HEMOGLOBIN SWITCHING

Plank, J., Soucy, J., Dale, R., and Dean, A.
Laboratory of Cellular and Developmental Biology, NIDDK, NIH, Bethesda, MD

Mammalian erythropoiesis is coordinated by a series of sequential events and precise regulation of a gene expression program. The Ldb1 complex, consisting of transcription factors Tal1 and Gata1, the Lim domain protein Lmo2, and bridging protein Ldb1, regulates expression of key genes during erythropoiesis. The transcriptional co-repressor Eto2 has been shown to associate with the Ldb1 complex to inhibit transcription of target genes. Mice lacking Eto2 exhibit decreased proliferation of hematopoietic stem cells and defective murine T-cell development, however, the role of Eto2 in erythropoiesis is unclear. We find an expansion of immature erythroid cells from the murine fetal liver at the expense of mature erythrocytes in *Eto2* null animals. Similarly, human erythroid progenitor cells lacking Eto2 exhibit impaired erythroid differentiation. We also sought to characterize the role of Eto2 in human hemoglobin switching, the transition from expression of the fetal b-globin gene (g) to expression of the adult b-globin gene (b), which is of clinical importance for developing therapies for b hemoglobinopathies. We established a model of human hemoglobin switching using differentiating umbilical cord blood cells. These cells initially expression g and "switch" to predominately express b. Using this model combined with shRNA depletion of Eto2, we demonstrate that Eto2 is a negative regulator of both g expression and hemoglobin switching. Finally, we show that Eto2 target genes have increased H3 acetylation in the absence of Eto2, suggesting that Eto2 regulates target gene expression through recruitment of histone deacetylases. Together, our data demonstrate that Eto2 is required for normal erythropoiesis in murine and human cells.

P-54

POLO-LIKE KINASE 1 (PLK-1) IS REQUIRED FOR NUCLEAR ENVELOPE REMODELING DURING C. ELEGANS EARLY EMBRYONIC DIVISIONS

Rahman, M.M., Lee, B., and Cohen-Fix, O.
Cell Cycle Regulation & Nuclear Structure Section, NIDDK, NIH, Bethesda, MD

Animal cells undergo open mitosis, where nuclear envelope breakdown (NEBD) allows microtubules emanating from centrosomes to connect to the duplicated chromosomes. At the end of mitosis, the nuclear envelope reforms around the segregated chromosomes, generating a single nucleus in each daughter cell. Polo-like kinase 1 (PLK-1) is a conserved kinase involved in multiple steps of mitosis and meiosis. However, a critical role in NEBD has yet to be shown. In *C. elegans,* complete inactivation of *plk-1* gene function leads to a failure in meiotic progression. However, we observed that a partial inactivation of *plk-1*, using a temperature sensitive (ts) mutant allele grown at a semi-permissive temperature, led to the appearance of embryos with a pair of nuclei in each cell. In these embryos, both nuclear pore complexes (NPC) and the nuclear lamina persisted around the chromatin throughout mitosis, suggesting a defect in NEBD. Normally, following fertilization, the maternal and paternal pronuclei meet, their nuclear envelopes disassemble and their chromosomes align on a single metaphase plate. In *plk-1* ts embryos, however, after fertilization the nuclear envelopes of the maternal and paternal nuclei failed to disassemble completely, forming two distinct nuclei attached to each other. Interestingly, failure in NEBD did not prevent progression through mitosis in early embryonic cells. Rather, the maternal and paternal nuclei underwent separate mitoses, creating daughter cells with paired nuclei. We hypothesized that the defect in NEBD in *plk-1* cells is due to defect in disassembly of one of more NE components. If that were the case, then a reduction in NE components by RNAi would facilitate NEBD and re-establish the formation of a single nucleus in *plk-1* cells. Indeed, we found that a reduction in certain NE components, such as, Nup98, Nup107, Nup35, suppressed the paired-nuclei phenotype of *plk-1* cells. Whether these are direct PLK-1 phosphorylation targets is under investigation. This study is the first demonstration of an involvement of PLK-1 in NEBD in an intact organism.

P-55

IN VIVO STOICHIOMETRY AND ASSEMBLY OF POLYCOMB REPRESSIVE COMPLEX 1 ON CHROMATIN REVEALED BY SINGLE-MOLECULE IMAGING

Tatavosian, R. and Ren, X.
Department of Chemistry, University of Colorado, Denver, CO

Chromatin in eukaryotes is the convergent platform of controlling gene expression. The organization of chromatin is mediated by genetic factors and epigenetic regulators. Polycomb repressive complex 1 (PRC1) is epigenetic complex of transcription regulatory that represses gene expression by acting on chromatin. There is little mechanistic insight into how PRC1 is assembled on native nucleosomes. Here we investigate the assembly of PRC1 on native nucleosomes by using single molecule fluorescence TIRF microscopy. In this procedure, PRC1 subunit fused with fluorescence protein was stably and inducibly expressed in ES cell line lack of PRC1 gene. Native PRC1-nucleosome complexes were purified from these cell lines and immobilized on the coverslip with antibody against either histone protein or PRC1 subunit. The quantity of the PRC1 proteins on native mononucleosome was counted at the single molecular level by using TIRF microscopy. Our results indicate that the number of PRC1 complex on native mononucleosome does not correspond to the number of histone tails of mononucleosome. We also reveal that differentiation of ES cells induces alteration of density of PRC1 on nucleosomes. Thus, our findings provide new insights to mechanisms of how PRC1 complexes act on chromatins.

P-56

CAJAL BODIES SHAPE GENOME CONFORMATION

Sawyer, I.A.[1,2], Sung, M-H.[2], Sturgill, D.M.[2], Wang Q.[1,2], Shevstov, S.P.[1], Pegoraro, G.[3], Hager, G.[2], and Dundr, M.[1]
[1]Department of Cell Biology, Rosalind Franklin University of Medicine & Science, North Chicago, IL; [2]Laboratory of Receptor Biology and Gene Expression and [3]High-Throughput Imaging Facility, Center for Cancer Research, NCI, NIH, Bethesda, MD

Prevailing thoughts regarding the nuclear domain known as the Cajal body (CB) propose a reactionary, passive region which catalyzes the biogenesis of small nucle(o)lar RNAs (sn(o)RNAs) and recycling of essential spliceosomal components after splicing. This domain, which is primarily found in aneuploid transformed cells, is known to specifically form at sites of small RNA transcription and has been observed to interact with several classes of snRNA genes. We hypothesized that CBs may cluster small RNA genes from several chromosomes simultaneously, as well as histone genes through the physically associated Histone locus body, providing an optimized environment for target RNA production and spliceosome assembly. Here, we examined the non-random positioning of CBs, major nuclear bodies involved in efficient spliceosomal assembly, and their role in human genome organization.

We find that CBs are predominantly located at the periphery of chromosome territories at the interface of multiple chromosomes. Genome-wide chromatin conformation capture analysis (4C-seq) revealed that CB-associated regions are enriched in highly expressed genes, including histone loci, and all categories of small U RNA loci. CB-associated genes form inter-chromosomal gene clusters in the 3D space. Analysis of the CB associated with chromosome 1 showed that the CB is necessary for configuring the entire chromosome into a rosette-like structure which envelopes the CB. RNAi-mediated disassembly of CBs leads to a substantial disruption of the CB-targeting gene clusters and widespread suppression of small U RNA and histone genes. We also observed discrete changes in gene expression and global increase in splicing noise even outside of the CB-proximal genomic regions.

We conclude that CBs are not passive byproducts of specific gene activity, but play an active role in organizing the genome in 3D, likely to accelerate snRNA processing steps but also to influence the expression and splicing of RNA pol II-driven genes in Cajal body proximal regions.

P-57

SYSTEMATIC IDENTIFICATION OF GENOME POSITIONING FACTORS BY HIGH-THROUGHPUT IMAGING SCREENING

Shachar, S.[1], Voss, T.C.[2], Pegoraro, G.[2], Sciascia, N.[1], and Misteli, T.[1]
[1]Laboratory of Receptor Biology and Gene Expression and [2]High-Throughput Imaging Facility, Center for Cancer Research, NCI, NIH, Bethesda, MD

Genomes are non-randomly arranged in the 3D space of the cell nucleus. The location and local environment of a genomic locus affects its transcription efficiency, replication timing and repair. The molecular nature of the cellular factors that establish and maintain the 3D location of gene loci are largely unknown. Here we have developed a high-precision, high-throughput automated fluorescent in situ hybridization (FISH) imaging pipeline that enabled us to conduct an unbiased siRNA screen to identify factors involved in genome organization in human cells. Using this high-throughput imaging positioning mapping (HIPMap) approach we identified 50 candidate genes that are required for proper positioning of target genes. Genome positioning factors included chromatin remodelers, histone modifiers and nuclear envelope and pore proteins. Components of the replication and post-replication chromatin re-assembly machinery were particularly enriched and affected multiple gene loci of diverse nature. We show that timely progression of cells through replication is required for correct genome positioning. Re-positioning occurred during S and G2 phases of the cell cycle and did not require passage through mitosis. Our results establish high-throughput FISH as a method for the identification of molecular mechanisms involved in genome positioning and they provide a compendium of nuclear genome organization factors.

P-58

DYNAMICS OF HISTONE TAILS AND LINKER DNA IN NUCLEOSOMES: MICROSECOND MOLECULAR DYNAMICS SIMULATIONS

Shaytan, A.K.[1], Armeev, G.A.[2], Goncearenco, A.[1], Zhurkin, V.B.[3], Landsman, D.[1], and Panchenko, A.R.[1]
[1]National Center for Biotechnology Information, NLM, NIH, Bethesda, MD; [2]Faculty of Biology, M.V. Lomonosov Moscow State University, Russia; [3]Laboratory of Cell Biology, Center for Cancer Research, NCI, NIH, Bethesda, MD

Nucleosomes are elementary units of chromatin compaction, which play crucial role in genome functioning. X-ray crystallographic studies of the nucleosome core consistently revealed a compact structure that has approximately 147 DNA base pairs wrapped in a 1.7 superhelical turn around histone octamer. However, at the same time nucleosomes are dynamic entities with conformational plasticity at various levels and timescales, which is necessary to fulfill their functions.

We have constructed a full nucleosome atomistic model with the linker DNA segments and histone tails based on the available X-ray structure of the nucleosome core. We perform multiple molecular dynamics (MD) simulations on microsecond time scale at different ionic conditions. Here we report a detailed analysis of nucleosome dynamics, conformational changes with respect to the available X-ray structure of the core, and protein-DNA interactions. We find that histone tails condense onto DNA, including the linker DNA, may undergo order-disorder conformational transitions and may affect the conformation, stability and dynamics of both nucleosomal and linker DNA. The linker DNA is flexible enough to accommodate various bent conformations within the thermal fluctuation limit. DNA binding sites in the nucleosomal core show varying stability and potential for rearrangement of DNA path within the core. We discuss a hypothesis whereby stable association of histone tails with nucleosomal and liner DNA may result in and additional level of cross-talk between histone post-translational modifications and effector protein binding. Overall our findings suggest a complex interplay between nucleosome conformational dynamics and modes of histone-DNA interactions influenced by positions of histone tail domains and presence of linker DNA.

AS was supported by the US-Russia Collaboration in the Biomedical Sciences NIH visiting fellows program. Development of nucleosome visualization algorithms was supported by the Russian Science Foundation (RSF grant № 14-24-00031).

P-59

DNA HYPOMETHYLATION CONTRIBUTES TO THE NEOPLASTIC PROCESS IN INTESTINAL CANCER

Sheaffer, K.L., Elliott, E.N., and Kaestner, K.H.
Department of Genetics and Institute for Diabetes, Obesity and Metabolism, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA

Abstract withdrawn.

P-60

MBD3 REGULATES CHROMATIN ACCESSIBILITY AT ACTIVE PROMOTERS

Shimbo, T.[1], Lavender, C.[2], Grimm, S.A.[2], Doi, M.I.[4], Henriques, T.[1], Cannady, K.R.[1], Murphy, K.J.[3], Gilchrist, D.A.[1], Burkholder, A.[2], Hayes, J.J.[3], Adelman, K.[1], Archer, T.K.[1], Zaret, K.S.[4], and Wade, P.A.[1]

[1]Epigenetics and Stem Cell Biology Laboratory and [2]Integrative Bioinformatics, NIEHS, NIH, Research Triangle Park, NC; [3]Department of Biochemistry and Biophysics, University of Rochester, Rochester, NY; [4]Institute for Regenerative Medicine, Epigenetics Program, Department of Cell and Developmental Biology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA

Chromatin structure is tightly regulated in cells and its dysregulation is associated with diseases such as cancer. The Mi-2/NuRD (Nucleosome Remodeling Deacetylase) complex is postulated to organize chromatin structure using its nucleosome remodeling and histone deacetylase activities. MBD3 is an integral component of NuRD, which potentially targets the complex to the specific sites of the genome. Recently, we have demonstrated that MBD3/NuRD targets regulatory elements of active genes, a finding diametrically opposed to historical models depicting NuRD as a co-repressor localized to transcriptionally non-permissive chromatin. Moreover, MBD3 co-localized with RNA polymerase II (pol II) at loci where promoter-enhancer loops are formed. Although our previous findings shed light on the role of NuRD in the regulation of active genes, the detailed mechanism of how NuRD is involved in transcriptional regulation is yet not fully understood. To investigate this question, we have developed a next generation, tagmentation based chromatin immunoprecipitation method followed by massively parallel sequencing (ChIP-nexo), which substantially increased the resolution of the MBD3/NuRD mapping. ChIP-nexo defined MBD3/NuRD localization at high resolution, showing accumulation of MBD3 at active promoters with a dip at transcription start site, suggesting an active role of NuRD in modulation of the cell-type specific transcriptional network. To interrogate the detailed functions of NuRD at active promoters, we conducted MNase-seq using conditions which measures both nucleosome positioning and sensitivity, in MBD3 depleted cells. Depletion of MBD3 changed the accessibility of nucleosomes at promoters, while maintaining overall nucleosome positioning. These data indicate an active regulatory function of NuRD in fine tuning the transcriptional network by modulating transcription rates of pol II. We propose that NuRD may be involved in establishing cell type specific gene expression patterns in diverse cell types by organizing promoter nucleoprotein architecture.

P-61

EFFECT OF RPA-BINDING SMALL MOLECULES ON RPA-STIMULATED DNA UNWINDING BY HUMAN RECQ1 AND FANCJ HELICASES

Sommers, J.A.[1], Khan, I.[1], Kabukuru, A.[1], Wold, M.[2], Waterson, A.G.[3], Fesik, S.[3], Chazin, W.J.[3], Turchi, J.J.[4], and Brosh Jr., R.M.[1]
[1]Laboratory of Molecular Gerontology, NIA, NIH, Baltimore, MD; [2]Department of Biochemistry, Carver College of Medicine, University of Iowa, Iowa City, IA; [3]Department of Pharmacology, Vanderbilt University, Nashville, TN; [4]Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN

Replication Protein A (RPA) is a single-stranded DNA (ssDNA) binding protein known to play a key role in DNA metabolic pathways including DNA repair and replication. RPA's importance as a DNA damage signaling molecule makes it an intriguing target for cancer therapy. Several novel RPA-binding small molecules have recently been discovered that either disrupt RPA's ability to bind to ssDNA or are predicted to interfere with its protein interactions. In this study we analyzed the effect of several known RPA-interacting small molecules on RPA-stimulated double-stranded DNA unwinding catalyzed by the human Fanconi Anemia Complementation Group J (FANCJ) or RECQ1 helicases. Either FANCJ or RECQ1 acting alone only marginally unwound a 75 base pair (bp) forked DNA substrate, whereas RPA stimulated both of these helicases to unwind the long duplex DNA substrate in an efficient manner. However, the presence of RPA-interacting compounds TDRL505 (ref. 1) or 8 (ref. 2) inhibited RPA-stimulated and ATP-dependent FANCJ or RECQ1 helicase activity on the 75 bp forked DNA substrate in a dose-dependent manner, effectively eliminating any detectable helicase-catalyzed DNA unwinding at the highest drug concentration tested. The apparent IC_{50} values for inhibition of RPA-dependent FANCJ helicase activity on the 75 bp DNA substrate were approximately 8 μM for either compound. To confirm that the effects of compounds 8 and TDRL505 were not due to direct inhibition of helicase activity, they were tested for their ability to inhibit FANCJ or RECQ1 helicase activity on a short (19 bp) forked duplex DNA substrate that both enzymes efficiently unwind in the absence of RPA. Compounds TDRL505 or 8 failed to inhibit FANCJ or RECQ1 helicase activity on the 19 bp forked DNA substrate, suggesting that they specifically disrupted the functional interaction of RPA with the human DNA repair helicase by interfering with RPA ssDNA binding (compound TDRL505) or RPA protein interaction (compound 8).

Because small molecule 8 binds to an RPA70 N-terminal domain suggested to be important for mediating RPA protein interactions, we performed enzyme linked immunosorbent assays (ELISA) to test for disruption of the FANCJ-RPA physical interaction. Immobilized RPA bound FANCJ in a specific manner, whereas the presence of compound 8 in the binding incubation mixture significantly reduced the FANCJ-RPA interaction. Based on the ELISA data, we conclude that compound 8 interferes with the ability of RPA to physically bind FANCJ, suggesting its mode of inhibition of RPA-dependent FANCJ helicase activity relies on preventing the helicase to physically coordinate with RPA during processive unwinding of long DNA duplexes. Thus, key partnerships between RPA and an interacting DNA helicase can be disrupted by a small molecule which either perturbs the ability of RPA to bind ssDNA or to physically interact with the helicase protein. These studies provide new insights to the mechanisms whereby RPA-interacting compounds potentially suppress proliferation of cancer cells, cause apoptosis, or induce DNA damage during cellular replication.

References: Frank et al., J. Med. Chem. (2013) 56, 9242-50; Shuck et al., Cancer Res. (2010) 70, 3189-98.

P-62

SP1 BINDS TO CENTROCHROMATIN TO MAINTAIN THE CENTROCHROMATIN LANDSCAPE AND PRESERVE CHROMOSOME STABILITY

Sowash, A.R. and Azizkhan-Clifford, J.
Department of Biochemistry and Molecular Biology, Drexel University College of Medicine, Philadelphia, PA

Chromosomal instability (CIN) is a dynamic and continual gain or loss of whole chromosomes, or parts of chromosomes, during cell division at an elevated rate. It is associated with poor patient outcome in multiple cancer types, as well as tumor heterogeneity and resistance to multiple chemotherapeutics, underscoring its clinical importance. Despite its prevalence and clinical importance, the exact mechanism(s) that leads to CIN remain to be determined.

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 recruits different factors to chromatin 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, as well as lagging chromosomes and anaphase bridges, all of which are phenotypes consistent with CIN.

New preliminary data is the first to show that Sp1 localizes and binds to the centromere during mitosis. This localization is dependent on ATM activity, and does not require the Sp1 DNA binding domain. Loss of Sp1 results in disrupted centrochromatin, including changes in histone modifications and transcription of α-satellite arrays. Further, loss of Sp1 results in defects in chromosome cohesion, as well as a decrease in centromeric protein A (CENP-A) deposition at the centromere, suggesting that Sp1 may be important for maintaining the structure and function of this region. We hypothesize that Sp1 contributes to faithful chromosome segregation through a novel function(s) at the centromere during mitosis, thereby preventing CIN.

P-63

EPIGENOME METHOD DEVELOPMENT AND ITS APPLICATION TO AGING OF HEMATOPOIETIC STEM CELLS

Sun, D.
Institute of Biosciences & Technology, Texas A&M University Health Science Center, Houston, TX

The fate decision of Hematopoietic Stem Cell (HSC) between self-renewal and differentiation and its linkage with cancer were examined from epigenetic angles. In order to investigate the stem cell biology of cancer, two strategies were used. One is aging which is the most important demographic risk factor of cancer. And another one is DNA methylome perturbation by knockout of DNMT3A and DNMT3B. Three types of high throughput sequencing data, WGBS for methylation, ChIP-Seq for histone modification H3K4me3 and H3K27me3, and RNA-Seq for transcriptome, were generated from 4 month, 12 month, 24 month HSCs in aging study, and from DNMT3A Knockout and DNMT3A-DNMT3B double-knockout HSCs in methylation perturbation study. In order to get most out of the sequencing data, one novel concept and two bioinformatics software packages were created for DNA methylation analysis of base-resolution bisulfite sequencing data. These tools were integrated into a comprehensive software solution, MOABS, which is efficient in run time and computing resources, to do the data mining of the first epigenome dataset of mouse HSC. MOABS introduces a novel strategy to combine statistical p-value and biological difference into a single metric, termed credible methylation difference (CDIF), and has enough power to detect single-CpG resolution differential methylation in small regulatory regions, such as transcription factor binding sites (TFBSs), with as low as 4-10 fold coverage. Our simulation study reveals superior performance of MOABS over other leading algorithms, such as Fisher's exact test. Using real whole genome BS-seq data, we demonstrate that MOABS improves the detection of allele-specific DNA methylation as well as differential methylation underlying TFBSs, especially at low sequencing depth. In addition, MOABS analysis can be easily extended to more complicated scenarios, such as differential 5hmc analysis using a combination of RRBS and oxBS-seq. The HSC epigenome findings discovered by MOABS during aging process and during DNA methylation perturbation provide clues to cancer progression and treatment from the epigenetic perspective.

P-64

PROFILING PROTEIN OCCUPANTS OF THE GENOME: IS TF FOOTPRINTING READY FOR PRIME TIME?

Sung, M.H., Baek, S., Guertin, M., and Hager, G.L.
Laboratory of Receptor Biology and Gene Expression, Center for Cancer Research, NCI, NIH, Bethesda, MD

High-throughput sequencing technologies have allowed many gene locus-level molecular biology assays to become genome-wide chromatin profiling methods. DNA cleaving enzymes such as DNase I have been used to probe accessible chromatin. The accessible regions contain functional regulatory sites, including promoters, insulators, and enhancers. Chromatin mapping studies have revealed the dynamic and cell state-specific nature of accessibility *in vivo*. Deep sequencing of DNase-seq libraries and computational analysis of the cut profiles have been used to infer protein occupancy in the genome at the nucleotide-level, which was introduced as "digital genomic footprinting". The approach has been proposed as an attractive alternative to ChIP-seq of hundreds of transcription factors, and overcomes antibody issues, poor resolution, and batch effects. Recent reports have uncovered some limitations of the DNase-based genomic footprinting approach that significantly reduce the scope of detectable protein occupancy, especially for transcription factors whose binding to chromatin is short-lived. Moreover, transposase-accessible chromatin using sequencing (ATAC-seq) has recently been introduced as a new chromatin accessibility assay that can be performed on a small number of cells. Amid these new developments, the genomics community is grappling with issues concerning the utility of genomic footprinting and the distinction between the potential and robust deliverables of the proposed approaches. Here we summarize the consensus emerging from the recent reports and describe the remaining issues and hurdles for genomic footprinting. We conclude that the enzyme-based protein occupancy profiling approach represents an evolving methodology that requires significant improvements to mature into a powerful tool for advancing the genomics of chromatin regulation.

P-65

STEROID RECEPTORS CAN FACILITATE THE BINDING OF THE PIONEER FACTOR FOXA1 IN BREAST CANCER CELL LINES THROUGH A DYNAMIC ASSISTED LOADING (DynALoad) MECHANISM

Swinstead, E.E.[1], Miranda, T.B.[1], Songjoon, B.[1], Hawkins, M.[1], Sohyoung, K.[1], Grøntved, L.[2], and Hager, G.L.[1]
[1]Laboratory of Receptor Biology and Gene Expression, Center for Cancer Research, NCI, NIH, Bethesda, MD; [2]Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense M, Denmark

The estrogen (ER) and glucocorticoid receptors (GR) and the forkhead box protein 1 (FoxA1) all play an important role in breast cancer development. The ER, GR, and FoxA1 status in breast cancer is a significant factor for determining the outcome of the disease. However, the cellular interactions between ER, GR, and FOXA1 and the role these interactions play in the progression of breast cancer are not well understood. Our previous studies have shown that both ER and GR direct one another's binding to the chromatin landscape in a mammary cell line. In addition, FoxA1 has been implicated in ER binding patterns; however, the effects of ER on the function of FoxA1 has been controversial. Furthermore, the molecular interplay between GR and FoxA1 is also poorly understood. We have further characterised GR, ER, and FoxA1 crosstalk in three estrogenic breast cancer cell lines utilizing chromatin immunoprecipitation followed by high-throughput sequencing. Upon single and dual activation of receptors these studies show that GR and ER can both alter the binding of each other at a subset of sites, by a mechanism termed dynamic assisted loading (DynALoad). However, there is little crossover observed in the binding patterns for the differing cell lines. This indicates that while the DynALoad mechanism is functioning in all three cell lines, the sites altered are cell specific. Furthermore, and contrary to previous findings, activated ER and GR have the ability to alter the genomic response of the well-established pioneer factor FoxA1. Genome-wide analysis of FoxA1 binding upon hormone treatment shows that both ER and GR can recruit FoxA1 to specific binding sites within the genome, also through the DynALoad mechanism. In addition, analysis of DNase hypersensitivity sequencing in these cell lines under the differing hormone treatments demonstrates there is an increase in chromatin accessibility at ER, GR, and FoxA1 DynALoad binding sites. This indicates there is chromatin reorganization upon activation of ER and GR. Most importantly, there is a lack of a ER, GR, and FoxA1 DNase footprint in these cells suggesting the binding patterns of these factors is highly dynamic with a short DNA residence time. These findings do not support a model wherein a specific set of pioneer factors which bind to closed chromatin and establish the binding landscape for other transcription factors (TFs). These results suggest rather that many TFs in a given cell have the potential to affect the binding landscape of other TFs, depending on the chromatin context. In addition, this study has shifted our classical understanding of pioneer factors in breast cancer, demonstrating that activated GR and ER have the capability to recruit and alter the response of FoxA1. Overall, this has provided information on a previously unknown complexity to FoxA1 action in breast cancer cells.

P-66

CG METHYLATED MICROARRAYS IDENTIFY NOVEL METHYLATED SEQUENCES BOUND BY CEBPG, CEBPE HOMODIMERS AND HETERODIMERS OF ATF4 WITH CEBP FAMILY MEMBERS

Syed, K.S., He, X., Tillo, D., and Vinson, C.
Laboratory of Metabolism, Center for Cancer Research, NCI, NIH, Bethesda, MD

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 (PBMs). Previously, we showed that ATF4|CEBPB heterodimer preferentially binds to methylated CGAT|GCAA and this sequence is bound by both ATF4 and CEBPB *in vivo*. We extended this analysis to additional ATF4 heterodimers with CEBP family memebers. We show that CG methylation enhanced the binding of ATF4 heterodimers with CEBP family members to CGAT|GTAA better when methylated. ATF4 heterodimers with cJUN, JUND and FOS did not preferentially bind to methylated sequences. Additionally we find novel methylated motifs that are bound by CEBPG and CEBPE homodimers 5 fold better when methylated, but not with CEBPA, CEBPB and CEBPD. ATF4 ChIP-seq data using primary female mouse dermal fibroblasts with 50X methylome coverage indicate that the methylated sequences well-bound by ATF4 heterodimers on the PBMs are bound *in vivo*.

P-67

GATA3-MEDIATED CHROMATIN ESTABLISHMENT IN BREAST CANCER CELLS

Takaku, M.[1], Grimm, S.A.[2], Shimbo, T.[1], Perera, L.[3], Machida, S.[4], Kurumizaka H.[4], and Wade, P.A.[1]
[1]Epigenetics and Stem Cell Biology Laboratory, [2]Integrative Bioinformatics Group, and [3]Genome Integrity and Structural Biology Laboratory, NIEHS, NIH, Research Triangle Park, NC; [4]Graduate School of Advanced Science & Engineering, Waseda University, Tokyo, Japan

GATA transcription factors are zinc-finger class of DNA-binding proteins that is required for the development of diverse tissues. One such gene, GATA3, is a key regulator of multiple cellular programs, including T lymphocyte development, trophoblast development, and mammary epithelial cell differentiation. Recent comprehensive genomic analyses have identified GATA3 as one of the most frequently mutated genes in breast cancer. While recent findings strongly suggest that GATA3 has a critical role in tumorigenesis, the molecular mechanisms utilized by GATA3 to regulate gene expression in breast cancer cells is not fully described. GATA3 is known to participate in a complex regulatory network with FOXA1 and ER-alpha, governing the transcriptional program in luminal tumors. Biochemical analyses indicate that: (1) GATA3 binds to chromatin in an estrogen-independent manner, (2) GATA3 can act upstream of FOXA1. These studies suggest GATA3 may act as a pioneer factor, independently associating with closed chromatin and modulating chromatin structure to recruit additional transcription factors such as ER-alpha and FOXA1. To experimentally assess the capacity of GATA3 to function as a pioneer transcription factor, we chose the MDA-MB-231 human breast adenocarcinoma cell line, in which GATA3, FOXA1 and ER-alpha are undetectable, and established stable cell lines expressing wild-type GATA3. Consistent with previous results, GATA3-expressing cells presented a pseudo-epithelial phenotype at the cellular and molecular level. In order to explore whether GATA3 can mediate reprogramming of chromatin configuration, we investigated histone modifications and chromatin structure at GATA3 binding regions throughout the genome. We will present out latest data and discuss the role of GATA3-mediated chromatin licensing in the initiation of the epithelial transcriptional program.

P-68

AN EPIGENETIC FOCUSED siRNA SCREEN IDENTIFIES NOVEL DRUGGABLE TARGETS THAT INHIBIT GROWTH AND INDUCE DIFFERENTIATION IN NEUROBLASTOMA

Veschi, V.[1], Liu, Z.[1], Wang, C.[1], Voss, T.[2], Ozbun, L.[2], Hager, G.[2], Giannini, G.[4], and Thiele, C.[1]
[1]Pediatric Oncology Branch, [2]High-throughput Imaging Facility, and [3]Laboratory of Receptor Biology and Gene Expression, Center for Cancer Research, NCI, NIH, Bethesda, MD; [4]Molecular Medicine Department, Sapienza University of Rome, Italy

Neuroblastoma (NB), the most common extracranial solid tumor of childhood, originates from the neural crest precursors. It accounts for 15% of all pediatric oncology deaths and less than 50% of the high-risk patients experience long-term survival. NexGen sequencing identified few novel druggable mutations in NB and these were mainly in chromatin and epigenetic regulators. Drug discovery targeting epigenetic regulators is a dynamic area of research, however which epigenetic enzymes drive NB tumorigenesis is unknown. To identify epigenetic regulators of NB cell growth and differentiation, we used a high through-put format in which a focused Dharmacon Smartpool siRNA library of 400 known modulators of chromatin structure and function were reversed transfected into 2 NB cell lines (SY5Y-GFP & SK-N-BE) in 384-well plates. After 3 days at 37°C, plates were fixed and stained with Hoechst 33342. Cells were analyzed using an Opera High-Content Screening system in which 12 fields per well with five z-stacks 1um apart were imaged using a 20x objective lens. QQ-plot analyses identified 10 genes with oncogenic activity (defined as siRNAs causing a decrease in Nuclei number (NN) and an increase in Neurite length (NL)) that statistically deviated from the normally distributed bulk population of siRNA measurements. IPA analysis revealed the oncogenes are involved in embryonic and tissue development, cell cycle, DNA replication, recombination and repair ($p= 10^{-5}$). Thirty percent of hits are associated with poor survival in stage 4 NB patients (R2 database). A second round of screening was performed with 4 deconvoluted siRNAs from the 12 highest priority hits. Two hits, CENPE and BRD4, have already been shown to be therapeutic targets in NB. EZH2, another hit, is dysregulated and represses tumor suppressor gene expression in NB. To verify EZH2, we transduced a TET-inducible EZH2shRNA into NGP cells and found that decreases in EZH2 expression were associated with decreased tumor growth *in vitro*. The TET-inducible EZH2shRNA NGP NB cells were implanted into mice and when tumor size reached 200-300mm^3, mice were stratified into 2 groups; 1which received normal chow and the other group which received DOX-chow. The growth of tumor xenografts was significantly inhibited in mice receiving DOX-chow and their survival was significantly prolonged murine (p=0.01). Western analyses of xenografts indicated that the tumors from mice receiving DOX had decreased levels of EZH2. Additional hits, which include SETD8, INCENP, KDM4B, TRIM28, CHAF1A and members of the HMGN family are under analysis. This approach has identified epigenetic targets that are important in regulating NB.

P-69

SETBP1 INDUCES LEUKEMIA DEVELOPMENT THROUGH REPRESSION OF RUNX1

Vishwakarma, B.A.[1], Gudmundsson, K.O.[1], Makishima, H.[2], Honsono, N.[2], Nguyen, N.[1], Negi, N.[1], Oakley, K.[1], Maciejewski, J.P.[2,*], and Du, Y.[1,*]
[1]Department of Pediatrics, Uniformed Services University of the Health Sciences, Bethesda, MD; [2]Department of Translational Hematology and Oncology Research, Taussig Cancer Institute, Cleveland Clinic, Cleveland, OH. [*]These authors contributed equally to this work.

Abnormal activation of *SETBP1* through overexpression or missense mutations is highly recurrent in various myeloid malignancies; however, it is unclear whether such activation alone is able to induce leukemia development. Here we show that *Setbp1* overexpression in mouse bone marrow progenitors through retroviral transduction is capable of initiating leukemia development in irradiated recipient mice. Before leukemic transformation, *Setbp1* overexpression significantly enhances the self-renewal of hematopoietic stem cells (HSCs) and expands granulocyte macrophage progenitors (GMPs). Interestingly, *Setbp1* activation also causes transcriptional repression of tumor suppressor gene *Runx1* and this effect is crucial for *Setbp1*-induced transformation. *Runx1* repression is induced by Setbp1-mediated recruitment of Hdac1 to *Runx1* promoters and can be relieved by treatment with histone deacetylase (HDAC) inhibitors entinostat and vorinostat. Moreover, treatment with these inhibitors caused efficient differentiation of *Setbp1*-induced myeloid leukemia cells and immortalized myeloid progenitors in culture and significantly extended the survival of mice with *Setbp1*-induced myeloid neoplasm, suggesting that HDAC inhibition could be an effective strategy for treating myeloid malignancies with *SETBP1* activation.

Funding source: NIH RO1CA143193

P-70

INHERITANCE OF DNA METHYLATION IN THE MOUSE

Grimm, S.A, Shimbo, T., Fargo, D.C., and Wade, P.A.
Epigenetics and Stem Cell Biology Laboratory, NIEHS, NIH, Research Triangle Park, NC

DNA methylation is an essential epigenetic mark, intimately involved in mammalian gene regulation. The pattern of DNA methylation is dramatically reprogrammed at multiple points between generations in mammals. While local DNA sequence and overall genetic background are known to influence histone modification patterns, the extent to which DNA methylation patterns are impacted by genetics remains incompletely understood. We addressed this issue by intercrossing inbred mouse strains and analyzing DNA methylation at the base-pair level across the genome in somatic tissue from parents and age-matched offspring of multiple families. Loci at which CpG methylation differed between strains were generally located far from transcription start sites, were associated with regulatory DNA elements and offspring had methylation levels intermediate between parental levels. In the vast majority of cases, differential CpG methylation patterns observed in parents were preserved on the relevant parental alleles in offspring. At differentially methylated regions, DNA sequence differences between strains frequently occurred within enriched transcription factor binding sites where deviations from consensus at invariant residues were associated with increases in local DNA methylation. These results support a framework to understand the manner in which genetic differences between inbred strains, or between individuals in outbred populations including humans, impact the epigenetic features that dictate gene expression patterns characteristic of health and disease.

P-71

HISTONE H2AX IS A NOVEL REGULATOR OF EPITHELIAL TO MESENCHYMAL TRANSITION

Weyemi, U., Redon, C.E., Choudhuri, R., Maeda, D., Kasoji, M., Abrams, N., and Bonner, W.M.
Laboratory of Molecular Pharmacology, Center for Cancer Research, NCI, NIH, Bethesda, MD

The vast majority of deaths from cancer are due to its progression from primary tumor to metastatic disease. Understanding how some cells can migrate from the primary tumor to seed new tumors throughout the body is a longstanding challenge in developing novel cancer treatments. Current models propose that these migratory cells acquire an invasive phenotype through a process known as the epithelial to mesenchymal transition (EMT), characterized by the loss of cell polarity and cell-to-cell adhesion, and the acquisition of migratory and invasive properties. Such changes allow the cells to invade the extracellular matrix and migrate throughout the body, aided by the formation of lamellipodia, filopodia and invadopodia. In a converse transition, the MET, mesenchymal cells may revert back to the epithelial phenotype and establish tumors at distant sites. We have preliminary observations showing that when one member of the H2A family of histones, H2AX, is silenced or inactivated in HCT116 cells, these cells exhibit mesenchymal-like characteristics including increased invasiveness. Furthermore, genome-wide expression analysis implicates the critical EMT transcription factors, SLUG and ZEB1, as mediators of H2AX loss-induced EMT. To make these studies more rigorous, we generated a novel HCT116 cell line with H2AX destroyed. Our findings show that ectopic expression of H2AX in H2AX-null cells reverses the invasiveness induced by H2AX inactivation. Moreover, in 233 human colon cancer samples, a strong correlation is found between the levels of H2AX and SLUG/ZEB1 expression. These observations lead us to hypothesize that H2AX is a key regulator of EMT and plays a critical role in the downstream events leading to increased metastasis.

P-72

EPSTEIN-BARR VIRUS ONCOPROTEIN SUPER-ENHANCERS CONTROL B CELL GROWTH

Willox, B., Zhou, H., Jiang, S., Schmidt, S., Bernhardt, K., Liang, J., Johannsen, E.C., Kharchenko, P., Gewurz, B.E., Kieff, E., and Zhao, B.
Department of Medicine, Brigham and Women's Hospital, Department of Microbiology and Immunobiology, Harvard Medical School, Boston, MA

Super Enhancers are recently identified genomic regulatory regions that are principal determinants of cell identity and oncogenesis, yet to be implicated in host-pathogen interactions. Here, we discover that EBV transcription factors and EBV-activated NF-κB transcription factors converge to thousands of enhancer sites in EBV transformed lymphoblastoid cell lines (LCLs). Of these, 187 enhancers had markedly higher and broader signals for H3K27ac histone modifications, characteristic of Super Enhancers. These EBV Super Enhancers associated with various genes essential for LCL growth and survival, including MYC and BCL2. ~96% of EBV Super Enhancers and their nearest associated genes occurred in the same Hi-C topological domains. The Bromodomain inhibitor, JQ1, disrupts EBV Super Enhancers and induces LCL growth arrest. Although EBV Super Enhancers were already primed in pre-transformation Resting B Lymphocytes (RBLs), we find differences in enhancer usage between LCLs and RBLs. In summary, these findings allow new insights into how EBV hijacks nuclear epigenetic processes to drive cell proliferation.

P-73

KRAB ZINC FINGER PROTEINS ARE EVOLUTIONARILY ADAPTIVE REPRESSORS OF PARASITIC DNA ELEMENTS

Wolf, G.[1,2], Yang, P.[1], Füchtbauer, A.C.[2], Füchtbauer, E.M.[2], Silva, A.M.[2], Park, C.[1], Wu, W.[1], Nielsen, A.L.[3], Pedersen, F.S.[2], and Macfarlan, T.S.[1]
[1]Program in Genomics of Differentiation, NICHD, NIH, Bethesda, MD; [2]Department of Molecular Biology and Genetics and [3]Department of Biomedicine, Aarhus University, Denmark

Retroviruses have been invading mammalian germ lines for millions of years, accumulating in the form of endogenous retroviruses (ERVs) that account for nearly one-tenth of the mouse and human genomes. To protect their genomic integrity, mammals have developed a number of defense mechanisms that include epigenetic silencing of ERVs during development. Given the vast number and sequence diversity of ERVs and other parasitic DNA elements, this epigenetic repression system is believed to rely on sequence-specific and evolutionary adaptive target recognition factors. Recent advances in the field have indicated that at least some Krüppel-associated box zinc finger proteins (KRAB-ZFPs), the largest single transcription factor family in mammals, with a unique repertoire of several hundred members in each mammalian species, have evolved to repress parasitic DNA. KRAB-ZFPs bind large target motifs via usage of tandem arrays of up to dozens of C2H2-type zinc finger domains, each capable to specifically bind three nucleotides of DNA. Epigenetic silencing is mediated via the KRAB domain which recruits the potent corepressor KAP1. Intriguingly, KRAB-ZFPs are highly diversified in mammals and frequently show signs of positive selection, possibly indicating adaptive evolution in an arms race against ERVs that have invaded the germ-line.

Here we demonstrate that ZFP809, a member of the KRAB-ZFP family, initiates silencing of a defined subset of ERVs in a sequence-specific manner via recruitment of heterochromatin inducing complexes. ZFP809 knock-out mice develop normally but display highly elevated levels of ZFP809-targeted ERVs in all tested somatic tissues. ERV reactivation is accompanied by an epigenetic shift from repressive to active histone modifications and the loss of DNA methylation. Importantly, using conditional alleles and rescue experiments, we demonstrate that ZFP809 is required to initiate ERV silencing during embryonic development, but becomes largely dispensable in somatic tissues. Finally, we show that ZFP809 silences a handful of non-viral host genes that have acquired ZFP809 binding sites via ancient ERV insertions. To investigate whether other KRAB-ZFPs may also function as ERV repressors, we determined the genome-wide binding profiles of several previously uncharacterized murine KRAB-ZFPs using ChIP-seq technology. We show that the majority of these proteins bind to ERV sequences in a sequence-specific manner. Importantly, these KRAB-ZFP binding sites are also bound by the KAP1 corepressor and downstream acting histone modifiers, and show signatures of repressive histone methylation marks.

In sum, these data strongly support the hypothesis that possibly hundreds of KRAB-ZFPs evolved in response to germ-line colonization events by ERVs and other transposable elements. Together with our observation that ancient ERVs have altered the expression profiles of cellular genes via ZFP809 recruitment, this indicates that the ongoing arms race between ERVs and KRAB-ZFPs may have repeatedly re-wired transcriptional networks during mammalian evolution.

P-74

STEMNESS FACTOR SALL4 IS REQUIRED FOR DNA DAMAGE RESPONSE IN EMBRYONIC STEM CELLS

Xiong, J.[1,3], Todorova, D.[1], Su, N.[1], Lee, P.[2], Shen, Z.[2], Briggs, S.P.[2], and Xu, Y.[1]
[1]Section of Molecular Biology, [2]Section of Cell and Developmental Biology, Division of Biological Sciences, University of California, San Diego, La Jolla, CA; [3]Center for Molecular Medicine, NHLBI, NIH, Bethesda, MD

Mouse embryonic stem cells (ESCs) are genetically more stable than somatic cells, thereby preventing the passage of genomic abnormalities to their derivatives including germ cells. The underlying mechanisms, however, remain largely unclear. Here we show that the stemness factor Sall4 is required for activating the critical Ataxia Telangiectasia Mutated (ATM)-dependent cellular responses to DNA double-stranded breaks (DSBs) in mouse ESCs and confer their resistance to DSB-induced cytotoxicity. Sall4 is rapidly mobilized to the sites of DSBs after DNA damage. Furthermore, Sall4 interacts with Rad50 and stabilizes Mre11-Rad50-Nbs1 complex for the recruitment and activation of ATM. Sall4 also interacts with Baf60a, a member of the SWI/SNF ATP-dependent chromatin-remodeling complex, which is responsible for recruiting Sall4 to the site of DNA DSB damage. Since the expression of Sall4 in human cancers is correlated with drug resistance, our findings provide novel mechanisms to coordinate stemness with genomic stability of ESCs and the drug resistance of Sall4-expressing cancers.

P-75

CHARTING EPIGENOMES: APPLYING A PRINCIPLED APPROACH FOR ChIP-SEQ OPTIMIZATION

Xue, C., Gladden, A., Kelliher, E., Bochicchio, J., Farjoun, Y., Nusbaum, C., Busby, M., and Goren, A.
Broad Technology Labs, The Broad Institute of MIT and Harvard, Boston, MA

ChIP-seq is a powerful method that utilizes immunoprecipitation by antibodies targeting specific DNA-associated proteins, followed by sequencing of the DNA fragments that are pulled down. This approach enables charting the genomic localization of DNA-associated proteins such as transcription factors and modified histones. ChIP-seq is an invaluable tool used to address a wide range of biological and medical questions. Despite its usefulness, there are various steps of the ChIP-seq method that can introduce error or variability for unknown reasons. ChIP-seq currently yields significant failure rates, has both intra-assay and inter-assay variability and results that are not easily reproduced. The lack of a standardized ChIP-seq protocol has restricted its use to a limited number of specialized laboratories. Here, we describe our efforts leveraging a principled approach to revolutionize ChIP-seq methodology, aimed at empowering the scientific community with a standardized and robust technology.

We designed an experimental system that enables rigorous statistical analysis, including: (*i*) splitting the sheared chromatin between all conditions, so tested antibodies are evaluated on the same initial sample; (*ii*) performing 4 replicates for each condition; and (*iii*) implementing automation to ensure precise and consistent liquid handling and to control for human error. Analysis was carried out using both human and mouse samples. Leveraging this approach, we evaluated a set of 2 monoclonal antibodies targeting key histone modifications, and demonstrate that these monoclonal reagents are as specific as the 'gold standard' polyclonal antibodies for these epitopes, and further have an improved signal to noise ratio. Our work shows that polyclonal antibodies can be supplanted by monoclonals for use in ChIP-seq and similar affinity-based methods, resulting in increased reproducibility and robustness.

P-76

CHANGES IN THE METHYLATION PATTERN OF p53 GENE PROMOTER IN THE MEGALOBLASTIC BONE MARROW DISEASE

Yadav, M.K.[1], Manoli, N.N.[2], Aman, I., Madhunapantula, S.V., Manjunath, G.V., and Natraj, S.M.
Department of Pathology, Centre of Excellence in Molecular Biology and Regenerative Medicine (CEMR), Department of Biochemistry, Jagadguru Sri Shivarathreeshwara Medical College, Jagadguri Sri Shivarathreeshwara University, Karnataka, India

Megaloblastic anaemia, a disease originated by faulty DNA synthesis leading to abnormal maturation of haematopoietic cells, is characterized by the presence of abnormal, very large sized megaloblasts harboring a fine reticular nuclear structure. Prior studies have shown the deficiency of cobalamin (Vitamin B12 – VitB12) and Folic Acid (FA), which are essential for DNA biosynthesis, is the primary cause for megaloblastic bone marrow diseases. However, detailed mechanism describing how these vitamin deficiencies transform a normoblast in to megaloblast are currently lacking. In addition, it is also unknown whether these vitamin deficiencies induce changes in promoter DNA methylation of genes, such as p53 (the guardian of the genome, involved in regulating cell cycle and apoptosis. Therefore, in this study we have estimated and compared the VitB12 and Folic Acid levels of megaloblastic bone marrow patients with that of control group and determined the changes in methylation pattern of P53 gene in bone marrow aspirate using methylation specific PCR and expression level of p53 gene in paraffin block cell by immunohistochemistry. Preliminary findings identified low and very low levels of FA and VitB12 in megaloblastic anaemic patients compared to control individuals (FA >5.38 ng/ml, VitB12 – 211-911 pg/ml). The p53 promoter methylation and expression data will be presented at the time of presentation.

P-77

microRNA TRANSFERASE FUNCTION OF AUF1 p37

Yoon, J.[1], Jo, M.[2], White, E.J.F.[3], De, S.[1], Hafner, M.[4], Zucconi, B.E.[3], Abdelmohsen, K.[1], Yang, X.[1], Tuschl, T.[5], Becker, K.G.[1], Wilson, G.M.[3], Hohng, S.[2], and Gorospe, M.[1]
[1]Laboratory of Genetics, NIA, NIH, Baltimore, MD; [2]Department of Physics and Astronomy, Institute of Applied Physics, National Center for Creative Research Initiatives, Seoul National University, Seoul, Korea; [3]Department of Biochemistry and Molecular Biology and Marlene and Stewart Greenebaum Cancer Center, University of Maryland School of Medicine, Baltimore, MD; [4]Laboratory of Muscle Stem Cells and Gene Regulation, NIAMS, NIH, Bethesda, MD; [5]Howard Hughes Medical Institute and Laboratory of RNA Molecular Biology, Rockefeller University, New York, NY

Eukaryotic gene expression is tightly regulated posttranscriptionally by RNA-binding proteins (RBPs) and microRNAs. Silencing the RBP AU-rich-binding factor (AUF) 1 reduced the interaction of microRNAs with Argonaute 2 (Ago2), the catalytic component of the RNA-induced silencing complex (RISC). Analysis of this effect revealed that the AUF1 isoform p37 displayed high affinity for the microRNA let-7b, promoted the interaction of let-7b with Ago2, and enhanced Ago2-let-7-mediated mRNA decay. Our findings uncover a novel mechanism whereby microRNA transfer from AUF1 p37 to Ago2 facilitates microRNA-elicited gene silencing.

P-78

QUANTITATIVE ANALYSIS OF TRANSCRIPTION FACTOR DYNAMICS IN YEAST GENOME

Zaidi, H.A. and Bekiranov, S.
Department of Biochemistry and Molecular Genetics, University of Virginia School of Medicine, Charlottesville, VA

This poster presents our work on the quantitative analysis of binding dynamics in a competition-ChIP assay of TBP in yeast. We use a physical model characterized by three parameters: the in-vivo association and disassociation rates of a transcription factor and the induction rate of the competing allele. Numerical work shows that our method captures the essence of competition-ChIP well, and we are finalizing the pipeline to conduct a rigorous analysis of publicly available data. We are also extending the locus specific CLK method to the whole genome (CLK-seq) and have developed a computational analysis pipeline to extract TF-chromatin dynamic parameters. We will compare competition ChIP and CLK-seq derived parameters for TBP across the yeast genome.

P-79

THE ROLE OF WDR18 PROTEIN IN CENP-A DEPOSITION PATHWAY

Zasadzinska, E. and Foltz, D.R.
Department of Biochemistry and Molecular Genetics, University of Virginia Medical School, Charlottesville, VA

Epigenetic memory that controls gene expression and defines unique chromosome domains is encoded in posttranslational modifications of histones or the incorporation of nucleosomes containing histone variants. Centromeres are unique domains on each chromosome, which define the site of kinetochore formation during mitosis therefore ensuring equal chromosome segregation and genetic stability. Centromeric identity is epigenetically regulated by the incorporation of the centromere specific CENP-A histone H3 variant. In contrast to the canonical H3.1 histone variant, CENP-A deposition is independent of DNA replication, and occurs in early G1. The mechanism of CENP-A deposition is cell cycle regulated, and depends on CENP-A specific histone chaperone Holliday Junction Recognition that has been shown to be a *bone fide* chromatin assembly factor for CENP-A. Deposition of CENP-A is also regulated by the activity of the Mis18 complex (comprising of Mis18α/β/BP1). These proteins accumulate during late G2 and into mitosis and CENP-A deposition ensues in early G1. We hypothesize that the stability of these proteins must be both negatively and positively regulated. HJURP and Mis18 must be protected from degradation during G2 and mitosis and negative regulation is required for degradation of these proteins following CENP-A deposition. In humans, negative regulation of Mis18 stability involves Cul1 mediated ubiquitylation and subsequent proteasomal degradation of Mis18β during interphase but not mitosis. In contrast, ubiquitylation has been recently shown to protect CAL1 (the HJURP functional homolog in Drosophila) from proteasomal degradation. However it remains unclear what mechanism governs HJURP levels in humans. We found the WD-repeat containing protein WDR18 is associated with both Mis18α and HJURP by co-immunoprecipitation experiments. Moreover WDR18 depletion results in a significant increase of HJURP degradation as assessed by the pulse chase experiments and MG132 treatment. Depletion of WDR18 by shRNA significantly reduced cellular levels of HJURP and centromeric levels of CENP-A and Mis18α, showing that WDR18 is required for the stability of the CENP-A deposition pathway proteins. We hypothesize that the WDR18 protein contributes to the mechanism regulating the stability of HJURP in the preassembly complex providing a mechanism of how the human CENP-A deposition pathway is positively regulated by controlling protein degradation prior CENP-A incorporation.

P-80

FUNCTIONALLY REDUNDANT AND COMPENSATORY BINDING OF HMGN VARIANTS TO NUCLEOSOMES AFFECT DNaseI HYPERSENSITIVITY OF CHROMATIN AND GENE EXPRESSION IN MOUSE B LYMPHOCYTES

Zhang, S.[1], Zhu, I.[2], Deng, T.[1], Rochman, M.[1], Furusawa, T.[1], Casellas, R.[3], Landsman, D.[2], and Bustin, M.[1]
[1]Laboratory of Metabolism, Center for Cancer Research, NCI, NIH, Bethesda, MD; [2]Computational Biology Branch, National Center for Biotechnology Information, NLM, NIH, Bethesda, MD; [3]Laboratory of Molecular Immunogenomics, NIAMS, NIH, Bethesda, MD

The dynamic features of chromatin allow cells to alter gene expression in response to various environmental or endogenous cues. The chromatin structure is regulated by dynamic interactions between DNA elements and numerous chromatin-associated factors. A key challenge in the field of gene regulation is to characterize these factors. Here we report that HMGN proteins affect the DNaseI hypersensitivity patterns of chromatin and modulate the fidelity of transcriptional profile in mouse B lymphocytes. HMGN is a family of ubiquitous proteins that bind dynamically to chromatin. B lymphocytes contain two major variants named HMGN1 and HMGN2 and a minor variant named HMGN3. Our ChIP-seq results revealed that all the HMGN variants are strongly enriched at CpG island containing promoters of transcriptionally active genes. At silenced genes, HMGN variants mark the 'poised' genes that are activated in response to specific stimuli. Interestingly, despite of the strong overlapping between HMGN binding sites and DNaseI hypersensitivity sites, no significant changes in the DNaseI hypersensitivity or gene expression were detected in *Hmgn1-/-/Hmgn3-/-* double knockout B cells. However, simultaneous loss of HMGN1 and HMGN3 induced a dramatic increase in the binding of HMGN2 at the promoter regions, suggesting that HMGN2 may functionally compensate for loss of HMGN1 and HMGN3. To further validate the compensatory function of HMGN2, we examined the transcriptomic profiles in *Hmgn1-/-/Hmgn2-/-* double knockout cells using RNA-seq. Indeed, we found that simultaneous loss of HMGN1 and HMGN2 induced significant changes in the expression of 389 genes. Strikingly, analysis of DNase-seq data revealed that ~ 40% DNaseI hypersensitivity sites were lost in *Hmgn1-/-/Hmgn2-/-* double knockout cells as compared with wild type controls. This study demonstrated a role for HMGN proteins in maintaining DNaseI hypersensitivity of chromatin and in modulating gene-regulatory networks. Given the ubiquitous presence of HMGN1 and HMGN2 in all vertebrate cells, it is likely that similar compensatory mechanisms are widely spread.

P-81

THE BINDING LANDSCAPE OF CENP-A/H4 DIMER REVEALS THERMODYNAMIC DISTINCTIONS FROM H3/H4 DIMER

Zhao, H.[1,2], Dalal, Y.[2], and Papoian, G.A.[1]
[1]Biophysics Program, Institute for Physical Science and Technology, Department of Chemistry and Biochemistry, University of Maryland, College Park, MD; [2]Laboratory of Receptor Biology and Gene Expression, Center for Cancer Research, NCI, NIH, Bethesda, MD

Centromere protein A (CENP-A) is a centromere-specific variant of histone H3 and shares ~50% amino acid identity with canonical H3 protein. CENP-A is required to package the centromere, and to separate sister chromatids during mitosis. Despite their discrete functions, previous reported co-crystal structures reveal surprising similarities exists between CENP-A/H4 and H3/H4 dimers. In this work, to distinguish features of CENP-A/H4, which might be unique, we use molecular dynamics simulations to map the binding free energy landscape for CENP-A/H4 and H3/H4 dimers. The Associated memory, Water mediated, Structure and Energy Model (AWSEM) and umbrella sampling were applied for each simulation to obtain two-dimensional free energy profiles of monomeric protein association and folding. Interestingly, our calculations revealed significant thermodynamic distinctions between the dimerization profiles of CENP-A/H4 and of H3/H4 pairs. Furthermore, the free energy landscape of CENP-A/H4 dimer is significantly remodeled in the presence of its cognate chaperone HJURP. These results are in general agreement with the available experimental data and provide new thermodynamic insights into the mechanisms underpinning chaperone-mediated histone variant CENP-A nucleosomes assembly *in vivo*.

Participant List

Lara Abramowitz
NIDDK
lara.abramowitz@nih.gov

Natalie Abrams
NCI
natalie.abrams@nih.gov

Amanda Ackermann
Children's Hospital of Philadelphia
ackermanna@email.chop.edu

Tolga Acun
NCI
tolga.acun@nih.gov

Sheera Adar
University of North Carolina, Chapel Hill
sheera@live.unc.edu

Sunita Agarwal
NIDDK
sunitaa@mail.nih.gov

Susie Ahn
NCI
ahnsu@mail.nih.gov

Alexandra Ainsztein
NIGMS
ainsztea@nigms.nih.gov

Mirit Aladjem
NCI
aladjemm@mail.nih.gov

Bogi Andersen
University of California, Irvine
bogi@uci.edu

Thorkell Andresson
NCI
andressont@mail.nih.gov

Jaclyn Andricovich
George Washington University
jandricovich@gwu.edu

Aishe Angeletti Sarshad
NIAMS
aishe.angelettisarshad@nih.gov

Olga Aprelikova
NCI
apreliko@mail.nih.gov

Jesse Arbuckle
NIAID
jesse.arbuckle@nih.gov

Trevor Archer
NIEHS
archer1@niehs.nih.gov

Jonathan Arias
NIH/Center for Scientific Review
ariasj@csr.nih.gov

Grace Ault
NCI
aultg@mail.nih.gov

James Austin
NIAID
james.austin2@nih.gov

Maggie Baker
Johns Hopkins University
mbaker41@jhmi.edu

Devaiah Ballachanda
NCI
ballachandad@mail.nih.gov

Krishna Banaudha
FDA
krishna.banaudha@fda.hhs.gov

Russell Bandle
NCI
bandler@mail.nih.gov

Deblina Banerjee
NCI
deblina.banerjee@nih.gov

Laura Baranello
NCI
baranellolf@mail.nih.gov

Meghan Barnhart-Dailey
University of Virginia
mcb3g@virginia.edu

Frederic Barr
NCI
barrfg@mail.nih.gov

Jemima Barrowman
NCI
jemima.barrowman@nih.gov

Nizar Batada
Ontario Institute for Cancer Research
nizar.batada@oicr.on.ca

Tasneem Bawa-Khalfe
University of Houston
tbawa-khalfe@uh.edu

Kevin Becker
NIA
beckerk@grc.nia.nih.gov

Matthias Becker
Wurzburg University
matthias.becker@uni-wuerzburg.de

Kate Beishline
Wistar Institute
KBeishline@Wistar.org

Marie-Jose Belanger
NIH/Center for Scientific Review
belangerm@csr.nih.gov

Shelley Berger
University of Pennsylvania
bergers@upenn.edu

Eric Bernhard
NCI
bernhardej@mail.nih.gov

Diana Bernstein
University of Pennsylvania
dianaber@mail.med.upenn.edu

Emily Bernstein
Mount Sinai Hospital
emily.bernstein@mssm.edu

Kapil Bharti
NEI
kapilbharti@nei.nih.gov

Sanjay Bharti
NIA
bhartisk@mail.nih.gov

Nisan Bhattacharyya
NIDCR
bhattacharyyan@nidcr.nih.gov

Juraj Bies
NIH/Center for Scientific Review
biesj@mail.nih.gov

Sven Bilke
NCI
bilkes@mail.nih.gov

Hans Bjornsson
Johns Hopkins University
hbjorns1@jhmi.edu

Olivier Blondel
NIDDK
blondelol@niddk.nih.gov

Max Boeck
University of North Carolina, Chapel Hill
maxboeck@email.unc.edu

Brenda Boersma
NCI
boersmab@mail.nih.gov

James Bone
EpiCypher - SBIR
jim@epicypher.com

Samiksha Borkar
Georgetown University
sab311@georgetown.edu

Alex Bortvin
Carnegie Institution for Science
bortvin@ciwemb.edu

Greg Bowman
Johns Hopkins University
gdbowman@jhu.edu

Leslie Boyer
Massachusetts Institute of Technology
lboyer@mit.edu

Lesley Brown
NICHD
lebrown@mail.nih.gov

Minh Bui
NCI
buim@mail.nih.gov

Michael Bustin
NCI
bustinm@mail.nih.gov

Beata Buzas
NIAAA
bbuzas@mail.nih.gov

Colleen Byrnes
NIDDK
byrnesc@mail.nih.gov

Yi Cai
Johns Hopkins University
kelvincaiyi@gmail.com

Elsa Callen
NCI
callene@mail.nih.gov

Viviane Callier
NCI
callierv@nih.gov

Bertram Canagarajah
NIDDK
bertram@mail.nih.gov

France Carrier
University of Maryland, Baltimore
fcarr001@umaryland.edu

Chanelle Case Borden
NCI
casec@mail.nih.gov

Rafael Casellas
NIAMS
casellar@mail.nih.gov

Venkat Chalamcharla
NCI
chalamcharlavr@mail.nih.gov

Panagiotis Chandris
NIBIB
panagiotis.chandris@nih.gov

Dhruba Chattoraj
NCI
chattoraj@nih.gov

Ritu Chaudhary
NCI
ritu.chaudhary@nih.gov

Chris Cheadle
Elsevier
c.cheadle@elsevier.com

Brian Chen
NIA
brian.chen@nih.gov

Hegang Chen
University of Maryland
hchen@epi.umaryland.edu

Huimin Chen
NCI
huimin.chen@nih.gov

Ping Chen
NEI
chenp3@mail.nih.gov

Ron Chen
University of Cambridge
rac83@cam.ac.uk

Wei-Dong Chen
NCI
wei-dong.chen@nih.gov

Weiping Chen
NIDDK
weipingchen@niddk.nih.gov

Xin Chen
Johns Hopkins University
xchen32@jhu.edu

Zhen-Xia Chen
NIDDK
zhen-xia.chen@nih.gov

Yuzhong Cheng
NICHD
yuzhongcheng@mail.nih.gov

Razvan Chereji
NICHD
razvan.chereji@nih.gov

Katherine Chiappinelli
Johns Hopkins University
kchiapp1@jhmi.edu

Lisa Chong
Science Magazine
lchong@aaas.org

Supratim Choudhuri
FDA
Supratim.Choudhuri@fda.hhs.gov

David Clark
NICHD
clarkda@mail.nih.gov

Sean Clark
NICHD
sean.clark@nih.gov

Anthony Conley
NIAID
conleyto@niaid.nih.gov

Richard Conroy
NIBIB
conroyri@mail.nih.gov

Julie Cooper
NCI
julie.cooper@nih.gov

Kairong Cui
NHLBI
cuik@nhlbi.nih.gov

Yamini Dalal
NCI
dalaly@mail.nih.gov

Ryan Dale
NIDDK
dalerr@niddk.nih.gov

Charles Danan
NIAMS
charles.danan@nih.gov

Hien Dang
NCI
danghi@mail.nih.gov

Rina Das
NIMHD
dasr2@mail.nih.gov

Mary Dasso
NICHD
dassom@mail.nih.gov

Sean Davis
NCI
sdavis2@mail.nih.gov

Sandip De
NICHD
des22@mail.nih.gov

Ann Dean
NIDDK
anndean@helix.nih.gov

Maria DeBernardi
NIH/Center for Scientific Review
debernardima@csr.nih.gov

Rosamma Decastro
NCI
decastrr@mail.nih.gov

Tao Deng
NCI
dengt@mail.nih.gov

Wulan Deng
HHMI
dengw@janelia.hhmi.org

Allison Dennis
NIH/Johns Hopkins
allison.dennis@nih.gov

Christina DeStefano Shields
Johns Hopkins University
cdestef1@jhmi.edu

Mrinal Dewanjee
NEI
dewanjeem@nei.nih.gov

Andrea Di Francesco
NIA
andrea.difrancesco@nih.gov

John Didion
NHGRI
john.didion@nih.gov

Sabine Dietmann
Wellcome Trust-Medical Research
Council Cambridge Stem Cell Institute
sabine.dietmann@gmail.com

Michael Difilippantonio
NCI
difilipm@mail.nih.gov

Shailaja Divekar
Georgetown University
sdd7@georgetown.edu

Douglas Dluzen
NIA
douglas.dluzen@nih.gov

William Donegan
Drexel University College of Medicine
wed25@drexel.edu

Gang Dong
NIAID
gdong@niaid.nih.gov

Frank Donovan
NHGRI
donovanfx@mail.nih.gov

Katie Driest
NCI
katie.driest@nih.gov

Barbara Dunn
NCI
dunnb@mail.nih.gov

Bill Dunty
NIAAA
duntyw@mail.nih.gov

Daniel Durocher
Lunenfeld-Tanenbaum Research Institute
durocher@lunenfeld.ca

Tara Dutka
NIAMS
tara.dutka@nih.gov

Hari Easwaran
Johns Hopkins University
heaswar1@jhmi.edu

Joseph Ecker
HHMI/Salk Institute
ecker@salk.edu

Iris Eke
NCI
iris.eke@nih.gov

Karl Ekwall
Karolinska Institute
Karl.Ekwall@ki.se

Mariam Eljanne
NCI
eljannem@mail.nih.gov

Laura Elnitski
NHGRI
elnitski@mail.nih.gov

Michael Erdos
NHGRI
mikee@mail.nih.gov

Aysegul Ergen
NCI
ergena@mail.nih.gov

Peter Eriksson
NICHD
erikssop@mail.nih.gov

Michael Graham Espey
NCI
SP@nih.gov

Luis Espinoza
NIH/Center for Scientific Review
espinozala@csr.nih.gov

Moriah Eustice
NIDDK
moriah.eustice@nih.gov

Joshua Farber
NIAID
jfarber@niaid.nih.gov

Gary Felsenfeld
NIDDK
gary.felsenfeld@nih.gov

Lijuan Feng
Johns Hopkins University
lfeng6@jhu.edu

Patricia Fernandez Ferri
NCI
fernandezferrp@mail.nih.gov

Debbie Figueroa
NHLBI
Debbie.Figueroa@nih.gov

Kipper Fletez-Brant
Johns Hopkins University
cafletezbrant@gmail.com

Marco Foiani
IFOM and University of Milan
marco.foiani@ifom.eu

H. Diego Folco
NCI
folcohd@mail.nih.gov

Daniel Foltz
University of Virginia
dfoltz@virginia.edu

Jean-Philippe Fortin
Johns Hopkins University
fortin946@gmail.com

Daniel Fowler
NCI
dhfowler@helix.nih.gov

Carlos Eduardo Frigerio Domingues
NIDCD
carloseduardo.frigeriodomingues@nih.gov

Haiqing Fu
NCI
fuha@mail.nih.gov

Jianfei Fu
NHLBI
jian-fei.fu@nih.gov

Priscilla Furth
Georgetown University
paf3@georgetown.edu

Takashi Furusawa
NCI
furusawt@mail.nih.gov

Pamela Gallagher
NCI
pamela.gallagher@nih.gov

Guanjun Gao
NCI
gaogu@mail.tsinghua.edu.cn

Kai Ge
NIDDK
kai.ge@nih.gov

Claudia Gebert
NICHD
gebertcl@mail.nih.gov

Rob Genuario
Illumina, Inc.
rgenuario@illumina.com

Pavol Genzor
Johns Hopkins University/Carnegie Institution
pgenzor1@jhu.edu

Rajib Ghosh
Johns Hopkins University
rghosh7@jhmi.edu

Ido Goldstein
NCI
ido.goldstein@nih.gov

Daniel Gossett
NIDDK
daniel.gossett@nih.gov

Susan Gottesman
NCI
gottesms@helix.nih.gov

Erin Green
University of Maryland, Baltimore County
egreen@umbc.edu

Shiv Grewal
NCI
grewals@mail.nih.gov

Joost Gribnau
Erasmus MC
j.gribnau@erasmusmc.nl

Tal Grinshtein
University of Maryland, University College
talgrin@gmail.com

Berkley Gryder
NCI
berkley.gryder@nih.gov

Bjorg Gudmundsdottir
NHLBI
bjorg@mail.nih.gov

Kristbjorn Orri Gudmundsson
Uniformed Services University of the Health Sciences
kristbjorn.gudmundsson.ctr@usuhs.edu

Max Guo
NIA
max.guo@nih.gov

Astrid Haase
NIAMS
astrid.haase@nih.gov

Markus Hafner
NIAMS
markus.hafner@nih.gov

Gordon Hager
NCI
hagerg@exchange.nih.gov

Carol Hamelink
NIH/Center for Scientific Review
hamelinc@csr.nih.gov

YiXing Han
NCI
yi-xing.han@nih.gov

Yoo-Jeong Han
University of Chicago
yjhan@medicine.bsd.uchicago.edu

Christin Hanigan
Johns Hopkins University
clhanigan@gmail.com

Sean Hanlon
NCI
hanlonse@mail.nih.gov

Kasper Hansen
Johns Hopkins University
khansen@jhsph.edu

Nazmul Haque
NHLBI
nazmul.haque@nih.gov

Jennifer Harr
Johns Hopkins University
jharr1@jhmi.edu

Bing He
NCI
bing.he@nih.gov

Ximiao He
NCI
Ximiao.He@nih.gov

Yunlong He
NCI
heyunlong5@gmail.com

Carley Heck
Johns Hopkins University
Check4@jhmi.edu

Dale Hereld
NIAAA
hereldd@mail.nih.gov

Martin Hetzer
Salk Institute for Biological Studies
hetzer@salk.edu

Edward Hinchcliffe
University of Minnesota
ehinchcliffe@hi.umn.edu

Jackson Hoffman
NIEHS
jackson.hoffman@nih.gov

Jingjun Hong
NCI
hongj2@mail.nih.gov

Julie Hong
NCI
hongj@mail.nih.gov

Paul Hook
Johns Hopkins University
phook2@jhmi.edu

Dave Hoon
John Wayne Cancer Institute
hoond@jwci.org

Izumi Horikawa
NCI
horikawi@mail.nih.gov

Bruce Howard
NICHD
howard@helix.nih.gov

Kevin Howcroft
NCI
Howcrofk@mail.nih.gov

Peggy Hsieh
NIDDK
peggyh@niddk.nih.gov

Iawen Hsu
NCI
iawen.hsu@nih.gov

Yu-Jie Hu
University of Massachusetts
YU-JIE.HU@UMASSMED.EDU

Jiaqiang Huang
NCI
jiaqiang.huang@nih.gov

Xiaoqin Huang
aecom
xiaoqinhuang2013@gmail.com

Paula Hyland
NCI
hylandpl@mail.nih.gov

Masahiko Imashimizu
NCI
imashimizum@mail.nih.gov

Maria Ivaldi
NIDDK
maria.ivaldi@nih.gov

Sucharitha Iyer
NIDDK
sucharitha.iyer@nih.gov

Steven Jacobsen
HHMI/University of California, Los Angeles
jacobsen@ucla.edu

Pawan Jain
FDA
pawan.jain@fda.hhs.gov

Deepika Jaiswal
University of Maryland
deepikamhg16@gmail.com

Sonia Jakowlew
NCI
jakowles@mail.nih.gov

Marta Jakubaszek
University of Virginia
jakubaszek.marta@gmail.com

Kennison James
NICHD
Jim_Kennison@nih.gov

Tess Jeffers
Princeton University
tjeffers@princeton.edu

Chamelli Jhappan
NCI
jhappanc@mail.nih.gov

Xinhua Ji
NCI
jix@mail.nih.gov

Xing Jian
NIDDK
jianx2@mail.nih.gov

Yuan Jiang
NCI
yujiang@mail.nih.gov

Wenfei Jin
NHLBI
wenfei.jin@nih.gov

Ron Johnson
NCI
rjohnso2@mail.nih.gov

Yan Kai
George Washington University
smilekai@gwmail.gwu.edu

Vasumathi Kameswaran
University of Pennsylvania
vask@mail.med.upenn.edu

Tomohiko Kanno
NIAID
kannot@mail.nih.gov

Yuka Kanno
NIAMS
kannoy@mail.nih.gov

Eric Karlins
NCI
karlinser@mail.nih.gov

Tatiana Karpova
NCI
karpovats@gmail.com

Judy Kassis
NICHD
jkassis@mail.nih.gov

Sukhbir Kaur
NCI
kaurs@mail.nih.gov

Scott Kelly
NCI
scott.kelly@nih.gov

Irfan Khan
NIA
khanis@mail.nih.gov

Javed Khan
NCI
khanjav@mail.nih.gov

Simran Khurana
NCI
khuranas2@mail.nih.gov

Jeongkyu Kim
NCI
jeongkyu.kim@nih.gov

Jung-Woong Kim
NEI
kimj26@nei.nih.gov

Okjin Kim
University of the District of Columbia
okjin.kim@udc.edu

Rinho Kim
University of Pennsylvania
rinhokim@mail.med.upenn.edu

Soo-Young Kim
NEI
kims16@mail.nih.gov

Sathyan Kizhakke Mattada
University of Virginia
sk8fz@virginia.edu

Amar Klar
NCI
klara@mail.nih.gov

Preeti G. Kochar
NLM
kocharp@mail.nlm.nih.gov

Xiangqian Kong
Johns Hopkins University
xkong9@jhmi.edu

Fedor Kouzine
NCI
kouzinef@mail.nih.gov

Ivan Krivega
NIDDK
krivegai@niddk.nih.gov

Danuta Krotoski
NICHD
krotoskd@mail.nih.gov

Michael Kruhlak
NCI
kruhlakm@mail.nih.gov

Dubravka Kufrin
FDA
dubravka.kufrin@fda.hhs.gov

Ajit Kumar
George Washington University
akumar@gwu.edu

Daman Kumari
NIDDK
damank@helix.nih.gov

Gita Kumari
NIA
gita.kumari@nih.gov

Binbin Lai
NIDDK
binbin.lai@nih.gov

Marikki Laiho
Johns Hopkins University
mlaiho1@jhmi.edu

Ashish Lal
NCI
ASHISH.LAL@NIH.GOV

Andre Larochelle
NHLBI
larochea@nhlbi.nih.gov

Daniel Larson
NCI
dan.larson@nih.gov

Csilla Lazar
NEI
csilla.lazar@nih.gov

Hee-Sheung Lee
NCI
leehes@mail.nih.gov

Hungoo Lee
Rutgers University
btwall@gmail.com

Jae Wook Lee
NHLBI
leejw4@mail.nih.gov

Jong Joo Lee
NIDDK
jongjoo.lee@nih.gov

Maxwell Lee
NCI
leemax@mail.nih.gov

Nathan Lee
NCI
nathan.lee@nih.gov

Nicholas Lee
NCI
nicholas.lee@nih.gov

Terry Lee
NIDDK
tl100s@nih.gov

Elissa Lei
NIDDK
leielissa@niddk.nih.gov

Peter Lemkin
NIDDK
plemkin@lemkingroup.com

Tineke Lenstra
NCI
tineke.lenstra@nih.gov

David Levens
NCI
levens@helix.nih.gov

Elaine Lewis
NIA
elainelewis@nia.nih.gov

Aiguo Li
NCI
liai@mail.nih.gov

Hongchuan Li
NCI
lihongchu@mail.nih.gov

Hualing Li
NIA
hualing.li@nih.gov

Peng Li
NHLBI
peng.li@nih.gov

Quentin Li
NCI
liquenti@mail.nih.gov

Rui Li
NIAID
williamlirui@gmail.com

Shan Li
NCBI
shan.li@nih.gov

Zhihao Li
Johns Hopkins University
pppeirain@gmail.com

Michael Lichten
NCI
mlichten@helix.nih.gov

Hee Woong Lim
University of Pennsylvania
heewlim@mail.med.upenn.edu

Sophie Limou
Leidos Biomedical Research, Inc.
sophie.limou@nih.gov

Billy Lin
NIDDK
billy.lin@nih.gov

Shuye Lin
Beijing Jiaotong University
linshuye@foxmail.com

Xiaoying Lin
Thermo Fisher Scientific
xiaoying.lin@thermofisher.com

Yuquan Lin
NCI
yuquan.lin@nih.gov

Jason Little
Diagenode
jason.little@diagenode.com

Hong Liu
NIDDK
liuhong@mail.nih.gov

Limin Liu
University of Virginia
ll9ec@virginia.edu

Mengying (Mona) Liu
Wayne State University
ed9354@wayne.edu

Poching Liu
NHLBI
pcliu@nhlbi.nih.gov

Xiuhuai Liu
NIAID
liuxiuhuai@mail.nih.gov

Yie Liu
NIA
liuyie@mail.nih.gov

Ying Liu
NICHD
yingl@mail.nih.gov

Zhihui Liu
NCI
liuzhihu@mail.nih.gov

Hernando Lopez-Bertoni
Kennedy Krieger Institute
lopez-bertoni@kennedykrieger.org

Hong Lou
NCI
louho@mail.nih.gov

Xing-Jian Lou
NCI
loux@mail.nih.gov

Ed Luk
Stony Brook University
ed.luk@stonybrook.edu

Teresa Luperchio
Johns Hopkins University
trl@jhmi.edu

Todd Macfarlan
NICHD
todd.macfarlan@nih.gov

Robert Mahon
NIAID
robert.mahon@nih.gov

Kate Malchester
NIDCR
kate.malchester@nih.gov

Mariam Malik
NCI
malikm@mail.nih.gov

Safia Malki
Carnegie Institution for Science
malki@ciwemb.edu

Conrad Mallia
NIAID
cmallia@niaid.nih.gov

Mariana Mandler
NCI
mariana.mandler@nih.gov

Sudhir Manickavel
NIAMS
sudhir.manickavel@nih.gov

Gennady Margolin
NHGRI
gennady.margolin@nih.gov

Ewy Mathe
NIAMS
mathee@mail.nih.gov

Michael Matunis
Johns Hopkins University
mmatunis@jhsph.edu

Ignacio Mazon
Diagenode
ignacio.mazon@diagenode.com

Alison McBride
NIAID
amcbride@nih.gov

Michael McManus
University of California, San Francisco
michael.mcmanus@ucsf.edu

Matt Meiners
EpiCypher - SBIR
matt@epicypher.com

Daniel Melters
NCI
daniel.melters@nih.gov

Paul Meltzer
NCI
pmeltzer@mail.nih.gov

Claudia Mendez-Catala
NIAID
mendezcatalacf@niaid.nih.gov

Jennifer Miller
NCI
millerj@mail.nih.gov

Kyle Miller
University of Texas, Austin
kyle.miller@austin.utexas.edu

Louis Miller
NIAID
lmiller@niaid.nih.gov

Maria Miller
NCI
mariami@mail.nih.gov

Eric Milliman
NIEHS
millimanej@niehs.nih.gov

Tina Miranda
NCI
mirandatb@mail.nih.gov

Lalji Mishra
FDA
lalji.mishra@fda.hhs.gov

Tom Misteli
NCI
mistelit@mail.nih.gov

Khadijah Mitchell
NCI
khadijah.mitchell@nih.gov

Sarah Mitchell
NIA
sarah.mitchell@nih.gov

Suman Mitra
NHLBI
mitras@mail.nih.gov

Takeshi Mizuguchi
NCI
mizuguchit@mail.nih.gov

Amir Moarefi
NYU Medical Center
amir.moarefi@med.nyu.edu

Suresh Mohla
NCI
mohlas@nih.gov

Stephanie Morris
NCI
morriss2@mail.nih.gov

Jianbing Mu
NIAID
jmu@niaid.nih.gov

Kathrin Muegge
NCI
Kathrin.Muegge@nih.gov

Seid Muhie
NCI
seid.muhie@nih.gov

Sanjit Mukherjee
NCI
sanjit.mukherjee@nih.gov

Benedetta Naglieri
NIDDK
benedetta.naglieri@nih.gov

Kyster Nanan
NCI
kyster.nanan@nih.gov

Ezequiel Nazer
NIDDK
ezequiel.nazer@nih.gov

George Nelson
Leidos Biomedical Research, Inc.
nelsong@mail.nih.gov

Paula Nersesian
NINR
paula.nersesian@nih.gov

Lawrence Ng
NIH/Center for Scientific Review
ngkl@csr.nih.gov

Mike Nickerson
NCI
nickersonml@mail.nih.gov

Zuqin Nie
NCI
niezu@mail.nih.gov

Ekaterina Nizovtseva
Fox Chase Cancer Center/Rutgers University
katrinaniz@gmail.com

Ji Heon Noh
NIA
jiheon.noh@nih.gov

Davood Norouzi
NCI
davood.norouzi@nih.gov

Andre Nussenzweig
NCI
nussenza@mail.nih.gov

Jonathan Nye
NCI
jon.nye@nih.gov

Philipp Oberdoerffer
NCI
philipp.oberdoerffer@nih.gov

Shalini Oberdoerffer
NCI
shalini.oberdoerffer@nih.gov

Josefina Ocampo
NICHD
ocampoj@mail.nih.gov

Angelica Ochoa
Georgetown University
ao508@georgetown.edu

Hana Odeh
Johns Hopkins University
hodeh2@jhmi.edu

Young Oh
NHLBI
yoh@mail.nih.gov

Paul Okano
NCI
okanop@mail.nih.gov

Atsushi Onodera
Chiba University
a-onodera@faculty.chiba-u.jp

Andras Orosz
NIAAA
orosza@mail.nih.gov

Ville Paakinaho
NCI
ville.paakinaho@nih.gov

Teresita Padilla-Benavides
University of Massachusetts
Teresita.Padilla@umassmed.edu

Haihui Pan
NHLBI
haihui.pan@nih.gov

Igor Panyutin
NIH/Clinical Center
ip2q@nih.gov

Manikandan Paramasivam
NIA
manikandan.paramasivam@nih.gov

Ki-Sun Park
NICHD
parkk5@mail.nih.gov

Kyeyoon Park
NINDS
parkky@mail.nih.gov

Young-Kwon Park
NIDDK
young-kwon.park@nih.gov

Abbas Parsian
NIAAA
parsiana@mail.nih.gov

Bill Pavan
NHGRI
bpavan@mail.nih.gov

Mike Pazin
NHGRI
pazinm@mail.nih.gov

Alex Pearlman
NIDDK
alexander.pearlman@nih.gov

Michael Pendrak
NCI
pendrakm@mail.nih.gov

Sam Peng
NINDS
pengs@mail.nih.gov

Katherine Peterson
NEI
petersonk@nei.nih.gov

Hanna Petrykowska
NHGRI
petrykowska@mail.nih.gov

Richard Piekarz
NCI
rpiekarz@nih.gov

Genay Pilarowski
Johns Hopkins University
gpialro1@jhmi.edu

Katarzyna Placek
NHLBI
katarzyna.placek@nih.gov

Jennifer Plank
NIDDK
jennifer.plank@nih.gov

Gyongyi Plucer-Rosario
University of Maryland, University College
gyongyi@asia.umuc.edu

Thomas Pohida
NIH/CIT
pohida@nih.gov

Jonathan Pollock
NIDA
jpollock@mail.nih.gov

Yves Pommier
NCI
pommier@nih.gov

Lisa Postow
NHLBI
lisa.postow@nih.gov

Diego Presman
NCI
presmandm@mail.nih.gov

Peter Preusch
NIGMS
preuschp@nigms.nih.gov

Brendan Price
Dana-Farber Cancer Institute
brendan_price@dfci.harvard.edu

Rinki Ratna Priya
NEI
rinki.ratnapriya@nih.gov

Kim Pruitt
NLM
pruitt@ncbi.nlm.nih.gov

Elena Pugacheva
NIAID
epugacheva@niaid.nih.gov

Jiang Qian
Johns Hopkins University
jiang.qian@jhmi.edu

Jin Qiu
NIAID
qiuj2@mail.nih.gov

Yun Qiu
University of Maryland
yqiu@som.umaryland.edu

Delphine Quenet
NCI
delphine.quenet@nih.gov

Mohammad Rahman
NIDDK
mohammad.rahman@nih.gov

Ganesha Rai
NCATS
bantukallug@mail.nih.gov

Sherry Ralls
NICHD
rallssher@mail.nih.gov

Sanjida Rangwala
NCBI
rangwala@ncbi.nlm.nih.gov

Mahadev Rao
NCI
raomahad@mail.nih.gov

Payal Ray
NICHD
payal.ray@nih.gov

Prabhakara Reddi
University of Virginia
ppr5s@virginia.edu

Karen Reddy
Johns Hopkins University
kreddy4@jhmi.edu

Matthew Reilly
NIAAA
reillymt@mail.nih.gov

Jakob Reiser
FDA
Jakob.Reiser@fda.hhs.gov

Xiaojun Ren
Univeristy of Colorado, Denver
xiaojun.ren@ucdenver.edu

Alan Renton
NIA
alan.renton@nih.gov

Thomas Ried
NCI
riedt@mail.nih.gov

Rebecca Riggins
Georgetown University
rbr7@georgetown.edu

Samuel Rivero-Hinojosa
NIAID
samuel.riverohinojosa@nih.gov

Gertraud Robinson
NIDDK
traudl@nih.gov

Ana Robles
NCI
roblesa@mail.nih.gov

Isaac Rodriguez-Chavez
NIDCR
isaac@nidcr.nih.gov

Joseph Rodriguez
NCI
Joseph.Rodriguez@nih.gov

Virginia Rodriguez
NCI
vrodrigu@mail.nih.gov

Sharon Ross
NCI
rosssha@mail.nih.gov

Yazmin Rovira Gonzalez
FDA
yazmin.roviragonzalez@fda.hhs.gov

Arpita Roy
University of the District of Columbia
arpita.roy@udc.edu

Valya Russanova
NICHD
russanov@mail.nih.gov

Denise Russo
NICHD
Drusso1@mail.nih.gov

Lynn Rust
NIAID
lrust@niaid.nih.gov

Kirsten Sadler Edepli
Mount Sinai Hospital
kirsten.edepli@mssm.edu

Layla Saleh
NIH/Clinical Center
layla.saleh@nih.gov

Dalia Salem
NCI
salemda@mail.nih.gov

Morgan Salmon
NHLBI
msa5m@virginia.edu

Maayan Salton
NCI
saltonm@mail.nih.gov

Sheryl Sato
NIDDK
ss68z@nih.gov

John Satterlee
NIDA
satterleej@nida.nih.gov

Iain Sawyer
NCI
iain.sawyer@nih.gov

Paola Scaffidi
Francis Crick Institute
Paola.Scaffidi@cancer.org.uk

Michael Schmidt
NCI
mschmidt@mail.nih.gov

Cara Schornak
University of Illinois, Urbana-Champaign
schorna2@illinois.edu

Mary Schueler
NIH/Center for Scientific Review
marygs@mail.nih.gov

Michael Seidman
NIA
seidmanm@grc.nia.nih.gov

Roza Selimyan
Johns Hopkins University
rselimyan@jhu.edu

Eric Selker
University of Oregon
selker@uoregon.edu

Zeynep Sercan
NCI
zeynep.sercan2@nih.gov

Sigal Shachar
NCI
shachars@mail.nih.gov

Raghavendra Shamanna
NIA
shamannara@mail.nih.gov

Neel Sharma
NEI
neel.sharma@nih.gov

Vivek Sharma
NCI
vivek.sharma@nih.gov

Barbara Ramsay Shaw
Duke University
barbara.r.shaw@duke.edu

Alexey Shaytan
NLM
alexey.shaytan@nih.gov

Karyn Sheaffer
University of Pennsylvania
karyns@mail.med.upenn.edu

Chringma Sherpa
NCI
chringma.sherpa@nih.gov

Han-Yu Shih
NIAMS
han-yu.shih@nih.gov

Takashi Shimbo
NIEHS
shimbot@niehs.nih.gov

Vivek Shukla
NCI
vivek.shukla@nih.gov

Ulrich Siebenist
NIAID
us3n@nih.gov

Dinah Singer
NCI
singerd@mail.nih.gov

Maxine Singer
NCI
msinger@carnegiescience.edu

Joshua Sommers
NIA
SommersJ@grc.nia.nih.gov

Joseph Soucy
NIDDK
joseph.soucy@nih.gov

Aislinn Sowash
Drexel University College of Medicine
ars345@drexel.edu

Hans Spiegel
NIAID
hans.spiegel@niaid.nih.gov

Lisa Steele
NIH/Center for Scientific Review
LISA.STEELE@NIH.HHS.GOV

Kimberly Stephens
Johns Hopkins University
kstephe3@jhu.edu

Todd Stukenberg
University of Virginia
pts7h@virginia.edu

David Sturgill
NCI
davidsturgill@mail.nih.gov

Deqiang Sun
Texas A&M University Health Science Center
dsun@tamu.edu

Jian Sun
aecom
sunjian1949@gmail.com

Lu Sun
Stony Brook University
HIDER1990@GMAIL.COM

Wenyue Sun
NCI
wenyue.sun@nih.gov

Zu-Wen Sun
EpiCypher - SBIR
zwsun@epicypher.com

Myong-Hee "Mia"Sung
NCI
sungm@mail.nih.gov

Michael Surace
NIAID
mjsurace@vcu.edu

Martha Susiarjo
NIEHS
Susiarjo@mail.med.upenn.edu

Anand Swaroop
NEI
swaroopa@nei.nih.gov

Erin Swinstead
NCI
erin.swinstead@nih.gov

Khund Sayeed Syed
NCI
syedsk@mail.nih.gov

Alan Tackett
University of Arkansas
ajtackett@uams.edu

Salman Tajuddin
NIA
salman.tajuddin@nih.gov

Motoki Takaku
NIEHS
motoki.takaku@nih.gov

Wei Tang
NCI
tangw3@mail.nih.gov

Yong Tao
Johns Hopkins University
ytao10@jhmi.edu

Sean Taverna
Johns Hopkins University
staverna@jhmi.edu

Abdul Tawab-Amiri
NCI
atawab@mail.nih.gov

Belete Teferedegne
FDA
belete.teferedegne@fda.hhs.gov

Leyla Teos
NIDCR
teosly@mail.nih.gov

Sam Tetlow
EpiCypher - SBIR
sam@epicypher.com

Marla Tharp
Johns Hopkins University
mtharp1@jhu.edu

Morgan Thenoz
NCI
morgan.thenoz@nih.gov

Carol Thiele
NCI
ct47a@nih.gov

Danielle Thierry-Mieg
NLM
thierryd@mail.nih.gov

Jean Thierry-Mieg
NLM
mieg@ncbi.nlm.nih.gov

Gobi Thillainadesag
NCI
gobi.thillainadesan@nih.gov

Jeremy Thorpe
Johns Hopkins University
thorp2jj@gmail.com

Desiree Tillo
NCI
desiree.tillo@nih.gov

Salih Topal
University of Massachusetts
salih.topal@umassmed.edu

Cecelia Trainor
NIDDK
ceceliat@mail.nih.gov

Michael Tramantano
Stony Brook University
miketram608@gmail.com

Andy Tran
NCI
andy.tran2@nih.gov

Khoa Tran
University of Maryland, Baltimore County
trkhoa1@umbc.edu

Cyndi Trang
NCI
t_cyndi@yahoo.com

Chon Hwa Tsai-Morris
NICHD
morrisch@mail.nih.gov

Laxminath Tumburu
NIDDK
lax.tumburu@nih.gov

Anne-Marie Turner
NIAID
anne-marie.turner@nih.gov

Mudit Tyagi
George Washington University
tmudit@email.gwu.edu

Karen Usdin
NIDDK
ku@helix.nih.gov

Golnaz Vahedi
NIAMS
vahedig@mail.nih.gov

Manuel Valenzuela
Meharry Medical College
mvalenzuela@mmc.edu

Haritha Vallabhaneni
FDA
hvallab1@hotmail.com

Haico van Attikum
Leiden University
h.van_attikum@lumc.nl

Garima Varma
NCI
garima.varma@nih.gov

Jaira Vasconcellos
NIDDK
jaira.vasconcellos@nih.gov

Michelle Vaz
Johns Hopkins University
mvaz1@jhmi.edu

Jose Velazquez
NIA
jvelazqu@mail.nih.gov

Veronica Veschi
NCI
veronica.veschi@nih.gov

Charles Vinson
NCI
vinsonc@mail.nih.gov

Bandana Ajay Vishwakarma
Uniformed Services University of the Health Sciences
bandana-ajay.vishwakarma@usuhs.edu

Jodi Vogel
NIAID
jvogel@niaid.nih.gov

Nadarajen Vydelingum
NCI
vydelinn@mail.nih.gov

Paul Wade
NIEHS
wadep2@niehs.nih.gov

Anil Wali
NCI
walia@mail.nih.gov

Yihan Wan
Carnegie Institution for Science
wan@ciwemb.edu

C.-L. Albert Wang
NIH/Center for Scientific Review
cl.wang@nih.gov

Difei Wang
NCI
wangdi@mail.nih.gov

Julia Wang
University of Pennsylvania
yuewang1@mail.med.upenn.edu

Lan-Hsiang Wang
NHLBI
Lw72f@nih.gov

Limin Wang
NIDDK
limin.wang@mail.nih.gov

Shuang Wang
Mount Sinai Hospital
shuang.wang@mssm.edu

Solita Wang
NIH/Center for Scientific Review
chiayeng.wang@nih.gov

Weixin Wang
NIH/Clinical Center
wangw6@mail.nih.gov

Josh Waterfall
NCI
waterfalljj@mail.nih.gov

Jocelyn Weissman
NCI
jdw@mail.nih.gov

James Welch
NIDDK
james.welch@nih.gov

Sossou Urbain Weyemi
NCI
urbain.weyemi@nih.gov

Brad Willox
Brigham and Women's Hospital/Harvard University
BWILLOX@partners.org

Gernot Wolf
NICHD
gernot.wolf@nih.gov

Kyoung Jae Won
University of Pennsylvania
wonk@upenn.edu

Bin Wu
Headway Workforce Solution
binwusuccess@gmail.com

Bingruo Wu
Albert Einstein College of Medicine
bingruo.wu@einstein.yu.edu

Carl Wu
NCI
carlwu@helix.nih.gov

Roy Wu
NCI
wur@ctep.nci.nih.gov

Warren Wu
NICHD
warren.wu@nih.gov

Yuaner Wu
NINR
yuaner.wu@nih.gov

Andrea Wurster
NIAID
wurstera@mail.nih.gov

Limin Xia
Johns Hopkins University
lxia7@jhmi.edu

Tiaojiang Xiao
NIDDK
xiaotiao@niddk.nih.gov

Wenbing Xie
Johns Hopkins University
wxie6@jhmi.edu

Jianhua Xiong
NHLBI
jianhua.xiong@nih.gov

Jin Xu
University of Maryland, Baltimore
jinxu@umaryland.edu

Zuoyu Xu
NIAID
ZX15P@NIH.GOV

Catherine Xue
Montgomery Blair High School
cathysxue@gmail.com

Manish Kumar Yadav
JSS Medical College
manishdr088@gmail.com

Sharda Yadav
NEI
yadavsh@mail.nih.gov

Chul Min Yang
NIDDK
chulmin.yang@nih.gov

Fan Yang
NCI
fyang@mail.nih.gov

Hyun-Jin Yang
NEI
hyun-jin.yang@nih.gov

Liming Yang
NCI
lyang@mail.nih.gov

Peng Yang
NICHD
peng.yang@nih.gov

Rose Yang
NCI
royang@mail.nih.gov

Xi Yang
University of Maryland, Biotechnology Institute
yyangxi2002@gmail.com

Yanqin Yang
NHLBI
yanqin.yang@nih.gov

Ray-Whay Chiu Yen
Johns Hopkins University
ryen1@jhmi.edu

Je-Hyun Yoon
NIA
yoonjehyun@gmail.com

Matthew Young
NCI
youngma@mail.nih.gov

Richard Young
Whitehead Institute
young@wi.mit.edu

Jing Jie Yu
West Virginia University
jyu@hsc.wvu.edu

Hussain Zaidi
University of Virginia
haz4z@virginia.edu

Linda Zane
NCI
zanelk@mail.nih.gov

Manzoor Zarger
NIH/Center for Scientific Review
zargerma@csr.nih.gov

Ewelina Zasadzinska,
University of Virginia
ez4e@virginia.edu

Wei Zhang
NCI
zhangwe@mail.nih.gov

Chi Zhang
Mount Sinai Hospital
zhangchiyy@gmail.com

Hongliang Zhang
NCI
hongliangzhang@mail.nih.gov

Shaofei Zhang
NCI
zhangs9@mail.nih.gov

Shile Zhang
NCI
zhangs6@mail.nih.gov

Ya Zhang
NCI
zhangy15@mail.nih.gov

Bo Zhao
Brigham and Women's Hospital/Harvard
Medical School
bzhao@partners.org

Haiqing Zhao
NCI
haiqing.zhao@nih.gov

Bin Zhou
Albert Einstein College of Medicine
bin.zhou@einstein.yu.edu

Zhuo Zhou
NIDDK
zhouz2@mail.nih.gov

Feng Zhu
NCI
zhuf2@mail.nih.gov

Jun Zhu
NHLBI
jun.zhu@nih.gov

Lenan Zhuang
NIDDK
lenan.zhuang@nih.gov

Daniel Zilberman
University of California, Berkeley
danielz@berkeley.edu

Adriana Zingone
NCI
adriana.zingone@nih.gov