CEEHRC NETWORK
Canadian Epigenetics, Environment and Health Research Consortium Network

THE 3RD CANADIAN CONFERENCE ON EPIGENETICS

EPIGENOMICS in DEVELOPMENT and DISEASE

18-21 September 2016
Estérel Resort · Estérel, QC, Canada

EPIGENETICS CANADA
GenomeCanada
CIHR IRSC
ESTÉREL RESORT

Welcome!

Dear Colleagues,

It is our great pleasure to welcome you to the Estérel Resort for the 3rd Canadian Conference on Epigenetics: "Epigenomics in Development and Disease".

The field of epigenetics has seen explosive growth in recent years. This is due in part to the realization of a link between environmental- and age-related changes to epigenetic patterning and modes of disease progression. Despite this insight, we have not yet translated environmental changes predisposing to disease into robust molecular signatures. Additionally, although model organism studies have revealed that epigenetic processes are highly conserved, epigenomic mechanisms within other areas of the life sciences (e.g. livestock, agriculture, and aquaculture) have received less attention. There is therefore significant potential for continued growth in epigenomic research.

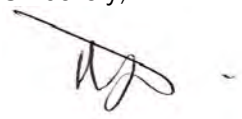
This symposium is intended to bring together a critical mass of Canadian epigenetics researchers, along with key international leaders in the field, to engage in cross-disciplinary dialogue on recent advancements in the field of epigenetics with a focus on the impact of epigenetics on human disease and development. The scientific program includes distinguished invited speakers in the fields of epigenetics and epigenomics covering topics including basic chromatin biology, epigenetic profiling, epigenetic markers, and new developments and advancements in epigenomics research. These topics will be covered over 8 sessions that include keynote lectures and short oral presentations, 2 workshops, and 2 poster sessions.

We especially encourage interaction and networking between young scientists and Principal Investigators, and hope to foster an atmosphere that encourages scientific collaboration and discussions about science and careers in the field of epigenetics and epigenomics.

This year's meeting is the first to be coordinated by the new Canadian Epigenetics, Environment and Health Research Consortium (CEEHRC) Network, in collaboration with the organizers of the successful "Epigenetics, Eh!" conferences. The aim of the Network is to connect Canadian epigenetics researchers and expand their reach to the broader health research community in Canada and beyond. We also facilitate access to epigenomics data; produce and curate epigenetic and epigenomics tools, software and protocols; and curate Canadian jobs, training opportunities and events listings. More information is available at **www.epigenomes.ca**. We welcome your feedback on what you'd like to see from future meetings, and from the Network in general.

On behalf of the Organizing Committee, we wish you a warm welcome to Estérel.

Sincerely,



Martin Hirst

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Organizing Committee

Dr. Cheryl Arrowsmith
University of Toronto

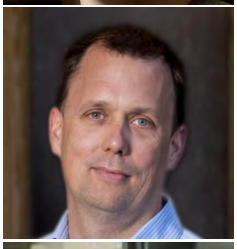
Dr. Nathalie Bérubé
Western University

Dr. Guillaume Bourque
McGill University

Dr. Cath Ennis
BC Cancer Agency

Dr. Leonard Foster
University of British Columbia

Dr. Steven Jones
BC Cancer Agency



Dr. Martin Hirst
University of British Columbia

Dr. Tony Kwan
McGill University

Dr. Mathieu Lupien
Princess Margaret Cancer Centre

Dr. Tomi Pastinen
McGill University

Ms. Sunshine Purificacion
BC Cancer Agency

Speakers

Invited Speakers

Cheryl Arrowsmith, PhD

Professor, Department of Medical Biophysics, University of Toronto

Dr. Arrowsmith is a Senior Scientist at the Princess Margaret Cancer Centre and Professor in the Department of Medical Biophysics at the University of Toronto, where she holds a Canada Research Chair in Structural Genomics. She received a Ph.D. in chemistry from the University of Toronto and carried out postdoctoral research at Stanford University in the area of protein NMR spectroscopy. Dr. Arrowsmith's research focuses on structural and chemical biology of chromatin and epigenetic regulatory factors especially as relates to cancer. She is the Chief Scientist of the Toronto Node of the Structural Genomics Consortium (SGC) where she leads the SGC's program to develop chemical probes to chromatin regulators for target validation.

Nathalie Bérubé, PhD

Associate Professor, Departments of Biochemistry and Paediatrics, Western University

Dr. Bérubé is an Associate Professor in the Departments of Paediatrics, Biochemistry and Oncology at Western University, and Scientist at the Children's Health Research Institute. Using a multipronged approach, research in her laboratory probes the function of epigenetic regulators involved in neurodevelopmental disorders and intellectual disability (ID). Her group discovered that inactivation of the ATR-X syndrome gene causes extensive genomic instability in neural stem cells due to DNA replication stress, telomere dysfunction and mitotic abnormalities. Studies in her laboratory continue to examine the connection between the ATRX, MeCP2 and CTCF chromatin proteins and the relationship that exists between various neurodevelopmental disorders, including schizophrenia, autism, Rett and ID syndromes.

Marjorie Brand, PhD

Professor, Department of Medicine, University of Ottawa

Marjorie started her lab at the Ottawa Hospital Research Institute (OHRI) in September 2004. She received a PhD in Molecular Biology (2001) from Université Louis Pasteur (Strasbourg, France) and did her postdoctoral studies (2001-04) at the Fred Hutchinson Cancer Research Center (Seattle). Marjorie is a Senior Scientist at the Sprott Center for Stem Cell Research in the OHRI and a Full Professor in the Department of Medicine at the University of Ottawa. She was elected to the Royal Society of Canada's College of New Scholars, Artists and Scientists in 2014.

Daniel De Carvalho, PhD

Assistant Professor, Department of Medical Biophysics, University of Toronto

Daniel De Carvalho is a former postdoctoral research fellow at USC Norris Comprehensive Cancer Center who now works at Princess Margaret Cancer Centre, University Health Network in Toronto, Canada. His research focuses on understanding the epigenetic mechanisms underlying tumorigenesis and translate this knowledge into more efficient approaches for epigenetic therapy. He uses truly multi-disciplinary approach to discover what mechanisms are driving cancer-specific epigenetic alterations and translates this knowledge into more efficient epigenetic interventions.

Yali Dou, PhD

Associate Professor, Dept of Pathology and Biological Chemistry, University of Michigan

Dr. Dou received a Bachelor's in Medicine from Peking University (1996) and a PhD (Biology, 2000) from the University of Rochester, New York, followed by postdoctoral training (2002-06) at The Rockefeller University. In 2006, she joined the Department of Pathology as an Assistant Professor. Dr. Dou has received a number of prestigious awards including the Leukemia & Lymphoma Society Scholar Award (2012), Stand Up to Cancer IRG Award (2011), AACR Gertrude B. Elion Cancer Research Award (2010).

Daniel Gaffney, PhD

CDF Group Leader, Wellcome Trust Sanger Institute

Dr. Daniel Gaffney earned his PhD in evolutionary genetics from Edinburgh University in 2006. Following postdoctoral studies at McGill University (2006-08) and the University of Chicago (2008-11), Daniel started as a Career Development Fellowship Group Leader at the Wellcome Trust Sanger Institute. His focus is to understand the molecular and cellular consequences of genetic changes in gene regulatory regions by combining statistical genetics with high-throughput experimental techniques in human cells. His group makes extensive use of human induced pluripotent stem cells as model systems to understand how genetic changes between individuals influence cellular phenotypes such as chromatin structure and transcription.

Nada Jabado, MD, PhD

Professor, Department of Human Genetics, McGill University

Dr. Nada Jabado is a clinician-scientist at the Research Institute of the McGill University Health Center studying brain tumors, the leading cause of cancer-related mortality and morbidity in the pediatric years. Her group established that pediatric high-grade astrocytomas (HGA) are molecularly and genetically distinct from adult tumors. They identified a new molecular mechanism driving pediatric HGA, recurrent somatic driver mutations that are tightly correlated with a distinct global DNA methylation pattern, as well as neuroanatomical and age specificities. She aims to identify events affected downstream of each mutation, and validate targets to better advise the use of experimental or pipeline drug(s) for rapid translation into clinical trials.

Sarah Kimmins, PhD

Associate Director, McGill Centre for the Study of Reproduction, McGill University

Sarah Kimmins was appointed in 2005 as an assistant professor in the department of Animal Science in the faculty of Agricultural and Environmental Sciences at McGill University in Montréal. She received her PhD in Reproductive Biology from Dalhousie University in 2003. Her post-doctoral training in spermatogenesis and transcription was undertaken at the Université Louis Pasteur, at the Institut de génétique et de biologie moléculaire et cellulaire in Strasbourg, France. Her research is focused on determining the role of epigenetics in germ cell development, fertility and offspring health.

Michael Kobor, PhD

Professor, Department of Medical Genetics, University of British Columbia

Dr. Michael S. Kobor is a Professor in the Dept. of Medical Genetics at UBC, and a Senior Scientist at the Centre for Molecular Medicine and Therapeutics in the Child and Family Research Institute (CFRI). He holds the Canada Research Chair in Social Epigenetics, is a Senior Fellow of the Canadian Institute for Advanced Research (CIFAR) Child and Brain Development Program, and is Director of the Program on Social Epigenetics at the Human Early Learning Partnership (HELP) at UBC's School of Population and Public Health. Dr. Kobor's own research program is focused on illuminating the developmental origins of health and disease through combining fundamental discovery research in model organisms with translational research in human populations.

Janine LaSalle, PhD

Professor, Dept of Medical Microbiology and Immunology, University of California, Davis

Dr. LaSalle is a Professor of Microbiology and Immunology at the University of California, Davis, with memberships in the Genome Center, and the MIND Institute. Dr. LaSalle serves as the Associate Director of Genomics at the UC Davis Genome Center, Chair of the Genes in Health and Disease study section for the NIH, and is on the editorial board of Human Molecular Genetics, Molecular Autism, and Environmental Epigenetics. Dr. LaSalle's laboratory focuses on epigenetics of neurodevelopmental disorders, including autism, Rett, Prader-Willi, Angelman, and Dup15q syndromes using genomic and epigenomic technologies to investigate the role of DNA methylation and MeCP2 in the pathogenesis of Rett syndrome and autism spectrum disorders.

John Lis, PhD

Professor, Department of Molecular Biology and Genetics, Cornell University

John Lis received his Ph.D. in Biochemistry from Brandeis University, did postdoctoral research at Stanford, joined the faculty at Cornell University in 1978, and is the Barbara McClintock Professor of Molecular Biology and Genetics. His research is focused on transcription regulation and developing technologies to study transcription in cells and genome-wide.

Matt Lorincz, PhD

Professor, Department of Medical Genetics, University of British Columbia

Matthew Lorincz is an Associate Professor in the Department of Medical Genetics at the University of British Columbia. He holds a Ph.D. in Genetics from Stanford University (1997) and carried out postdoctoral research at the Fred Hutchinson Cancer Research Center (Seattle) in epigenetics and transcriptional regulation. Dr. Lorincz joined UBC in 2005. His research is aimed at dissecting the interplay between DNA methylation and covalent histone modifications in transcriptional regulation of genes and transposable elements using the mouse as a model system. Dr. Lorincz is Head of the Molecular Epigenetics Group at the Life Science Institute at the UBC and co-PI on the CIHR CEEHRC Epigenomic Data Coordination Centre (EDCC).

Tim Reddy, PhD

Assistant Professor, Department of Biostatistics and Bioinformatics, Duke University

Tim Reddy is an Assistant Professor in the Duke Center for Genomic & Computational Biology and the Dept of Biostatistics & Bioinformatics. He holds a secondary appointment in Biomedical Engineering and the Dept of Molecular Genetics & Microbiology. His lab is focused on understanding how genetic variation that alters gene regulation contributes to human traits including risk for common diseases such as diabetes through an interdisciplinary combination of high-throughput genomics, computational biology, and traditional molecular biology and genetics experiments. The hope is to better understand the gene-regulatory bases for human evolution and human disease and reveal new pathways of disease that can ultimately be targeted for novel diagnostics and treatment.

Alex Ruthenburg, PhD

Assistant Professor, Depts of Molecular Genetics and Cell Biology, Biochemistry and Molecular Biology, University of Chicago

Dr. Alex Ruthenburg joined the Department of Molecular Genetics and Cell Biology at UChicago in July 2010. After completing his PhD in Structural and Chemical Biology at Harvard, Dr. Ruthenburg joined the laboratory of Dr. David Allis at Rockefeller University, where he characterized the structural and biophysical properties of histone binding protein complexes with a focus on multivalency and cooperative binding. He will continue his work on defining the molecular basis for recognition of modified histones by various nuclear regulators as he develops his research program at UChicago.

Yang Shi, PhD

Merton Bernfield Professor of Neonatology (BCH), Professor of Cell Biology, Harvard Medical School

Yang Shi joined Harvard Medical School as an assistant professor in 1991 and was appointed a Professor of Pathology in 2004. He is currently a Professor of Cell Biology. He is also the Merton Bernfield Professor of Neonatology in the Division of Newborn Medicine at Children's Hospital Boston. Yang Shi received his PhD from NYU and postdoctoral training at Princeton University.

Li-Huei Tsai, PhD

Director, The Picower Institute for Learning and Memory, Massachusetts Institute of Technology

Professor Li-Huei Tsai is the Director of the Picower Institute for Learning and Memory at MIT, a Picower Professor of Neuroscience, and an Associate Member of the Broad Institute. She is interested in elucidating the pathogenic mechanisms underlying neurological disorders that impact learning and memory. She takes a multidisciplinary approach to investigate the molecular, systems, and circuit basis of neurological disorders. Recent contributions include the identification of chromatin remodeling as a means to regulate memory gene expression and enhance cognitive function during neurodegeneration. Her lab also conducts epigenomic analysis of mouse and human Alzheimer's disease (AD) brain samples and has identified important contributions of dysregulated immune response genes in AD. Currently, the Tsai lab uses induced pluripotent stem cell (iPSCs) derived from human subjects to model AD and large scale imaging, optogenetics, and in vivo electrophysiology to study the brain circuitry affected by AD.

Workshop Speakers

Christopher Carlsten, MD, MPH

Associate Professor, Department of Medicine, University of British Columbia

Dr. Carlsten is an Associate Professor of Medicine, and Astra-Zeneca Chair and Canada Research Chair in Occupational and Environmental Lung Disease at The University of British Columbia. He also holds adjunct positions at the Peter Wall Institute for Advanced Studies, the UBC School of Population and Public Health and the James Hogg Centre for Heart Lung Innovation. He is the Director of the Occupational Lung Disease Clinic in the Vancouver General Hospital. The Carlsten laboratory focuses on the respiratory and immunological health effects of inhaled environmental and occupational exposures.

Gustavo Turecki, MD PhD

Professor and Chair, Department of Psychiatry, McGill University

Gustavo Turecki MD PhD is Professor and Chair of the Department of Psychiatry at McGill University. He is also the Director of the McGill Group for Suicide Studies (www.mgss.ca), the co-director of the Douglas Bell-Canada Brain Bank (www.douglasbrainbank.ca), and the director of the FRQS *Réseau québécois sur le suicide, les troubles de l'humeur et les troubles associés (www.reseausuicide.qc.ca)*. Gustavo's work uses functional genomic approaches to understand brain molecular changes that occur in major depression and molecular processes that explain treatment response. In addition, his work aims to elucidate the neurobiological basis of th e suicidal brain.

Misha Bilenky, PhD

Staff Scientist, Bioinformatics, BC Cancer Agency – Genome Sciences Centre

After being involved into a number of computational studies of the gene regulatory networks using phylogenetic footprinting, during last decade I worked on in the tool development and analysis of the Next-Generation-Sequencing experiments studying transcription factor DNA interaction, variety of histone modifications, DNA methylation, and gene expression by RNA-seq. The projects I was part of include early stages of ENCODE, the NIH Roadmap Project (REMC), and more recently CEEHRC - Canadian contribution to IHEC.

David Bujold

Project Manager & Software Developer in Bioinformatics, McGill University

David works in Dr. Guillaume Bourque's lab on software solutions in bioinformatics for organizing, visualizing and analyzing datasets produced by large-scale projects such as the International Human Epigenome Consortium (IHEC), which maps human epigenomes for a broad spectrum of cell types and diseases. He is also involved in the development of GenAP, a platform that leverages Compute Canada infrastructure to make bioinformatics analysis more accessible to non-bioinformaticians, and reduces data processing bottlenecks.

Selected Speakers

Carolyn Brown, PhD

Professor, Department of Medical Genetics, University of British Columbia

Dr. Brown obtained her PhD (1990) in Medical Genetics from the University of Toronto. During her PhD and postdoctoral work with Dr. Willard at Stanford and Case Western Reserve University, she studied the process of X chromosome inactivation, identifying genes expressed from the inactive X, including the XIST gene, which is a key initiator of X chromosome silencing. She joined the University of British Columbia (1994), where her research group studies X-chromosome inactivation, focusing on the role of the XIST RNA in initiating the heterochromatic changes that accompany X-chromosome silencing as well as the DNA elements that allow some genes to escape inactivation. As the process of X inactivation silences the orange or black X-linked coat colour alleles in cats, her cat, Helix, serves as a visible example of X-chromosome inactivation.

Jacques Côté, PhD

Professor, Department of Medical Genetics, Laval University

Dr. Côté obtained his PhD from Laval University after he characterized the structure and function of Endo G, an endonuclease involved in the replication of mitochondrial DNA and cell apoptosis. He did post-doctoral training with Dr. Jerry Workman at Penn State University where he identified and characterized chromatin remodeling complexes (SWI/SNF) involved in gene activation. He joined the Laval University Cancer Research Center (1997) where he holds the Canada Research Chair in Chromatin Biology and Molecular Epigenetics. His projects characterize the dynamic role of chromatin in the regulation of nuclear functions and cell proliferation. His work uses the yeast model system and human cells to define the structure, function and regulation of histone acetyltransferase complexes implicated in genome expression and maintenance.

Elin Grundberg, PhD

Assistant Professor, Department of Human Genetics, McGill University

Dr. Grundberg completed her PhD (2006) in Experimental Medicine at Uppsala University, Sweden and following post-doctoral training at the McGill University and Genome Quebec Innovation Centre. She joined Wellcome Trust Sanger institute in 2009 to lead the international MuTHER Consortium, a major resource of detailed genomics and epigenomics data from multiple disease-targeted tissues from twins. In 2012, she was appointed Assistant Professor at McGill University where her group is applying various next-generation sequencing approaches to understand genetic and epigenetic factors underlying complex disease susceptibility. Dr. Grundberg's main area of investigation concerns metabolic diseases and she leads two CEEHRC-CIHR team grants on this topic with partners in Canada and Europe.

Louis Lefebvre, PhD

Associate Professor, Department of Medical Genetics, University of British Columbia

Dr. Lefebvre obtained his BSc in biochemistry from the Université Laval (1987) before moving to the University of British Columbia for a PhD in biochemistry and molecular biology (1994) under the supervision of the Nobel Laureate Michael Smith, studying transcription regulation in yeast. He then completed two post-doctoral fellowships studying genomic imprinting in the mouse, first with Azim Surani at the Gurdon Institute, Cambridge (1998), then with Andras Nagy at the Lunenfeld-Tanenbaum Research Institute in Toronto (2003). He was recruited back at UBC in Vancouver in 2003, where he is an associate professor in Medical Genetics and held a Canada Research Chair (2003-2013). His research program mainly focuses on understanding the mechanism and function of genomic imprinting in mammals.

William Muller, PhD

Professor, Department of Biochemistry, McGill University

Dr. Muller is currently a Professor in Departments of Biochemistry and Medicine at McGill University. Dr. Muller is recognized as one of the leaders in the development of transgenic mouse models of human breast cancer. In recognition of his important contributions to the development and characterization of these transgenic mouse models of human breast cancer, Dr. Muller was recently awarded a CRC chair in Molecular Oncology at McGill University. Dr. Muller's extensive collaborations with numerous laboratories around the world have already provided important insight into the molecular basis for breast cancer progression.

Art Petronis, MD, PhD

Professor, Department of Pharmacology and Toxicology, University of Toronto

Art Petronis graduated from Kaunas Medical University, Lithuania, and he worked on his PhD at the Brain Research Institute in Moscow. Dr Petronis completed his post-doctoral training at the Clarke Institute of Psychiatry, Toronto, and since 1997 he has been a faculty at the Centre for Addiction and Mental Health, and the University of Toronto. Currently Dr. Petronis is Professor and Head of the Krembil Family Epigenetics Laboratory. He is also Tapscott Chair for Schizophrenia Studies at the University of Toronto. His research is dedicated to the elucidation of the role of epigenetic factors in complex non-Mendelian diseases such as schizophrenia, diabetes, and inflammatory bowel disease. Dr. Petronis published over 100 papers and book chapters (but only 5 of them are worth reading).

Short Talks

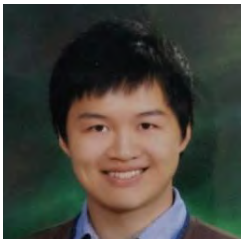
Julie Brind'Amour, PhD
Postdoctoral Fellow, Dept of Medical Genetics
University of British Columbia

Genevieve Deblois, PhD
Postdoctoral Fellow,
Department of Medical Biophysics
University of Toronto

Stephanie Dyke, PhD
Academic Associate,
Centre of Genomics and Policy
McGill University

David Labbé, PhD
Research Fellow,
Department of Medical Oncology
Harvard Medical School

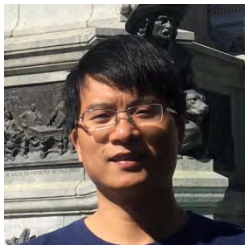
Jean-Philippe Lambert, PhD
Postdoctoral Fellow,
Department of Molecular Genetics
University of Toronto



Minggao Liang
Graduate Student,
Department of Molecular Genetics
University of Toronto

Simon Papillon-Cavanagh
Graduate Student,
Department of Human Genetics
McGill University

Davide Pellacani, PhD
Postdoctoral Fellow, Terry Fox Laboratory
British Columbia Cancer Agency



Xiaojian Shao, PhD
Postdoctoral Fellow,
Department of Human Genetics
McGill University

Xuefei Yuan
Graduate Student,
Department of Molecular Genetics
University of Toronto

Program Agenda

Sunday, September 18, 2016

4:00 pm - 7:00 pm	**Registration** *Reception Emotion*
7:00 pm - 9:00 pm	**BBQ Mixer** *Salon Dupuis / Terrasse*

Monday, September 19, 2016

7:00 am - 8:15 am	**Breakfast** *Bistro à Champlain / 260°*
8:15 am - 8:20 am	**Open Meeting** *Fridolin-Simard*
Session 1: High-Throughput Epigenome Mapping Chair: Martin Hirst *Fridolin-Simard*	
8:20 am - 9:00 am	Tim Reddy (Duke University) *Regulatory mechanisms of human disease*
9:00 am - 9:40 am	Alex Ruthenburg (University of Chicago) *Measuring patterns within the nucleosomal unit with Internal-standard calibrated ChIP (ICeChIP)*
9:40 am - 10:10 am	Carolyn Brown (University of British Columbia) *Using exceptions to identify human DNA elements regulating X-chromosome inactivation*
10:10 am - 10:25 am	Davide Pellacani (University of British Columbia) *Derivation of transcription factor networks from analyses of active enhancer states in different subsets of normal human mammary cells*
10:25 am - 10:40 am	**Coffee Break** *Foyer Fridolin-Simard*
Session 2: Regulation of the Epigenome During Development I Chair: Nathalie Bérubé *Fridolin-Simard*	
10:40 am - 11:20 am	Yang Shi (Harvard Medical School) *An RNA modification in the DNA damage response*
11:20 am - 12:00 pm	Sarah Kimmins (McGill University) *Determining the role of the sperm epigenome in offspring development and health*
12:00 pm - 12:15 pm	Xuefei Yuan (University of Toronto) *From mammals to fish and back again: discovering new regulators of early cardiac development*
12:15 pm - 12:30 pm	Julie Brind'Amour (University of British Columbia) *Impact of maternally inherited histone modifications on DNA methylation maintenance and enhancer activation in the early embryo*

12:30 pm - 2:00 pm	**Lunch** *Bistro à Champlain / 260°*
Workshop 1: Challenges of Clinical Epigenomics Chair: Martin Hirst *Fridolin-Simard*	
2:00 pm - 3:30 pm	Christopher Carlsten (University of British Columbia) *Exposures and Epigenomics: Promises and Pitfalls in the Context of Air Pollution* Gustavo Turecki (McGill University) *ncRNA predictors and mediators of antidepressant response*
3:45 pm - 4:00 pm	**Coffee Break** *Foyer Fridolin-Simard*
Session 3: Mechanisms of Epigenomic Perturbation in Disease Chair: Steven Jones *Fridolin-Simard*	
4:00 pm - 4:40 pm	Li-Huei Tsai (Massachusetts Institute of Technology) *Cell type specific epigenomic analysis in the neurodegenerating brain*
4:40 pm - 5:20 pm	Yali Dou (University of Michigan) *MLL in the control of cell identity and disease*
5:20 pm - 5:50 pm	William Muller (McGill University) *Polycomb Repressor Complex 2 is translationally upregulated by c-SRC and mTORC1 in ERBB2-driven breast cancer*
5:50 pm - 6:05 pm	Minggao Liang (SickKids) *Rewiring of enhancer-gene interactions drives PLAU overexpression in the pathogenesis of Quebec Platelet Disorder*
6:05 pm - 8:00 pm	**Dinner** *Bistro à Champlain / 260°*
Poster Session 1 *Salon Dupuis* illumina	
8:00 pm - 10:00 pm	See Poster Assignments (page 17)

Tuesday, September 20, 2016

7:00 am - 8:30 am	**Breakfast** *Bistro à Champlain / 260°*
Session 4: Epigenome-Wide Association Studies Chair: Guillaume Bourque *Fridolin-Simard*	
8:30 am - 9:10 am	Daniel Gaffney (Wellcome Trust Sanger Institute) *Mapping regulatory variants using ATAC-seq in stimulated immune cells*
9:10 am - 9:50 am	Michael Kobor (University of British Columbia) *Epigenetic embedding of early life environments*
9:50 am - 10:20 am	Elin Grundberg (McGill University)

	Capturing functional epigenomes for insight into metabolic disease susceptibility
10:20 am - 10:35 am	Xiaojian Shao (McGill University) *Dynamic DNA methylation changes upon smoking exposures revealed by large-scale next generation sequencing studies*
10:35 am - 10:50 am	**Coffee Break** *Foyer Fridolin-Simard*
Session 5: Cancer Epigenomics Chair: Mathieu Lupien *Fridolin-Simard*	
10:50 am - 11:30 am	Nada Jabado (McGill University) *'Oncohistones' in cancer: Where are we at?*
11:30 am - 12:10 pm	Daniel De Carvalho (University of Toronto) *DNA methylation: Biomarker and therapeutic target in cancer*
12:10 pm - 12:40 pm	Jacques Côté (Université Laval) *Oncogenic chromosomal translocations involving the NuA4/TIP60 and PRC2 complexes lead to aberrant chromatin landscape*
12:40 pm - 12:55 pm	Simon Papillon-Cavanagh (McGill University) *Impaired H3K36 methylation defines a subset of head and neck squamous cell carcinomas*
12:55 pm - 2:00 pm	**Lunch** *Bistro à Champlain / 260°*
Workshop 2: Epigenomics Tools and Resources Chair: Guillaume Bourque *Fridolin-Simard*	
2:00 pm - 3:30 pm	Misha Bilenky (BC Cancer Agency) *On the analysis and interpretation of the ChIP-seq data* David Bujold (McGill University) *Epigenomics Data Analysis using Public Datasets*
3:45 pm - 4:00 pm	**Coffee Break** *Foyer Fridolin-Simard*
Session 6: New Analytical and Experimental Approaches in Epigenomics Chair: Martin Hirst *Fridolin-Simard*	
4:00 pm - 4:40 pm	John Lis (Cornell University) *Elucidating transcriptional regulatory mechanisms in cells & across genomes*
4:40 pm - 5:20 pm	Cheryl Arrowsmith (University of Toronto) *Probing the epigenome using chemical biology*
5:20 pm - 5:35 pm	Genevieve Deblois (University of Toronto) *Epigenetic vulnerabilities arising from metabolic adaptation to taxane resistance in triple-negative breast cancer*
5:35 pm - 5:50 pm	Jean-Philippe Lambert (Lunenfeld-Tanenbaum Research Institute) *Functional proteomics characterization of the human acetyl lysine machinery*

6:00 pm - 8:00 pm	**Dinner** *Bistro à Champlain / 260°*
Poster Session 2 *Salon Dupuis*	
8:00 pm - 10:00 pm	See Poster Assignments (page 17)

Wednesday, September 21, 2016

7:00 am - 8:00 am	**Breakfast** *Bistro à Champlain / 260°*
Session 7: Regulation of the Epigenome During Development II Chair: Cheryl Arrowsmith *Fridolin-Simard*	
8:00 am - 8:40 am	Nathalie Bérubé (Western University) *Epigenetic regulation in neurodevelopmental disorders*
8:40 am - 9:20 am	Marjorie Brand (University of Ottawa) *Epigenetic regulation of hematopoiesis and leukemogenesis*
9:20 am - 9:50 am	Louis Lefebvre (University of British Columbia) *Regulation of genomic imprinting: from spreading to erasure*
9:50 am - 10:05 am	Stephanie Dyke (McGill University) *Epigenomics ELSI and the media*
10:05 am - 10:20 am	**Coffee Break** *Foyer Fridolin-Simard*
Session 8: Environmental Influences on the Epigenome Chair: Tomi Pastinen *Fridolin-Simard*	
10:20 am - 11:00 am	Janine Lasalle (University of California, Davis) *Epigenomic impact of multi-hit duplications and PCB exposures in autism spectrum disorder*
11:00 am - 11:40 am	Matt Lorincz (University of British Columbia) *Impact of LTR retrotransposons on the transcriptome, methylome & imprintome in the mouse germline*
11:40 am - 12:10 pm	Art Petronis (University of Toronto) *Circadian hallmarks of the aging epigenome*
12:10 pm - 12:25 pm	David Labbé (Harvard University) *Diet-induced obesity enhances MYC-driven prostate cancer through a coordinated metabolic and epigenetic rewiring*
12:25 pm - 12:35 pm	**Close Meeting**
CEEHRC Executive Meeting *Fridolin-Simard*	
12:45 pm - 2:00 pm	CEEHRC Awardees Annual General Meeting Chair: Tomi Pastinen
2:00 pm - 3:00 pm	Proposal of a National Centre of Excellence in Epigenetics Chair: Martin Hirst

Poster Assignments

Poster Session 1 Monday, September 19 8:00 – 10:00pm		Poster Session 2 Tuesday, September 20 8:00 – 10:00pm	
Number	**Name**	**Number**	**Name**
1	Julien Albert	2	Azad Alizada
3	Fiona Allum	4	Abdus Anwar
5	David Ashbrook	6	Swneke Bailey
7	Misha Bilenky	8	Virginie Bertrand-Lehouillier
9	Steve Bilodeau	10	Julie Brind'Amour
11	David Bujold	12	Darci Butcher
13	Donovan Chan	14	Lei Cao
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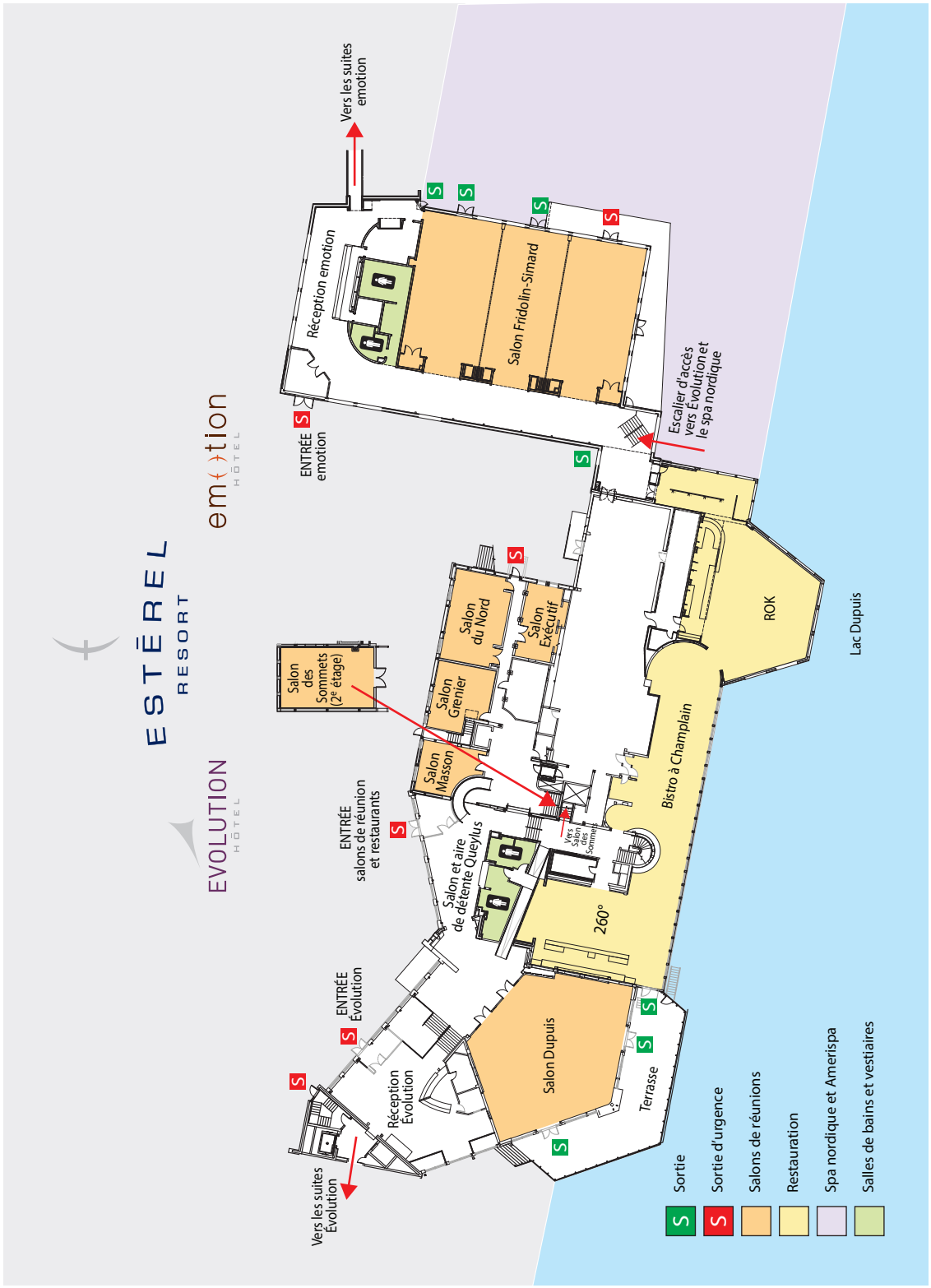

Selected Oral Presentations

Using Exceptions to Identify Human DNA Elements Regulating X-Chromosome Inactivation

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X-chromosome inactivation (XCI) epigenetically silences an X chromosome in mammalian females to provide dosage compensation between the sexes. However, a remarkable 15% of genes on the human inactive X chromosome escape inactivation, and the resulting over-expression in females is implicated in sexual dimorphisms, as well as in manifestations of sex-chromosome aneuploidies. By combining the results of three previous studies using four different methods to obtain the inactivation status of human genes, we assigned a consensus call to 639 genes, including 78% of X-linked protein coding genes that are expressed in females. Genes escaping XCI tend to cluster in humans but not mice, and while inactivation can spread to autosomal regions, it is less effective than on the X chromosome, leading to the hypothesis that there are intrinsic escape elements, boundary elements and waystations involved in the spread of facultative heterochromatin and euchromatin on the X chromosome. A further 15% of genes variably escape XCI between individuals and tissues, implicating additional epigenetic and/or genetic regulation. The variable escape genes are enriched at boundary regions, and clustering suggested a possible shifting of a boundary; however, preliminary results demonstrate that neighboring variable escape genes are independently regulated. The variable escape genes tend to have a broad range of methylation levels that do not correlate completely with their X-inactivation status, suggesting an epigenetic instability that we are studying further.

Studies on human XCI have been limited due to the lack of a developmental model, as human embryonic stem cells have very unstable epigenetic regulation of XCI. Therefore, we have targeted bacterial artificial chromosomes (BACs) containing human or mouse genes that escape X inactivation to the normally inactivated *Hprt* locus on the mouse X chromosome, to investigate the conservation of escape elements between mouse and human. The resulting BAC knock-in mice were bred to a strain carrying a deletion in *Xist* thus ensuring that our integrated BACs would always be on the inactive X. Tissues were analyzed for evidence of escape from the inactive X by measuring promoter CpG island methylation, as well as expression levels of RNA by RT-qPCR. Unexpectedly, the human escape gene *KDM5C* appeared subject to XCI while its mouse homologue, *Kdm5c*, was able to escape, suggesting that a regulatory element is missing in the *KDM5C* BAC, resulting in it being silenced. Preliminary analysis of a BAC transgene containing the human *RPS4X* escape gene and flanking genes subject to XCI reveals proper maintenance of both escape and silencing, suggesting the retention of human escape elements and boundaries recognized in the mouse. Thus knock-in human genes at the *Hprt* locus are a valuable complement to studies of human cells in the characterization of DNA elements regulating the spread of gene silencing or activation in human XCI.

[67] Derivation of transcription factor networks from analyses of active enhancer states in different subsets of normal human mammary cells

Davide Pellacani[1], Misha Bilenky[2], Nagarajan Kannan[1], Alireza Heravi-Moussavi[2], David J.H.F. Knapp[1], Sitanshu Gakkhar[2], Michelle Moksa[3], Annaick Carles[3], Richard Moore[2], Andrew Mungall[2], Marco A. Marra[2], Steven J.M. Jones[2], Samuel Aparicio[4,5], Martin Hirst[2,3*], Connie J Eaves[1*]

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The normal adult human female breast contains a continuous bilayered epithelial mammary gland surrounded by stromal fibroblasts and adipocytes. The cells in the outer "basal" layer of the mammary gland have myoepithelial features and are enriched in bipotent progenitors. The inner "luminal" layer comprises 2 phenotypically separable subsets with or lacking progenitor activity. Understanding the epigenomic features of these normal cell types, and how these impact on the regulation of their cellular functions is critical to elucidating the mechanisms controlling the breast epithelium throughout adult life (i.e. during menstrual cycle, pregnancy, lactation, involution, menopause, and aging), and how these are perturbed to result in breast cancer.

Here we have generated epigenomic profiles for 3 FACS-purified, functionally distinct, human mammary epithelial cell subpopulations: basal cells (BCs), luminal progenitors (LPs) and luminal cells (LCs), as well as the accompanying non-epithelial stromal cells (SCs) from 6 normal reduction mammoplasty tissues. 3 immortalized, but non-tumorigenic, human mammary epithelial cell lines widely used as substitute models of primary cells were also profiled. Genome wide profiles were obtained using mRNA-seq, miRNA-seq, ChIP-seq for H3K4me3, H3K4me1, H3K27me3, H3K27ac, H3K9me3 and H3K36me3, and whole genome bisulfite sequencing.

We found both the transcriptomes and chromatin states of all 4 primary cell types more closely resemble each other than any of the 3 cell lines and vice versa, despite the separate developmental origin of SCs. In contrast, the transcriptome datasets for the 4 primary cell types showed the SCs to be a distinct cell type and, within the 3 epithelial cell types, the LPs were intermediate between the LCs and BCs, but closer in this respect to the LCs. The chromatin states of each of the 4 primary cell types also contained many unique features affecting ~25% of the genome. Characterization of promoter bivalency (H3K4me3 and H3K27me3 co-occurrence) for the epithelial cell types showed that the bivalent promoter landscape of BCs resolves in LPs and is maintained in LCs, but with the gain of new bivalent promoters in the LCs. Analysis of the enhancer activation states showed extensive mammary cell-type specificity, with little similarity between LPs and LCs, suggesting that enhancer activation plays an important role in regulating their distinct transcriptional profiles. From these enhancer datasets we derived networks of cell-type specific transcription factors and identified novel candidates.

Together, these findings provide new epigenomic evidence for a hierarchical organization of the molecular and biological properties of the 3 major phenotypes that constitute the normal human mammary gland, and show how these datasets can be used to identify novel networks of transcription factors with candidate cell-specific regulatory functions.

[93] From mammals to fish and back again: discovering new regulators of early cardiac development

Xuefei Yuan[1,2], Mengyi Song[4], Anastasiia Aleksandrova[1], Patrick Devine[4], Benoit G Bruneau[4], Ian C Scott*[1,2], Michael D Wilson*[1,2],

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A comprehensive understanding of the mechanisms underlying heart formation is crucial for uncovering causes of congenital heart diseases and developing regenerative therapies. However, we still have an incomplete understanding of the gene regulatory networks that drive early cardiac lineage specification. Given the deep phylogenetic conservation of the cardiac transcription factors that drive cardiac gene expression, we tested whether a recently identified mouse enhancer (*Smarcd3*-F6) can mark early cardiac progenitors in zebrafish. We generated a stable *Tg(Smarcd3-F6:EGFP)* transgenic line and confirmed that the *Smarcd3*-F6 enhancer starts to drive GFP expression at 6 hours post fertilization (hpf) and by 13 hpf this population overlaps know cardiac marker *nkx2.5*. Furthermore, gene expression analyses using RNA-seq revealed that the *Smarcd3-F6:EGFP* labeled cells were enriched for cardiac and endoderm development-related pathways. In order to discover cardiac regulatory programs within the *Smarcd3*-F6+ population during early development, we conducted single-cell mRNA-seq expression analysis at the end of gastrulation (10hpf). We identified a cluster of cells that co-expressed known cardiac markers. Using *in situ* hybridization, we tested the expression of over 20 novel genes identified from the single-cell 'cardiac cluster'. We determined that the majority of our cardiac cluster genes and the *Smarcd3-F6 enhancer* itself are regulated by cardiac transcription factor Gata5/6. To discover early cardiac gene regulatory networks, we profiled open chromatin using ATAC-seq and identified 3838 *Smarcd3-F6:EGFP* specific open regions (ATAC-seq peaks). The 3838 *Smarcd3-F6:EGFP* enriched peaks were enriched for GATA, FOX and MECOM motifs. Interestingly, we found 129 out of 3838 *Smarcd3-F6:EGFP* enriched peaks overlap zebrafish conserved noncoding elements (zCNEs) and can be mapped back to human genome. 79 of the 129 sequence conserved peaks share open chromatin signatures between zebrafish and human. Those epigenetically conserved regions are highly enriched for transcription factors and heart development-related pathways. 8 out of 12 ultra-conserved regions consistently drove reporter gene expression in zebrafish hearts, suggesting they may play important roles in heart development. In addition to identifying a set of co-expressed genes that are active during early cardiac development in zebrafish, our cross species functional analyses have revealed a set of epigenetically active and ultra-conserved regulatory elements involved in vertebrate heart development.

[10] Impact of maternally inherited histone modifications on DNA methylation maintenance and enhancer activation in the early embryo

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Mammalian development comprises two distinct phases of epigenetic reprogramming, taking place in the pre-implantation embryo and in primordial germ cells. These two epigenetic reprogramming phases are characterized by global erasure and re-establishment of DNA methylation (DNAme), with DNAme levels reaching low-points in E13.5 primordial germ cells (PGCs) and in the blastocyst. In the germline, re-establishment of DNAme, including at imprinted gametic DMRs, is essential for normal development, and failure to establish or maintain DNAme is one of the underlying causes for pregnancy complications and many imprinting disorders.

In oocytes, global de novo DNAme by the DNA methyltransferase DNMT3A and its cofactor DNMT3L takes place postnatally in a manner broadly dependent on transcription. Interestingly, analyzing published DNAme data from the inner cell mass (ICM) of the blastocysts of F1 embryos, we find that much of this DNAme persists on the maternal genome, including at hundreds of CGIs methylated in oocytes, suggesting that inheritance of DNAme from the oocyte is not restricted to maternal imprints. The influence of maternally inherited histone modifications resistance to maternally inherited DNAme erasure during epigenetic reprogramming in the early embryo is still unknown. Moreover, the influence of persistent intergenic DNAme on the establishment of promoter (H3K4me3) or enhancer (H3K4me1/H3K27ac) marks in the early embryo and transcription from the maternal genome has not been addressed. In this project, we used ultra-low-input ChIP-seq in oocytes and in ICM cells obtained from reciprocal C57BL/6 x CASTEi/J mouse crosses to address these questions. We show that transcribed, *de novo* methylated genomic regions in oocytes are also marked with H3K36me3. Such DNAme/H3K36me3 marked regions include oocyte-specific intergenic transcription units, many of which encompass canonical CpG island promoters. Moreover, comparing oocytes and F1 ICMs DNAme with oocyte H3K9me2 profiles, we observe that, unlike what has been previously postulated, the regions marked by H3K9me2-marked domains in oocytes do not appear to be marked DNA methylated and thus do not correlate to those regions resist DNAme erasure in the early embryo. Taken together, these data suggest that the mechanism of interplay between histone modifications and DNAme during preimplantation development is distinct from other cell types.

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[62] Polycomb Repressor Complex 2 is Translationally Upregulated by c-SRC and mTORC1 in ERBB2-Driven Breast Cancer

Harvey W. Smith, Alison Hirukawa, Virginie Sanguin-Gendreau, Wafa B'Chir, Yutian Cai, Bin Xiao, Dongmei Zuo, Vincent Giguere, Ivan Topisirovic, and William J. Muller

Post-translational modifications of specific amino acids of core histones are a key mechanism of transcriptional regulation. Mutations or changes in the expression of writers, readers and erasers of these chromatin modifications are also central to the pathogenesis of many cancers. In breast cancer, the overexpression of EZH2, a component of Polycomb Repressor Complex 2 (PRC2), which mediates transcriptional silencing, is associated with poor prognosis. However, the mechanisms responsible are incompletely characterised and the role of PRC2 in breast cancer development is not fully understood. Here we have applied a genetic approach using transgenic mouse models to identify the tyrosine kinase c-Src as an important regulator of PRC2 in breast cancers driven by the ERBB2 oncogene. We show that ERBB2-driven mammary tumours lacking c-Src exhibit severely impaired growth, de-repression of genes normally silenced by PRC2, and loss of PRC2 component proteins, but not the corresponding mRNAs. This post-transcriptional regulation occurs through loss of c-Src-dependent activation of mammalian target of rapamycin complex 1 (mTORC1), leading to reduced translation of PRC2 components. Finally, we show that EZH2 is required for ERBB2-driven tumour cell proliferation and that its genetic ablation prevents ERBB2 from transforming mammary epithelial cells *in vivo*. These data indicate that tyrosine kinase signaling networks frequently activated in breast cancer can activate epigenetic programs required for mammary tumourigenesis. The translational control of PRC2 by mTORC1 may also point to previously unrecognised connections between the regulation of cellular metabolism and epigenetics.

[56] Rewiring of enhancer-gene interactions drives PLAU overexpression in the pathogenesis of Quebec Platelet Disorder

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Quebec platelet disorder (QPD) is a rare bleeding disorder caused by a unique gain-of-function defect in fibrinolysis. The hallmark of QPD is a marked increase in the expression of urokinase-type plasminogen activator (*PLAU*) in platelets and megakaryocytes, leading to accelerated clot degradation and delayed onset bleeding following hemostatic challenges. The genetic cause of QPD is a heterozygous tandem duplication of a 77 kb region of 10q22 that includes *PLAU* and its putative regulatory elements. However, the markedly increased (>100 fold) and cell-type specific expression of *PLAU* in QPD cannot be explained simply by a copy-number gain, implicating a megakaryocyte-specific regulatory defect in QPD.

To establish the molecular mechanism of *PLAU* overexpression in QPD, we performed transcriptomic (mRNA-seq) and epigenomic (ChIP-seq for histone modifications) profiling of primary blood cells (granulocytes and cultured megakaryocytes) from QPD patients and controls. Analysis of RNA-seq data showed that QPD *PLAU* transcripts were consistent with reference gene models, with a significantly greater fraction of reads originating from the disease chromosome in megakaryocytes than granulocytes. We identified a putative enhancer ~40 kb downstream of *PLAU* that is highly enriched for the histone modification H3K27ac in megakaryocytes compared to granulocytes. Chromosome confirmation capture experiments support that this enhancer interacts with the promoter of the downstream vinculin (*VCL*) gene. *VCL* is highly upregulated during normal megakaryocyte differentiation and the QPD duplication places one copy of *PLAU* downstream of this enhancer. We propose that the positioning of *PLAU* downstream of this enhancer results in aberrant enhancer-gene interactions that, in turn, drive overexpression of *PLAU* in QPD megakaryocytes.

Capturing Functional Epigenomes for Insight Into Metabolic Disease Susceptibility

Elin Grundberg[1] on behalf of the CEEHRC Team

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Obesity is a leading cause of death worldwide and significantly increases the likelihood of type 2 diabetes and cardiovascular diseases. Genetic factors are important but confer only a modest risk. Epigenomic aberrations may be key underlying factors but the specific regulatory pathways remain poorly understood. To this end, we aimed to generate novel insight into metabolic disease etiology by unraveling epigenetic signatures and mechanisms underlying risk of obesity and associated conditions. We are applying a state of the art, multidimensional approach intersecting unique clinical cohorts with in depth epigenomic analyses of pure cellular subpopulations isolated from adipose tissue and peripheral blood. By applying pipelines and protocols established within IHEC, we have successfully generated high-quality epigenomes of cells targeted to metabolic disease comprising NGS assays that are both established (RNA-Seq, WGBS, ChIP-Seq) and newly implemented (ATAC-Seq). First, we focused on the adipocyte and stromal vascular fraction of visceral and subcutaneous adipose tissue isolated from ten donors undergoing bariatric surgery. Results from these efforts allowed us to identify novel signatures of depot-specific adipocytes that may explain: 1) the different features of visceral vs. subcutaneous adipose tissue as indicator of cardiometabolic risk and 2) observed sex-specific effects on metabolic disorders and their complications. Second, we collaborated with EMC-McGill to perform reference epigenome mapping on adipocytes differentiated from bone marrow mesenchymal stem cells isolated from three healthy blood donors. These discovery phase epigenome mapping efforts were subsequently the basis of our translational studies in population-based and clinical cohorts for metabolic disease and tissue-targeted profiling of functional epigenetic landscapes. We used twins to identify key aspects of genetic vs. environmental regulated epigenetic (DNA methylation) signatures and large population-based WGBS analysis to highlight the importance of deep enhancer profiling for ideal capture of the dynamic and disease-linked epigenome. By analyzing both adipose and matched blood samples in populations we identified a substantial proportion of the variable methylome that is tissue-independent favoring epigenetic biomarker discovery in peripheral tissue samples. Our results from population-based WGBS analysis had implications for the scale-up to large-scale EWAS of metabolic diseases and inspired the implementation of an NGS-capture approach for targeted interrogation of functional methylomes (MCC-Seq). We have now applied MCC-Seq for simultaneous methylation (~2-5M CpGs) and genotyping profiling in adipose and blood cohorts (N=200) of obese individuals with variable cardiometabolic risks (as assessed by circulating lipids) – this integrated epigenetic/genetic map has the potential to provide new understanding of how genes and environments combine to impact metabolic traits. The maintenance of our program will allow further elucidation of the complex pattern in which inter-individual epigenetic variation interact with genetic and environmental factors and their prediction ability of risk of cardiometabolic diseases.

[78] Dynamic DNA Methylation Changes Upon Smoking Exposures Revealed by Large-scale Next Generation Sequencing Studies

Xiaojian Shao[1], Valérie Siroux[2], Dylan Aïssi[2], Pei-Chien Tsai[3], Jinchu Vijay[1], Elodie Boulier[1], Marie-Michelle Simon[1], Emmanuelle Bouzigon[4], Timothy Spector[3], Jordana Bell[3], Scott Wilson[5], Isabelle Pin[2], Florence Demenais[4], Tomi Pastinen[1], Elin Grundberg[1]

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Tobacco smoking remains the largest preventable cause of death in the world and has been recognized as a major risk factor for a variety of complex diseases including cancer, cardiovascular diseases and asthma. DNA methylation mechanisms have emerged as a potential mediator between smoking exposure and disease risk. Currently, epigenome-wide association studies of smoking traits have mainly utilized targeted array assay, e.g. the Illumina HumanMethylation450 BeadChip (450K), instead of comprehensive sequencing-based studies to deeply explore the relationship between DNA methylation and smoking status in a great detail. Here we apply our recently introduced MethylC-Capture Sequencing (MCC-Seq) approach to profile DNA methylation at more than 4M CpGs, almost an order of magnitude larger content than the 450K array, providing an unprecedented opportunity to investigate the impact of smoking exposure on the global DNA methylation landscape. We collected more than 1200 whole blood samples from two large cohorts and identified up to 359 smoking-associated epigenetic loci (differentially methylated CpGs or DMCs, q-value<0.05, current-past-never status). We observed that they are significantly depleted in promoter regions but enriched in CpG shores and enhancer elements, especially in the Type 2 helper T cells (Th2) DNase I hypersensitive sites. Among our DMCs, as many as 81.74% of them are identified in both cohorts and this replication rate is further strengthened if restricted to DMCs that are clustered in regions (DMRs) – i.e. 92.47%. Meanwhile, by comparing DMCs with before and after blood proportion adjustment, we found around 5.5% of smoking related DMCs are explained by cellular heterogeneity in whole-blood. Our results not only replicate majority of previously reported smoking loci (e.g. it covers 78% of well-reported sites reported in at least 5 studies), but also identify novel locus where on average only 9.8% of DMCs in the DMRs are covered in 450K array, suggesting MCC-Seq has the advantage to reveal the underline mechanisms. Specifically, we found more than 30 DMCs being novel and not even covered in 450K. Furthermore, our approach with comprehensive coverage also shows more detailed profiles around significant 450K-based loci, providing the opportunity for fine mapping and mechanistic insight. For instance, we fine-mapped the *AHRR* locus and identified a CpG 112 bp downstream of the well-reported 450K site (p-value=5.55e-16 vs. 4.59E-13, respectively) that showed improved predictive value (e.g. AUC score =0.90 vs. 0.79) for predicting current vs. never smokers using DNA methylation level only as a predictor, demonstrating that DNA methylation provides a potential biomarker for predicting outcome such as smoking status or disease status.

Oncogenic chromosomal translocations involving the NuA4/TIP60 and PRC2 complexes lead to aberrant chromatin landscape

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Mammalian NuA4 (also known as TIP60/p400) is an evolutionarily conserved multifunctional protein complex that possesses two catalytic functions – a histone acetyltransferase (HAT) activity and a histone exchange activity that deposits H2A.Z variant. The complex plays critical roles in transcriptional activation and DNA damage repair and is linked it to a variety of human diseases including cancer (haplo-insufficient tumor suppressor). Recent genomic studies have identified recurrent chromosomal translocations in patients with endometrial stromal sarcomas (ESS) and ossifying fibromyxoid tumors (OFMT) leading to an in-frame fusion of the NuA4 subunit EPC1 to PHF1, a protein associated with the polycomb repressive complex 2 (PRC2) complex. In contrast to NuA4, PHF1 and PRC2 proteins are linked to transcriptional repression of key developmental genes through methylation of histone H3 on lysine 27 (H3K27) by the EZH1/2 methyltransferases (HMT).

We present here the biochemical and functional characterization of the EPC1-PHF1 fusion. We show that the chimeric protein becomes incorporated into the NuA4 complex in place of EPC1, but also retains the ability to associates with PRC2, creating a hybrid super-complex harboring both HAT and HMT activities. Zinc-finger nuclease-mediated targeted integration of a cDNA encoding the fusion protein at the *AAVS1* locus allowed genome-wide analysis of its localization in parallel to its impact on chromatin modifications and gene expression. EPC1-PHF1 binding increased histone acetylation within promoters regulated by PRC2, notably within the HOXC and HOXD gene clusters. This is usually correlated with increased gene expression, consistent with the notion that EPC1-PHF1 mis-targets the HAT activity of NuA4/Tip60 to chromatin regions that are normally maintained in the silenced state. Increased H3K27 methylation was also detected, albeit at lower frequency, on specific loci normally bound by Tip60-EPC1 leading to decreased transcription. Interestingly, bivalent chromatin regions harboring both H3K4me3 and H3K27me3 are preferred targets of EPC1-PHF1 leading to increased local acetylation and expression.

Overall our data support the hypothesis that EPC1-PHF1 fusion promotes oncogenesis by joining two chromatin modifying activities with diametrically opposite functions, subverting the activity of the TIP60 complex and leading to the deregulation of PRC2 target genes. These findings further underline the importance of deregulated epigenetic modifiers in promoting oncogenesis.

[66] Impaired H3K36 methylation defines a subset of head and neck squamous cell carcinomas

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*These authors contributed equally to this work, #These authors jointly supervised this work

Human papillomavirus negative (HPV-) head and neck squamous cell carcinomas (HNSCC) are deadly and common cancers. Recent genomic studies implicate multiple genetic pathways including cell-signalling, cell-cycle and/or immune evasion in their development. Here, we analyze public datasets and uncover a previously unappreciated role of epigenome deregulation in the genesis of 13% HPV-HNSCCs. Specifically, we identify novel recurrent p.K36M mutations occurring in multiple histone H3 genes. We further validate their presence in multiple independent HNSCC datasets and show that, along with mutually exclusive, previously described *NSD1* mutations, they uniquely define a DNA methylation cluster. H3K36M and *NSD1* defects converge on altering H3K36 methylation, subsequently blocking cellular differentiation and promoting oncogenesis. Our data further indicate surprisingly limited redundancy for NSD family members in HPV-HNSCCs and suggest a potential role of impaired H3K36 methylation in their development. Further investigation of drugs targeting chromatin regulators is warranted in HPV-HNSCCs driven by aberrant H3K36 methylation.

[27] Epigenetic vulnerabilities arising from metabolic adaptation to taxane resistance in triple-negative breast cancer

Geneviève Deblois[1,2], Sayed Ali Madani[1,2], Yunchi Kao[1,2], Alexandra Fedor[1], Benjamin Haibe-Kains[1,2,3], Mathieu Lupien[1,2,3*]

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Breast cancer patients of the triple-negative (TNBC) subtype are treated using standard of care chemotherapy that includes taxane. However, therapeutic resistance to taxane commonly occurs in these patients limiting survival rates. Therapeutic resistance has been associated with adaptation of cellular metabolism and epigenetic alterations in various cancer types, including breast cancer. While the epigenetic machinery enzymes use metabolites as substrates and co-factors for methylation and acetylation reactions, the contribution of metabolic adaptations to the modulation of epigenetic landscapes in cancer, such as taxane-resistant breast cancer, remains unknown. We hypothesize that resistance to taxane results in metabolic adaptations that modulate the epigenetic landscapes in TNBC cells. Using a panel of taxane-resistant cell populations derived from TNBC cell lines, we identified a taxane-resistance gene expression signature significantly associated with metabolic adaptations potentially impacting on metabolites substrates and co-factors required for the activity of epigenetic enzyme machinery. We used quantitative metabolomics approaches to show that the resistant cells differentially utilize and synthesize metabolites involved in the activation of the epigenetic enzyme machinery compared to sensitive cells. We further show that the taxane-resistant cells display altered levels of specific histone modifications compared to the sensitive cells, a feature that makes them sensitive to modulation of epigenetic enzyme activity. Together our data suggest that metabolic adaptations inherent to the development of taxane resistance in TNBC cells contributes to the modulation of epigenetic profiles and creates epigenetic vulnerabilities that can be therapeutically exploited.

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[50] Functional proteomics characterization of the human acetyl lysine machinery

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Lysine acetylation (KAc) is one of the key post-translational modifications regulating chromatin. The KAc system is comprised of 19 lysine acetyltransferases (KATs), 18 deacetylases (KDACs) and of 43 proteins containing bromodomains (BRDs), the only protein domain known to bind acetyl lysine residues. Importantly, the BRD-KAc interactions are druggable, making them attractive for pharmaceutical intervention. Indeed, multiple compounds with different selectivity against BRDs are now available, several of which showing promising activity in preclinical and phase I clinical studies for the treatment of cancer and other health conditions.

To better characterize the recognition and specificity of the KAc machinery, we systematically characterized protein-protein interactions for the majority of KATs, KDACs and bromodomain-containing proteins stably expressed in human cells using proximity biotinylation (BioID) and affinity purification coupled to quantitative mass spectrometry (AP-MS). We also selected 60 of the interaction partners for reciprocal interaction proteomics screens. Together, this identified 5682 interactions with 2385 partners, the majority of which are novel. Our results demonstrate the expected enrichment for nuclear proteins in the interactome and a high degree of interconnectivity within the KAc machinery itself, but also define specific interactions for individual KAc machinery components. Notably, members of the transcription intermediary factor 1 (TIF1) family TRIM24, TRIM28 and TRIM33 all interact with each other but also establish drastically different interaction networks with transcriptional regulators (TRIM24), KRAB domain containing zinc finger proteins (TRIM28) and co-activators (TRIM33). As most BRD-containing proteins are large, multi-domains polypeptides, they harbour various regions that can mediate physical interactions. We thus performed additional characterization of the role of the different domains in the assembly of the interactome for the Bromo and Extra Terminal (BET) sub-family of BRD-containing proteins for which potent small molecule bromodomain inhibitors exist. We identified new BRD and ET domain dependent interaction partners for BET proteins. However, we also uncovered a surprising increase in association with the BET proteins of a group of proteins following treatment with small molecule BET bromodomain inhibitors, such as JQ1. Our work supports a model in which BET proteins, as well as other BRD-containing proteins, act as protein scaffolds at the level of chromatin, ensuring proper signaling integration for effective gene transcription.

Regulation of genomic imprinting: from spreading to erasure

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Most imprinted genes are embedded within large clusters of co-regulate genes showing parent-of-origin allele-specific expression as a consequence of a nearby CpG-rich element, an imprinting centre (IC), acquiring DNA methylation from a single parent. The distal end of mouse Chr 7 shares syntenic homology with 11p15 in human, a region associated with the imprinted growth disorders Beckwith-Wiedemann and Silver-Russell syndromes. Genes in this ~1Mb region are regulated by two gametic DMRs in mouse and human: one inherited from sperm in between *H19* and *Igf2* (IC1), and one inherited from the oocyte at the *Kcnq1ot1* promoter (IC2). We have characterized a GFP transgenic insertion, Tel7KI, next to the *Ins2-Igf2-H19* co-regulated cluster and showed that it behaves as a maternally imprinted gene. The paternal allele does not acquire DNA methylation from sperm and is not silenced in pre-implantation stage embryos. Like other endogenous imprinted genes, it acquires DNA methylation only on the paternal allele during the wave of post-implantation *de novo* methylation and is thereafter regulated by this somatic imprint. We have carried two different genetic experiments to identify the IC regulating Tel7KI. Surprisingly, our results show that Tel7KI is regulated by the distal IC2, which mediates silencing in *cis* via the lncRNA *Kcnq1ot1*. I will discuss our characterization of hybrid F1 trophoblast stem cell lines, showing that PRC2 and EZH2, which are known to be recruited by *Kcnq1ot1*, deposit H3K27me3 repressive marks all the way to the site of Tel7KI insertion. Finally, I will discuss our finding that a repressed paternal allele of Tel7KI is reactivated in the germ line, in a process known to occur in primordial germ cells. Our analysis of GFP-positive (reprogrammed) and GFP-negatice (silenced) gonadal PGCs by PBAT shows that Tel7KI is an excellent reported for the reprogramming of imprints in the germline, and allows studies on pure populations of PGCs at similar stages of reprogramming.

[31] Epigenomics ELSI and the Media

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Academic as well as media interest in the ethical, legal, and social implications (ELSI) of epigenomics highlights epigenomic research as an important, emerging area of science. As members of the International Human Epigenome Consortium (IHEC) Bioethics Workgroup, we sought to gain a better understanding of the promises and concerns associated with epigenomics that had been raised by ELSI scholars and the media.

In a scoping review of current ELSI literature (16 articles found with the keywords 'epigenetics/epigenomics/epigenome' AND 'legal/ethical/social/policy') we found that ELSI scholarship addressing epigenomics focused mainly on hypothetical issues involving institutional racism, discrimination, and the potential for new forms of eugenics, thereby raising concerns that epigenomic research may result in the rise of harmful social policies. The issue of transgenerational justice was consistently raised within other ELSI themes (e.g., privacy, public health, discrimination), based on evidence that epigenomic harm can be inherited and therefore does not only relate to the medical or social issues of the existing generation, but also to those of their descendants.

We also conducted a study of national news coverage of epigenomics in Europe and North America (Canada, France, Germany, UK, USA) and of select English-language press in Asia (Hong Kong, Japan, Singapore, South Korea). Content analysis of the reporting indicated that publics are receiving strong messages about the "fatality" of epigenetic harm, passed on through the generations, and about its serious impact in many areas of health. The main policy messages found in press reports were grouped under the themes of individual and parental responsibility for health, regulation, therapy and research, public health, and social justice. Members of the public were pushed to consider both personal and societal responsibility in preventing epigenetic harm. In particular, some of the stories hyped the consequences epigenetics might have for parenthood and its associated duties. However, the mainstream national press in the countries we looked at did not appear to be amplifying the dystopian views found in some of the ELSI literature on epigenetic discrimination.

As the risk messaging in media accounts was generally skewed by overstatement of our understanding of epigenetic changes, we further assessed the balance of the media reports and undertook a case study of how specific epigenomics scientific publications had been presented in the news. For example, early assessment indicates that the media do not discriminate between transient changes involved in gene regulation and heritable epigenetic changes in tissues and cells, leading to misinterpretation of the importance of reports on environment-genome interactions. Given the pivotal role of the media in raising awareness of ethical and social issues and in framing societal debate, we aimed to identify common misunderstandings to help epigenomic researchers and science journalists steer clear of pitfalls.

Circadian hallmarks of the aging epigenome

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Circadian rhythmicity is a fundamental "invention" of evolution, however its molecular mechanisms and biological ramifications are poorly understood. In this study, we performed a comprehensive analysis of DNA modification dynamics over a 24 hr period in human and mouse genomes and uncovered that daily epigenomic oscillations are closely related to aging.

First, we documented circadian cycling of DNA modifications using a variety of platforms in human and mouse tissues. Harmonic analysis showed that 8.5% of the analyzed human neutrophil DNA fragments have a 24 hr oscillation pattern, and dominant acrophases occurred (2.6-3.2x enrichment) between circadian time 7-10 hr. In a similar fashion, 8.3% and 35.4% of the 37K+ bisulfite sequenced CpGs in the mouse liver and lung DNA, respectively, were oscillating. Cyclic epigenomic oscillations were more common in the tissue-active genes compared to the suppressed genes ($p= 0.04 - 5.4x10^{-5}$) and were enriched for E-box motifs ($e = 8.3x10^{-12} - 5.2x10^{-152}$), which are regulatory sequences for circadian regulation. The oscillating cytosines were significantly overrepresented in among the cytosines exhibiting large variation in DNA modification ($p= 6.7x10^{-148}$). This finding indicates that circadian epigenetic oscillations can explain a substantial fraction of the inter-individual DNA modification variance, which traditionally has been interpreted as a predominantly stochastic process.

Second, we were interested if the circadian epigenome is related to aging. We mapped age dependent DNA modification changes in the mouse liver and lung. Both tissues exhibited a significant overlap between the cytosines showing circadian and aging effects (OR=2.33 and 1.37; $p = 2.6x10^{-24}$ and $1.3x10^{-5}$, for liver and lung, respectively). Interestingly, the epigenetic acrophases during the sleep phase (ZT0-12) were significantly overrepresented within loci that gained modification with age, while the acrophases of the wake phase (ZT12-24) were overrepresented within loci that lost modification (OR=67 and 394; $p=1.4x10^{-44}$ and $9.6x10^{-46}$, for liver and lung, respectively). The circadian-aging association was also detectable in the transcriptome.

This study provides strong evidence for the association of two time related phenomena: the circadian epigenome occurring during the Earth's rotation around itself and the aging epigenome accumulating over the lifespan.

[49] Diet-induced obesity enhances MYC-driven prostate cancer through a coordinated metabolic and epigenetic rewiring

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The lifetime risk of prostate cancer (PCa) is heavily influenced by extrinsic factors: in particular, men that are diagnosed with PCa, and adopt a Western dietary pattern or have excess body weight, are more likely to die of their disease. The genomic changes observed most frequently in primary PCa are 8q gain, or focal amplification of 8q24.21, both of which result in *MYC* amplification. A hallmark of MYC overexpression is a global metabolic reprograming that supports anabolic processes and cell growth, and in murine prostatic tissues *MYC* overexpression largely recapitulates the human disease; thus, MYC is believed to function as a driver oncogene in PCa.

In PCa, the landscape of epigenetic alterations that rely on metabolites as substrates or cofactors varies greatly as the cancer progresses, however, the interplay between metabolic and epigenetic rewiring in this disease remains unexplored. Here we use a MYC-driven mouse model of PCa to show that high fat diet-induced obesity (DIO) enhances the MYC transcriptional program through metabolic alterations that favor histone hypomethylation, which then leads to a DIO-dependent phenotype characterized by increased cellular proliferation and tumor burden. More specifically, DIO aggravates the global H4K20 hypomethylation that is triggered by MYC overexpression. This feature is greatly exacerbated by increased activity of the H4K20me1 histone demethylase PHF8 (a MYC transcriptional coactivator and regulator of proliferation) at the promoter region of MYC signature genes. Notably, we show that saturated fat intake in human prostatic tumors is also associated with an enhanced MYC signature, and that stratification of patients according to their saturated fat-induced MYC signature, irrespective of their MYC status, is associated with an increased risk of lethal PCa.

Our findings support an intricate crosstalk between DIO, metabolic, and epigenetic alteration geared toward an enhanced MYC signature, and suggest that in primary PCa, extrinsic risk factors such dietary fat intake support tumor progression by mimicking *MYC* amplification.

Abstracts

[1] Exploiting mouse hybrids, RNA- and ChIP-seq to explore the influence of SNPs and structural variants on gene expression.

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Despite conservation of transcription factor (TF) networks in human and mouse (~75 million years divergence) (Yue 2014), their *cis*-regulatory networks have undergone extensive rewiring (Vierstra 2014). Unfortunately, associating or pinpointing genetic differences in cis-regulatory DNA with gene expression across human and mouse samples is not informative due to confounding trans-effects (e.g. differential TF expression or strain-specific TFs). The mouse model organism offers a genetically diverse set of isogenic strains that span divergence times from a few generations to hundreds of thousands of years (Keane 2011). **We hypothesize that a significant portion of the complement of differentially expressed genes identified between two phenotypically and genetically distinct lab mouse strains can be explained by sequence variation in *cis*-regulatory regions.**

We identified *Mus musculus castaneus* and *domesticus* as prime candidates for our study because 1) they differ by ~20 million single nucleotide variants and 2) produce fertile F1 offspring (i.e. the two genomes are dissimilar and compatible). **By generating transcriptome data on F1 cells, i.e. that carry both parental genomes, confounding experimental and biological variation (*trans* effects) are minimized or eliminated.** Using a computational toolbox for allele-specific epigenomics analysis (Younesy 2013), I identified the complement of genes showing strain-specific expression bias in embryonic day 13.5 primordial germ cells extracted from F1 embryos generated by reciprocally crossing *castaneus* and *domesticus*. Ongoing ChIP-seq experiments for enhancer, promoter and repressive marks will profile regulatory sequences near genes showing strain-specific expression bias; which we predict are tightly correlated.

Allele-biased expression levels of candidate genes will be verified by qRT-PCR using allele-specific primers or pyrosequencing. Finally, I will analyze several candidate *cis*-element+gene pairs by generating constructs containing the putative *cis*-element from both strains, driving a luciferase reporter gene. Analysis of the relative expression levels of each construct in mouse embryonic stem cells will confirm whether naturally occurring genetic variation within the *cis*-regulatory element does indeed influence the expression of the neighboring gene, as predicted.

With the recent mapping of several human disease loci onto non-coding genomic regions, it is crucial that we define the extent to which natural genetic variation within these regions can modulate gene expression of nearby genes. Using the mouse model organism, this study will lead to a better understanding of how the transcriptome is shaped and how it can be disrupted, leading to disease.

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[2] Epigenetic Regulation and Evolution of Endothelial Cell Inflammation

Azad Alizada, Lina Antounians, Nadiya Khyzha, Minggao Liang, Huayun Hou, Jason Fish, Michael D. Wilson.

Vascular endothelial cells (ECs) line the interior of blood vessels and play important role during inflammation. ECs mediate responses essential for many inflammatory disorders such as atherosclerosis, ischemic heart disease, and thrombosis. During inflammation ECs respond to cytokines by expressing pro-inflammatory genes. The *cis*-regulatory elements (CREs) that control the expression of the pro-inflammatory genes have not been well characterized. The NF-κB complex is one of the major mediators of the pro-inflammatory gene expression in response to cytokines. A critical DNA binding subunit of this complex is RELA (p65). Detection of evolutionarily conserved inflammation-induced RELA binding sites in the genome can help us identify potential CREs that regulate inflammatory response in ECs. To do that we experimentally determined the genomic occupancy of RELA in TNFα-treated primary aortic ECs isolated from human, mouse, and cow. We found ~10,000 RELA binding sites in humans that are conserved with either mouse or cow. From this conserved fraction ~2000 sites are highly conserved in all three species. This highly conserved fraction enriches for pro-inflammatory response genes involved in biological pathways relevant to aortic EC inflammatory response, as well as Notch, angiotensin, and hypoxia-inducible factor (HIF) pathways. Moreover, the highly conserved RELA sites overlap known human regulatory disease mutations in processes related to the transforming growth factor beta (TGFB) signaling and angiogenesis. In order to test the function of CREs I am establishing CRISPR/Cas9 gene editing approach in an immortalized human aortic endothelial cell line (TeloHAEC). Overall our comparative epigenomics and CRISPR/Cas9 genome editing strategy can be used to identify and characterize functional CREs and has the potential to improve our understanding of EC biology and gene regulation in human disease.

[3] Epigenome-wide association studies of circulating lipid levels identify tissue-independent biomarkers for cardiometabolic risk

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Complex diseases such as obesity are under the control of genetic and environmental factors. Epigenetics (e.g. DNA methylation) is seen as the link connecting environment and genetics to phenotype and disease. Most large-scale epigenome-wide studies (EWAS) use targeted arrays or enrichment methods biased to CpG-dense regions, where methylation is mostly static. To address this, we developed MethylC-Capture Sequencing (MCC-Seq), a customizable, cost-effective approach to simultaneously profile functional methylomes and genotypes. Using an adipose-specific MCC-Seq panel (2.5M CpGs), we linked global methylation status in adipose to cardiometabolic risk through an EWAS of circulating lipid levels (triglycerides, HDL-C, LDL-C and total cholesterol) in 199 severely obese subjects (Université Laval). In total, 21,168 CpGs were significantly linked (5% FDR) to at least one lipid trait. Importantly, lipid-CpGs were found to be enriched within adipose enhancers but depleted in promoters, reinforcing the value of targeting dynamic enhancer regions in complex disease etiological studies. We performed a replication study in whole blood from a population-based cohort (CARTaGENE, N=137) to identify tissue-independent lipid-CpGs for potential biomarker use. We noted marked enrichment of replicated lipid-CpGs for all traits studied (5% FDR; >6.2-fold; $p<1.5x10^{-4}$). Using this dataset, we further corroborated and fine-mapped lipid-CpGs from a recent large-scale Illumina 450K EWAS in whole blood (N~3300, BIOS Consortium). Next, by linking genetic variation to adipose methylation (metQTL) we found enrichment of genetic regulation among adipose lipid-CpGs (metQTL $p<1.0x10^{-8}$; >5.1-fold; $p<2.2x10^{-16}$), which strengthened when restricting to tissue-independent sites (>8.0-fold; $p<2.5x10^{-11}$). Thereby, supporting that genetic factors are the main contributors limiting inter-tissue epigenetic drift. We then assessed the density of nearby lipid-CpGs and noted that genetically controlled lipid-CpGs (metQTL $p<1x10^{-8}$) tend to cluster in regions (77%) whereas environmentally driven sites ($p>0.01$) mostly occur as singletons (66%). Incorporating Global Lipids Consortium GWAS for the same lipid traits, we show that genetically controlled lipid-CpGs markedly overlap disease loci from lipid-GWAS (GWAS $p<5.8x10^{-8}$; 25.8-fold; $p<2.2x10^{-16}$). Our results represent the first large-scale adipose to whole blood replication of lipid-CpGs. We demonstrate the value of using disease-linked tissues as the starting material for increased resolution of biologically relevant biomarker discovery.

[4] Testing novel therapeutics to overcome T-ALL chemotherapy resistance in an *in vivo* mouse model"

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T-cell acute lymphoblastic leukemia (T-ALL) is characterized by aggressive hematological tumors, 40% – 60% of which express the TAL1 gene which can serve a potential therapeutic target. The clinical prognosis for this subtype of T-ALL is that less than 50% of patients survive after five years of treatment. This is because chemotherapy does not target tumors specifically and as a result fails to eliminate all the T-ALL cells causing them to become resistant. A drug screen was performed in the TAL1+ cell line Jurkat/TR and several drugs were identified that target various transcription factors involved in epigenetic modifications such as histone deacetylases and methylases. Comprehensive dose response experiments were then performed to determine optimal drug concentrations to target TAL1+ T-ALL. 25uM of the drugs identified in the screen significantly decreased the proliferation of the T-ALL cells after 36 hours. A similar dose response was also performed for the standard chemotherapeutic drugs and a similar decrease in T-ALL proliferation was observed. All combinations of the epigenetic and chemotherapeutic drugs were used at the GI_{50} concentrations obtained to understand their combinatorial effect on TAL1+ T-ALL proliferation. Drug combinations that were found to have the greatest combinatorial negative effect on T-ALL proliferation will be tested in a mouse model to determine their *in vivo* efficacy. This research is critical to identifying and determining the efficacy of potential therapeutics.

[5] Epigenetic impacts of stress priming of the neuroinflammatory response to sarin surrogate in a mouse model of Gulf War Illness

David Ashbrook

There is a great deal of interest in integrative approaches to understanding epigenetic-disease mechanisms in complex illnesses. Gulf War Illness (GWI) is an archetypal, medically unexplained, chronic condition characterised by persistent sickness behaviour, neuroimmune and neuroinflammatory components. An estimated 25-32% of the over 700,000 veterans of the First Gulf War fulfil the requirements of a GWI diagnosis. Patients with GWI continue to show debilitating symptoms 25 years after the conflict, a persistence consistent with the hypothesis that epigenetic modifications may contribute to disease pathology.

It has been hypothesised that the high physical and psychological stress of combat may have primed the immune system to over-react to the low level of the irreversible AChE inhibitor, sarin, that many veterans were exposed to in the theatre of war. Recent research in a mouse model has shown that pre-treatment with the stress hormone corticosterone (CORT) causes an increase in expression of specific chemokines and cytokines in response to diiso-propyl fluorophosphate (DFP), a sarin surrogate.

We are integrating transcriptome- and epigenome-wide approaches to further investigate combinations of exposure conditions associated with GWI in this mouse model. Our RNA-seq results show that genes differentially expressed in the frontal cortex after the combined CORT and DFP treatment, compared with either alone, are enriched for genes with immune related annotations.

Initial pyrosequencing results suggest that specific genes of interest, such as Tnf, may be differentially methylated with combined CORT and DFP treatment. We are now carrying out reduced representation bisulfite sequencing to investigate genome-wide changes in DNA methylation, and H3K27ac ChIP-seq to investigate histone changes indicative of genes that are transcriptionally activated in response to the exposures.

This project will show the epigenetic results of combined stress and exposure to an AChE inhibitor, potentially identifying pathways to understanding the etiology and treatment of GWI.

[6] Noncoding somatic and inherited single-nucleotide variants converge to promote *ESR1* expression in breast cancer

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Sustained expression of the oestrogen receptor alpha (ESR1) drives two-thirds of breast cancer (BrCa) and defines the ESR1-positive subtype. This transcription factor engages enhancers upon oestrogen stimulation setting an oncogenic expression program in BrCa cells. Somatic copy number alterations involving *ESR1* occur in approximately 1% of ESR1-positive BrCa, implying that other mechanisms underlie the persistent expression of *ESR1*. We report the significant enrichment of somatic mutations within the set of regulatory elements (SRE) converging on *ESR1* in 7% of ESR1-positive BrCa. These mutations regulate *ESR1* expression by modulating transcription factor binding to the DNA. The SRE includes a recurrently mutated enhancer whose activity is also affected by the functional inherited single nucleotide variant (SNV) rs9383590 accounting for the risk associated with the rs2046210, rs3734805 and rs9383938 BrCa risk-loci. Overall, our work highlights the importance of considering the combinatorial activity of regulatory elements as a single unit to delineate the impact of genetic alterations on single genes in cancer.

[7] FindER: A Sensitive Analytical Tool to Study Epigenetic Modifications and Protein-DNA Binding from ChIP-Seq data

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We present a versatile analysis tool developed to Find Enriched Regions (FindER v2.0.0) in ChIP-Seq datasets. FindER is intended to overcome sequence depth limitations of many existing tools and provides a common mechanism for identifying enrichment from localized (e.g. H3K4me3 histone modification, or DNA-protein binding) as well as dispersed (e.g. H3K27me3, H3K36me3) ChIP-Seq signal profiles, or mixed of two signal types (e.g. H3K4me1). After reading aligned IP-signal data and DNA Input control data in the BAM format, FindER computationally segments genome into nucleoseome-scale size bins defined by the local read density distribution in the IP data. Using these adaptive bins FindER first blacklists alignment artifacts (that are typically appear as spurious enriched regions) using Input DNA control (with an option to use external list of blacklisted regions as well).

FindER v2.0.0 uses a novel approach to find enrichment using DNA sequence specific classification of the identified genomic bins into enriched and non- enriched applying Otsu's method widely used in the image processing. Both the local sequence coverage of individual bins and the clustering of the bins along the genome are taken into account to assign significance. The final list of enriched regions is determined by applying a False Discovery Rate control process.

The approach is free from assumptions about underlying distributions of read density and common for different histone modifications. This allows for integrative multi-sample analysis for data with comparable signal-to-noise ratio, and is especially important in examining the effect of relative signal strength on the biology. FindER accepts aligned reads in a standard alignment format as input and generates a list of enriched genomic locations at a given significance (FDR) threshold genome wide. Crucially, FindER is a production grade application. It is a user friendly tool implemented in Java, and it has been tested on terabyte scale ChIP-Seq data. We present examples of application of FindER to the various ChIP-seq data.

[8] Specific alterations in the histone modification landscape as a consequence of transient *Dnmt1* deficiency in mouse ES cells

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Genome-wide demethylation and remethylation of DNA during early embryogenesis are essential for mammalian development and genome integrity. Through mostly unknown mechanisms, *imprinted* germline differentially *methylated* domains (gDMDs) are able to retain their methylation profiles during the reprogramming period. Using an embryonic stem (ES) cell line, in which *Dnmt1* is regulated by a *tet-off* system (*Dnmt1*$^{tet/tet}$ ES cells), we recently showed that a temporary lack of DNMT1 activity triggers the inherited loss of gDMDs and gDMD-like DNA methylation profiles, whereas most regions of the genome are able to recover original DNA methylation levels. Here, using the same *Dnmt1*$^{tet/tet}$ ES cell system, we investigate how the transient lack of DNMT1 activity influences global gene expression and epigenetic landscape. Our RNA-seq experiments highlight two noteworthy gene clusters following the transient lack of DNMT1; one set of genes that becomes strongly activated and one that becomes strongly inactivated. Included in these clusters are known gDMDs and gDMD-like associated genes that display inherited loss of DNA methylation profiles. However, a significant number of genes (including chromatin remodelers) within these two clusters does not exhibit alterations in promoter or gene body methylation. We then explore by ChIP-Seq if the transient lack of DNMT1 activity prompts rearrangements in the histone mark landscape (H3K4me3, H3K27me3, H3K27ac) associated to these two gene clusters. For some genes, we believe that alterations in the histone landscape impede the recruitment of DNMTs; suggesting that alterations in the histone modification landscape may prevent the proper recruitment of DNMT1 following its reactivation which results in altered gene expression. However, for other genes, we find that perturbations in histone modifications following temporary lack of DNMT1 occur with the absence of neighbouring inherited loss of DNA methylation. We believe that inactivation or activation of specific chromatin remodelers following the transient lack of DNMT1 permanently alters gene expression beyond known imprinted or imprinted-like genes. The present study presents new perspectives on how alterations in DNMT1-dependent methylation maintenance can alter DNA methylation profiles, histone modification cross-talk and gene expression as well as explain inherited epigenetic dysregulation events that could occur in abnormal cells and during early embryo development.

[9] FOXA and master transcription factors recruit Mediator and Cohesin to the core transcriptional regulatory circuitry of cancer cells

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Controlling the transcriptional program is essential to maintain the identity and the biological functions of a cell. The Mediator and Cohesin complexes have been established as central cofactors controlling the transcriptional program in normal cells. However, the distribution, recruitment and importance of these complexes in cancer cells have not been fully investigated. Here we show that FOXA and master transcription factors are part of the core transcriptional regulatory circuitry of cancer cells and are essential to recruit Mediator and Cohesin. Indeed, Mediator and Cohesin occupied the enhancer and promoter regions of actively transcribed genes and maintained the proliferation and colony forming potential. Through integration of publically available ChIP-Seq datasets, we predicted the core transcriptional regulatory circuitry of each cancer cell. Unexpectedly, for all cells investigated, the pioneer transcription factors FOXA1 and/or FOXA2 were identified in addition to cell-specific master transcription factors. Loss of both types of transcription factors phenocopied the loss of Mediator and Cohesin. Lastly, the master and pioneer transcription factors were essential to recruit Mediator and Cohesin to regulatory regions of actively transcribed genes. Our study proposes that maintenance of the cancer cell state is dependent on recruitment of Mediator and Cohesin through FOXA and master transcription factors.

[11] IHEC Data Portal 2016 update: datasets quality control, permanent sessions, public API

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The IHEC Data Portal (http://epigenomesportal.ca/ihec) is the integrative online resource to navigate through datasets produced by the International Human Epigenome Consortium. As of May 2016, the Portal hosts over 7500 human datasets, for which more than 5000 are in the IHEC core set of assays. With an average of 150 unique sessions weekly, it has become the central access point to visualize and obtain IHEC datasets. In order to increase information quality and accessibility, multiple new features have been added over the last year.

First, datasets quality assessment features have been implemented. These include a visual correlation tool that was introduced last year, now released on the main portal server. A quality control pipeline was also developed, verifying datasets quality by evaluating multiple metrics. Using only publicly accessible tracks, it identifies potential problems such as incomplete coverage, high background noise, and poor correlation to other tracks with similar metadata. This pipeline will soon be added to the Portal data integration workflow, and will allow dataset-producing consortia to be warned of inconsistencies that could be validated, prior to release. An IHEC-wide analysis using this pipeline has demonstrated that even with large inter-consortia variability in methods for library preparation and downstream analysis, and differences in cell types, diseases and other factors, a useful qualitative assessment can be offered to data producers and users.

Other improvements include the addition of bi-yearly persistent releases and permanent sessions. Publicly- accessible tracks are now being served directly from the Portal enabling reliable navigation sessions, when compared to the previous distributed model, as there is no more dependency on multiple remote servers statuses. Users can also save their Portal sessions permanently, allowing them to obtain an ID that links to their datasets selection and filtering options. These IDs can then be directly citable in papers making use of the IHEC Data Portal datasets. Lastly, a publicly accessible Web API allows users to query available datasets metadata, in both JSON and human-readable formats.

The IHEC Data Portal is a service hosted by GenAP (https://genap.ca), developed and maintained by the McGill Epigenomics Data Coordination Centre (http://epigenomesportal.ca). It is funded under the CEEHRC, by the CIHR and by Genome Quebec, with additional support from Genome Canada. The correlation matrix computation approach was developed by the Université de Sherbrooke, and funded by NSERC. The computing and networking infrastructure, and part of the software development, are provided by Compute Canada and CANARIE.

[12] Identification of Specific DNA Methylation Signatures in CHARGE and Kabuki Syndromes

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Mutations in epigenetic regulator genes (epigenes) are increasingly being reported as the cause of heritable genetic diseases with phenotypic features including intellectual disability, growth abnormalities and congenital anomalies. Epigenetic regulation normally determines where and when genes are expressed via a number of mechanisms including DNA methylation, histone modifications and chromatin remodelling. Accurate epigenetic programming is essential for normal organismal development and cellular function, and is controlled by an estimated 800 epigenes. CHARGE and Kabuki syndromes are caused by functional haploinsufficiency (nonsense, missense, or deletion) of two different epigenes, Chromodomain helicase DNA-binding protein 7 (CHD7) and Lysine (K) Methyltransferase 2D (KMT2D), respectively. Although these two syndromes are clinically distinct, there is clinical overlap in their phenotypic features, including delayed growth and development, genital hypoplasia and other congenital malformations (cardiac, ear and eye).

We have previously shown that mutations in epigenes can be associated with specific patterns of genome-wide DNA methylation. Therefore, we tested for DNA methylation alterations using the Illumina Infinium 450Methylation Beadchip array in blood cells from individuals with CHARGE and Kabuki syndromes. When compared to controls, samples from patients with functional mutations in CHD7 and KMT2D demonstrate unique DNA methylation signatures. Each of the DNA methylation signatures could be used to classify genetic variants of unknown significance (VUS) in CHD7 or KMT2D as either pathogenic or benign.

The specific DNA methylation signatures for CHARGE and Kabuki syndromes reveal alterations in DNA methylation at specific genes that may be relevant to the pathogenesis of each of these disorders. Altered DNA methylation, seen in both signatures, of CpG sites the promoter of *Homeobox A5* (*HOXA5*), may account for some of the common clinical features between the two disorders. There are also distinct DNA methylation alterations in each of the disorders that likely drive molecular pathways contributing to the distinct features in the clinical spectrum of each syndrome.

[13] Identifying environmentally susceptible sites in the human sperm methylome

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DNA methylation is the most studied epigenetic modification, occurring at approximately 30 million CpG dinucleotides across the genome. These patterns are established in germ cells: primarily set prenatally in males and postnatally during oocyte growth in females. While reprogramming takes place shortly following fertilization, not all marks are lost, leading to the possible transmission of normal/abnormal epigenetic information to the next generation. Bisulfite sequencing remains the gold standard for assessing DNA methylation; however, it requires good depth of coverage to accurately determine methylation at individual sites across the genome. Few studies have examined the human sperm methylome at great depth, with many looking at individual samples/donors. In this study, we used whole genome bisulfite sequencing (WGBS) to analyze DNA methylation patterns in a pool of spermatozoa from 30 individuals. A diverse group of donors, ranging in age, fertility status, smoking status, *MTHFR* 677 genotype and folic acid supplementation, were pooled to reveal possible regions of epigenetic variation. Equal amounts of DNA were pooled from each donor and processed for WGBS library preparation. Deep sequencing was achieved by the library being sequenced on 4 lanes of Illumina HiSeq 2000 sequencing. Approximately 27 million CpG sites were sequenced, with an average coverage of 23x and average methylation of 74.1%. The majority of CpG methylation sites were either lowly or highly methylated (15.5% and 71.0% of sites demonstrating <20% and >80% methylation, respectively). Interestingly, from our pool of donors, 7,763,944 CpGs (~29%) were invariable, showing either 100% or 0%. Examining CpGs with a minimum of 20x coverage (~15.3 million), a similar bimodal distribution of methylation was observed. As expected, regions of low methylation were over represented in promoter-transcription start sites (TSS); ~73% of CpGs found in promoter-TSS were <20% methylated. Sites with intermediate methylation levels (20-80%) were mainly located in intergenic and intronic regions, areas in the genome previously shown to regulate gene activity. Several samples from the pooled sperm DNA were previously analyzed by reduced representation bisulfite sequencing. Good correlation (coefficient >0.9) was observed between these two techniques; interestingly, the sample that was shown to have the greatest number of differentially methylated tiles, had the lowest correlation to the WGBS results (0.85). While WGBS allows for the analysis of nearly all CpG sites throughout the human genome, this study reveals that many CpG sites remain consistent in their DNA methylation even within a diverse pool of donors. A more targeted method, narrowed down to sites of interest (i.e. intermediate methylation; intergenic and intronic regions), that also delivers depth of sequencing, would be an ideal approach to the analysis of the human sperm DNA methylome. (Supported by CIHR)

[14] Mediation effects of DNA methylation on the impact of exposure to the prenatal maternal stress from the 1998 Quebec ice storm on metabolic outcomes at age 13½

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Animal and human studies suggest that prenatal exposure to stress is associated with adverse health outcomes such as type 2 diabetes, hyperglycemia, and insulin resistance in offspring. Epigenetic modification such as DNA methylation is considered one possible underlying mechanism. Given that women cannot randomly be assigned to experience depression or relationship difficulties in pregnancy, studies showing associations between maternal mood or psychosocial stressors and child outcomes cannot disentangle the genetic transmission of maternal traits from effects of the intrauterine environment and from conditions in the postnatal environment. The 1998 Quebec ice storm provides a unique opportunity to study a randomly assigned prenatal maternal stress (PNMS) on child outcomes. Project Ice Storm was conceived following one of Canada's worst natural disasters in history: The January 1998 Quebec ice storm and has been following a cohort of children whose mothers were pregnant during the ice storm. The objective of this study is to determine whether prenatal maternal exposure to objective hardship due to the ice storm influences children's metabolic outcomes (insulin and C-peptide secretion) at age 13½ and to determine whether DNA methylation of diabetes-related genes mediates these potential effects.

Five months after the ice storm we recruited women to assess their stress levels. In 2011, fasting and challenged blood samples were obtained from 31 adolescents at age 13½. Insulin secretion was estimated using the insulinogenic index and serum C-peptide levels were analyzed using chemiluminescent immunoassay. T cell DNA was extracted and methylation levels were analyzed. CpGs corresponding to genes from Type 1 and Type 2 diabetes mellitus pathways were selected for mediation analysis. We found that children whose mothers experienced higher objective hardship exhibited both higher insulin and C-peptide secretion in response to an oral glucose tolerance test. Furthermore, DNA methylation of type 1 and 2 diabetes-related genes had a positive mediating effect of objective hardship on both insulin and C-peptide secretion (i.e., the magnitude of objective hardship exposure was associated with an increase in DNA methylation, which in turn, was associated with an increase in insulin and C-peptide secretion).

Our findings suggest that DNA methylation could act as an intervening variable between prenatal stress and metabolic outcomes, highlighting the importance of epigenetic mechanisms in response to environmental factors.

[15] Defective epigenetic inheritance in Hutchinson-Gilford Progeria Syndrome vascular smooth muscle cells

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Hutchinson-Gilford Progeria Syndrome (HGPS) is a rare accelerated aging disorder in which affected children develop aging deficits and succumb to vascular complications in their teens due to deterioration of vascular smooth muscle cells (VSMCs). HGPS is caused by a mutation in *LMNA* gene that activates a cryptic splice site, leading to accumulation of mutant protein **Progerin** in the nuclear lamina.

Although mechanistic studies have not uncovered the HGPS disease process in HGPS VSMCs, HGPS fibroblasts exhibit increased DNA damage and global disruptions to epigenetic marks as well as gene expression. We have found that Progeria cells express high levels of reactive oxygen species (ROS), which is similar to increased ROS in aging vasculature, and could be an underlying cause for replicative stress in HGPS VSMCs. We hypothesize that high ROS in aged HGPS VSMCs drives replicative stress, causing defects in epigenetic inheritance. To test this hypothesis, we generated HGPS patient-specific iPSCs and differentiated them into VSMCs to model mechanisms driving epigenetic inheritance in these cells *in vitro*.

We found that HGPS VSMCs exhibit elevated levels of ROS and DNA damage during replication. Furthermore, we determined that the DNA damage response machinery and chromatin remodelers accumulated at sites of nascent replicated DNA, suggesting that HGPS VSMCs are undergoing replicative stress. In general, we hope to learn more about aging using HGPS model by assessing the molecular role of ROS and its link to epigenetics, and potentially uncover novel drug targets to treat the aging population.

[16] Implication of NuA4 histone acetyltransferase complex in DNA damage response pathways

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The NuA4 (TIP60/KAT5) histone acetyltransferase is a highly conserved multisubunit complex responsible for acetylation of nucleosomal histone H4 and H2A. It is important for gene expression but also the efficient repair of DNA double strand breaks (DSB). NuA4 is rapidly recruited to chromatin surrounding a DSB at the same time histone H2A(X) is phosphorylated in the neighboring region. While we have shown that NuA4 can interact with phosphorylated H2A(X), we speculated that another interaction was required for its initial recruitment to the break. Since an ATM-related factor is part of NuA4, it was tempting to postulate that DSB sensing factors known to recruit ATM, ATR or DNA-PKc could be responsible for NuA4 recruitment. In ChIP experiments using the inducible HO DSB, we show that deletion of homologous recombination factors Xrs2, Rad50 or Mre11, part of the MRX complex, strongly cripples NuA4 recruitment to DSB. Xrs2 FHA-BRCT domains directly interact with NuA4, but only when it is purified from cells treated with DNA damaging agents, in a phospho-dependent manner. Interestingly, after its initial recruitment, NuA4 spreads on each side of the DSB during DNA end resection. Using specific mutants, we demonstrate that nucleosomes around DSB are acetylated by NuA4, favoring resection and loading of Rad51. Interestingly, we also observed a delayed spreading of γH2A(X), indicating a link with checkpoint dynamics. Furthermore, we identified several key repair players such as RPA and CtIP/Sae2 as potential NuA4 acetylation substrates, indicating another layer of involvement in DNA damage response. Our data and subsequent analysis will shed light on the intricate function of NuA4 in DDR pathways, and its functional interactions with other chromatin modifying complexes to orchestrate chromatin based cellular processes during DNA repair.

[17] Accessing functional methylome through multi-layer allelic epigenomics

Warren Cheung, Andréanne Morin, Xiaojian Shao, Bing Ge, Tomi Pastinen

Background: Modern next-generation sequencing approaches provide us the opportunity to interrogate not only the genome and the transcriptome, but also the intertwining epigenetic layers. Cutting-edge analysis techniques allow us to distinguish individual-level genetic differences, resolving differences between individual chromosomes to provide an allelically-resolved view of epigenetic modifications and gene expression linked through personal genetics. Allele specific methylation (ASM), where one allele exhibits different methylation pattern compared to the other, can be found commonly across the genome with the majority of events being *cis*-regulated. ASM has been linked to the regulation of the allele-specific expression (ASE) of autosomal non-imprinted genes. Allele-specific histone (ASH) has also been linked to allelically-biased gene expression, with allelic enhancer signals correlated with ASE genes. ASM, ASE and ASH have each been shown individually to have genome-wide, autosomal associations for complex traits and particularly for complex disease. To date, however, the parallel investigation of ASM, ASH and ASE has only been carried in a limited set of samples.

Results: We have performed the first comprehensive characterisation of the relationship between multiple epigenetic layers and the functional transcriptome, evaluating 145 samples in 550 datasets where we linked ASM, ASE and ASH effects. We hypothesise the presence of genome-wide presence of allelic gene expression coordinated with allelic methylation rate and presence of histone marks, with the majority of significant effects concordant with the same non-allelic trends. We establish that our observed ASM are concordant with replicated by methylation QTL (meQTL) 95% of the time. We also noted that less than 10% of significant methylation to expression correlations overlapped between allelic and non-allelic analyses. We see that 76% of the CpGs with methylation significantly correlated to gene expression overlap with ASM or meQTL, indicating a high proportion of these correlations are under genetic control. We next correlated ASM and ASE, and observed concordance to non-allelic analysis ($R=47$, $p < 2.2x10^{-16}$). By linking ASM, ASE and ASH together, we are able to directly observe the allelic patterns coordinating the layers of epigenetics against the transcribed phenotype, with globally consistant patterns of coordinated allelic regulation. For activating H3K27ac, H3K4me1 and H3K4me3, we observed a clear pattern of high methylation and low histone mark corresponding to low expression as the strongest of four directional signals (67%, 48% and 66% respectively). For repressive H3K36me3 and H3K27me3, we see a weaker pattern of high methylation and high histone corresponding to low expression as the strongest signal instead (36% and 30% respectively). We also demonstrate, via a pediatric glioblastoma case study, that allelic analysis can reveal novel differences among disease causing variation, contrasting the allelic relationship in H3F3A.WT tumours against the disrupted pattern observed in H3F3A.K27M tumours.

Methods: We used MethylC-Seq for whole-genome (WGBS) or capture-based (MCC-Seq) methylation sequencing. These were linked to gene expression via total RNA sequencing (RNA-Seq) and chromatin immunoprecipitation sequencing (ChIP-Seq) for H3K27ac and H3K4me1 in purified naïve T-cells (nTC), monocytes and muscle cells.

[18] A functional DNA methylation signature for EZH2 mutations in Weaver syndrome

Sanaa Choufani

Overgrowth syndromes are a group of disorders including Sotos and Weaver Syndromes presenting with an increase in the size of the body or a part of the body pre- and postnatally making affected individuals at increased risk of developing cancers. They also present with distinct facial features and variable degrees of intellectual disability. Although clinically overlapping, Sotos syndrome is caused by mutations in the histone lysine methyltransferase gene, NSD1 (nuclear receptor-binding SET domain protein 1) which regulates transcription via interactions with H3-K36Me. Whereas Weaver Syndrome is caused by mutations in Enhancer of Zeste homolog 2 (*EZH2*) gene. EZH2 is the catalytic component of the polycomb repressive complex 2 (PRC2), which methylates histone H3 at lysine 27, resulting in a mark (H3K27me3) that specifies a transcriptionally repressive chromatin environment. We have shown that the functional effects of pathogenic mutations in *NSD1* can be identified using DNA methylation analyses (Choufani et al, 2015). Thus, we wanted to investigate whether mutations in *EZH2* also display a functional effect on the methylome in Weaver Syndrome patients. Therefore, we profiled DNA methylation from Weaver (n=7) cases and compared them independently to controls (n=50) using the Illumina Infinium450methylation BeadChip (450k array). DNA methylation analysis using non-parametric statistics coupled with permutation analyses identified large number of statistically significant differentially methylated CG sites between Weaver Syndrome and controls. Using unsupervised hierarchical clustering of the Weaver Syndrome specific CG sites, all Weaver cases clustered separately from controls and from Sotos Syndrome cases. Further elucidation of the specificity and sensitivity of the Weaver Syndrome signature is currently undergoing and will impact our understanding of the molecular pathophysiology of Weaver Syndrome and has the potential to identify specific molecular targets for the future development of novel therapies.

[19] The SWI/SNF complex is required for axon morphogenesis in the *Drosophila* mushroom body

Melissa Chubak

Defects in epigenetic regulation represent one of the most common causes of Intellectual disability (ID). In particular, the SWI/SNF chromatin remodelling complex is the most statistically over represented protein complex among the known dominant ID genes. The SWI/SNF complex is known to play a role in cellular differentiation and cancer, but recent studies have revealed a role for this complex in neurons. Genetic screens in *Drosophila* have identified several components of the SWI/SNF complex that are important for the regulation of dendrite morphogenesis. However, the role of this complex in axon morphogenesis remains unexplored. Furthermore, little is known about the role of this complex in the development of brain regions important for learning and memory. We have addressed these gaps by investigating the role of the SWI/SNF complex in the development of the *Drosophila* mushroom body (MB), a complex brain structure that is required for learning and memory in the fruit fly. Using targeted RNA interference (RNAi), we have knocked down the expression of each of the 15 SWI/SNF components specifically in the MB. Following knockdown, gross MB morphology was assessed using confocal microscopy to identify morphological defects. Initial results from this study have shown that several components of the SWI/SNF complex are essential for axon morphogenesis in the *Drosophila* MB, providing novel insight into the biological role of this core epigenetic regulatory complex. The results from this study will be used as a basis for further understanding the molecular mechanisms that are disrupted in the MB upon SWI/SNF knockdown. These findings may provide insight into the mechanisms that are disrupted in ID disorders that are caused by mutations effecting SWI/SNF components.

[20] Inactivation of SWI/SNF chromatin remodelling complex proteins in clinically aggressive dedifferentiated endometrial carcinoma

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In Canada, 1 in 36 women will develop uterine cancer, making it one of the most common gynecological malignancies. Dedifferentiated endometrial carcinoma (DDEC) is a subtype of uterine cancer where an undifferentiated carcinoma arises abruptly from a well-differentiated endometrioid adenocarcinoma. It is a highly aggressive disease with about 50% of patients succumbing to the disease within a year of diagnosis. In contrast to the well-differentiated component, the undifferentiated component lacks the expression of many epithelial and müllerian differentiation markers such as PAX8 and ER. It is also the more aggressive component, constituting the majority of extrauterine spread and is typically resistant to chemotherapy.

We recently identified mutually exclusive inactivating mutations involving several key subunits of the SWI/SNF chromatin remodeling complex (SWI/SNF CRC) that resulted in the loss of protein expression in the undifferentiated component of DDEC. These included BRG1 inactivation, ARID1A and ARID1B co-inactivation and INI1 inactivation, and such inactivation occurred preferentially occurs in a highly microsatellite instable (MSI-H) molecular context. The inactivation of these SWI/SNF CRC components coincides with the loss of PAX8 and ER expression. Gene expression profiling analysis comparing the SWI/SNF CRC protein-deficient undifferentiated component to the well differentiated component of DDEC showed increases in the level of expression of markers of stemness and epithelial to mesenchymal transition (EMT), results which have been further corroborated by immunohistochemical studies.

To test our hypothesis that loss of SWI/SNF CRC proteins contribute to an arrest of cellular differentiation and the maintenance of stem cell-like state, *in vitro* disease models of DDEC are currently being generated from endometrial cell lines using both shRNA-mediated knockdowns and CRISPR technology. Eleven endometrial cell lines were characterized through Western blot analysis, immunofluorescence studies and qRT-PCR assays and it was found that most were MSI-H cell lines with retained BRG1, INI1, ARID1B and PAX8 expression. These appear to represent ideal models for generating BRG1-deficient or ARID1A/1B co-deficient cell line models for further *in vitro* and *in vivo* (xenograft) characterization.

Understanding how inactivation of the different chromatin-remodeling proteins maintains undifferentiated gene expression programs in DDEC will provide valuable insights into how arrested differentiation accelerates tumor progression and likely unveil novel therapeutic approaches.

[21] Role of Histone H2A.Z in the regulation of mitochondrial function and impact on cellular respiration

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Histone variant H2A.Z is an important regulator of gene expression in all eukaryotic cells. In the yeast *Saccharomyces cerevisiae*, deletion mutants for *HTZ1* (*htz1*Δ), the gene encoding H2A.Z, are viable in rich medium but show marked deficiencies in adapting to several environmental stresses and carbon sources. We discovered that an unusually high proportion of *htz1*Δ cells exhibited a *petite* phenotype, and which we further demonstrated to be unable to grow on media containing a non-fermentable carbon source. This *petite* phenotype also occurs in wild type cells, but at a much lower frequency. Knowing that *petite* colonies are characterized by complete or partial loss of mitochondria DNA (mtDNA), we suspected H2A.Z to be involved in mtDNA stability. Hence, in this study, we aimed at investigating how H2A.Z may impact on mitochondrial genome stability, and subsequent cellular respiration.

We show that the frequency of *petite* colony formation in *htz1*Δ cells can be complemented by the expression of a wild type allele of *HTZ1*. We also show that *petite htz1*Δ mutants possessed non-functional mitochondria as determined by fluorescence microscopy by staining functional and unfunctional mitochondria. We next wanted to investigate the state of mtDNA in *petite htz1*Δ mutants by carrying out PCR assays on genes encoded by mtDNA. None of the genes tested are present in our mutant cells. We have next set up an assay where we are able to provide functional mitochondria to *petite* cells, and also induce *HTZ1* expression. By achieving this we find that addition of new functional mitochondria to *petite* cells with functional H2A.Z cannot reduce the frequency of *petite* cells to levels comparable to those observed in WT cells when growing in presence of EtBr. This is due to mutations cause by the initial genome instability in the *htz1Δ petite* strain that could not be repaired by introduction of a WT allele of *HTZ1,* and mitochondrial complementation.

These results suggest that H2A.Z is an essential component of the cellular respiration pathway in yeast, and we propose that it contributes to maintaining mitochondrial function by controlling the expression of genes involved in cellular respiration as well as exerting a role in genome stability.

Our approach has the potential to characterize regulators involved in mechanisms that lead yeast cells to choose between cellular respiration and fermentation since *S. cerevisiae* can easily be directed to either of these two pathways. By characterizing regulators and their mechanisms involved in the switch between cellular respiration and fermentation in yeast, it may help establish a link between those regulators and the Warburg effect that is observed in cancer cells.

[22] Inhibition of the lysine demethylase KDM4A by the oncometabolite 2-hydroxyglutarate activates mTOR signalling pathway.

Florence Couteau

The identification of cancer-associated mutations in the tricarboxylic acid (TCA) cycle enzymes isocitrate dehydrogenases 1 and 2 (IDH1/2) highlight the prevailing notion that aberrant metabolic function can contribute to tumorigenesis. IDH1/2 normally catalyze the oxidative decarboxylation of isocitrate into α-ketoglutarate (αKG). In gliomas and acute myeloid leukemia, IDH1/2 mutations confer gain-of-function leading to production of the oncometabolite *R*-2-hydroxyglutarate (2HG) from αKG. However the precise molecular basis of 2HG-stimulated oncogenesis remains unclear. Here we show that generation of 2HG by mutated IDH1/2 leads to the activation of the serine/threonine kinase mTOR. Due to the structural similarity between 2HG and αKG, 2HG acts as an inhibitor of αKG-dependent enzymes including the Jumonji (Jmj) family of lysine demethylases.

[23] Coordinated molecular events control histone H3K27 mono-methylation in plants

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In plants, the histone H3.1 K27 mono-methyltransferases ARABIDOPSIS TRITHORAX RELATED PROTEIN 5 AND 6 (ATXR5/6) regulate heterochromatic DNA replication (1) and this activity is regulated, in part, by the ability of ATXR5/6 to specifically methylate the canonical histone H3, H3.1, on K27 (H3.1K27) (2). In this study, we present structural and biochemical evidence showing that several molecular events control the activity of ATXR5/6 enzymes. First, we show that with the exception of R26 mono- and di-methylation (H3.1R26me1/2), post-transcriptional modifications of residues neighboring K27 are detrimental to ATXR5 activity. Structural studies reveal that ATXR5 binding to H3.1R26me2 is accommodated by the structural reorganization of H3.1R26me2 side chain to make novel hydrogen bonds with residues forming ATXR5 peptide binding cleft. Second, we demonstrate that ATXR5/6 preferentially methylate histone H3.1 when incorporated in a nucleosome core particle and that the presence of ATXR5 PHD domain is important for H3.1K27 methylation. The crystal structure of ATXR5 PHD domain in complex with a peptide corresponding to the first 10 residues of histone H3 tail shows that several hydrogen bond and van der Waals contacts coordinates the binding of histone H3. However, mutational analysis combined with steady-state kinetics show that substitution of residues interfacing ATXR5 PHD domain and histone H3.1 does not result in a significant decrease of the Km of ATXR5 for the nucleosome core particle but affect the Km of the enzyme for the cofactor S-adenosyl-L-methionine. These observations highlight a novel function of a PHD domain in integrating histone H3 binding, methylation and cofactor binding/release during the catalytic reaction. Overall, our results demonstrate that several molecular determinants regulate ATXR5/6 methyltransferase activity and epigenetic inheritance of H3.1K27me1 in plants.

[24] Epigenetic Mechanisms in the Development of Asthma

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Background: Asthma is a complex disease caused by a combination of genetic and environmental factors. The heritability of asthma is estimated to range between 0.48- 0.79. Despite the recent successes of genome wide association studies (GWAS), we still cannot explain the majority of the heritability of asthma and other complex traits. This has led to a quest to identify and locate "the missing heritability". The next steps toward understanding the genetic and environmental contributions to the etiology of common complex diseases are the study gene-environment interactions, parent-of-origin effects, and epigenetics. The last two are especially important for asthma and related traits. It has long been speculated that parent-of-origin effects (imprinting) and epigenetic regulators of methylation play a role in the development of asthma.

Hypothesis: We hypothesized that parent of origin effects and allele specific methylation (ASM) contribute to the etiology of asthma.

Methods: To investigate this hypothesis we used GWAS data from three Canadian family-based studies (two parents and one offspring). These studies are: 1) the Canadian Asthma Primary Prevention Study (CAPPS), a high-risk asthma birth cohort and, 2) the Study of Asthma Genes and Environment (SAGE), a population-based asthma birth cohort and 3) the Saguenay-Lac-Saint-Jean asthma familial collection (SLSJ), a founder population of French-Canadians. Investigate parent-of-origin effects in each study and a meta-analysis.

Results: Meta-analysis results identified two SNPs with significant parent-of-origin effects (*SLC39A10* and *LNX2/POLR1D)*. In tThe joint analysis of CAPPS and SAGE, we identified a parent-of-origin effect at a known imprinted gene, *CTNNA3*. This gene was previously identified in a GWAS study of occupational asthma. Of all significant results in those two analyses ($p<10^{-5}$), 12 of 20 of the SNPs were in or near lLong non-coding (lnc)RNA genes. LncRNAs are known to be involved in genomic imprinting and gene regulation.

Future Directions: Identification of long non-coding RNA's has poised a challenge to the team, many long non-coding RNA's do not produce an RNA product and are not "functional" as they do not produce an RNA product, and secondly and long-non coding RNA's can be located a long distance from the genes that they regulate. Given that we wish to validate our statistical findings by evaluating methylation at regions identified to be associated with asthma, ASM, and parent-of origin effects, we are developing of suite of bioinformatic tools to aid in the investigation of the long non-coding RNA's and the genes that they regulate.

[25] Paternal exposure to contaminants alters the sperm epigenome and induces negative pregnancy outcomes in an inter- and transgenerational manner

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Due to natural weather trajectories, the Arctic food chain is contaminated with **P**ersistent **O**rganic **P**ollutants (POPs). This pollution may be related to the major health discrepancy between Inuit and non-Aboriginal Canadians, which includes poor fetal growth, placental abnormalities, stillbirths, congenital defects, culminating in a 10-year shorter lifespan. The levels of neonatal and postnatal infant death are substantially elevated in Inuit communities. A recent study of pregnant Inuit women from Arctic Quebec revealed that prenatal exposure to POPs is correlated to shorter pregnancy duration, which, in turn, is associated with reduced infant birth weight, length and head circumference. POPs are also a general public health problem because some are still or again widely used to combat malaria and zika virus vectors. While it is well known that maternal exposures can affect child health and development, recent research indicates that the effects of environmental exposures can be transmitted to future generations via the fathers.

We use a rat model to test the hypothesis that early life exposure of fathers to environmentally-relevant levels of Arctic contaminants induces negative pregnancy outcomes and developmental defects, similar to what occurs in Inuit, in his offspring and subsequent generations. We observed that F1 males that were exposed to POPs early in life as well as their F2 sons were subfertile. Also in the lineage of POPs-exposed F1 fathers, *situs* anomalies appeared in F2 (20%) and F3 (15%) generations and 9.5% F3 pups exhibited hydrocephalus. Since the paternally-exposed lineage of F2 and F3 generations also demonstrated significant placental defects, reduced fetal growth, neonatal and postnatal death and other congenital anomalies, it seems that our model induces adverse health effects that are alarmingly similar to those experienced by Inuit populations. This experimental approach may represent a good prognostic tool to mimic human health problems, particularly in populations exposed to high POPs levels.

We further hypothesize that early-life paternal exposure to the POPs alters the methylation of his sperm DNA and also that of his sons and grandsons. Reduced represention bisulfite sequencing and pyrosequencing, revealed that an early paternal POPs exposure indeed modifies sperm epigenome through the F3 generation, and the sites conserved across 3 generations provide evidence for the concepts of inter- and transgenerational epigenetic transmission.

This study provides novel insight to the molecular foundations of a father's environment on the health of his future generations, including paternally-mediated inter- and transgenerational epigenetic transmission via his sperm methylome.

[26] Nucleosomal Response Pathway and Gene Programming

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Signaling cascades regulate multiple processes in the cell, including the expression of immediate-early genes (IEGs) in response to different stimuli. Mitogen- and stress-activated kinase 1/2 (MSK1/2), acting downstream of the MAPK signaling pathway, are involved in the regulation of IEGs. In mammalian cells, stimulation of RAS-MAPK-MSK1/2 by EGF, TPA or TNF alpha results in the phosphorylation of H3 at S10 and S28 (the nucleosomal response) and remodeling of the IEG regulatory regions. ChIP assays of human and mouse cells show the transient increase of H3S10ph or H3S28ph at the regulatory regions of IEGs. There is also a transient increase and decrease of H3K4me3 in the IEG coding region. To identify IEGs regulated by the MSK, we have completed transcriptome analyses of mouse and human cell lines stimulated with TPA, EGF and TNF alpha ± H89, a potent MSK inhibitor. Imaging and sequential ChIP assays demonstrate that an IEG epi-allele is phosphorylated at H3S10 or H3S28, but not at both sites. The level of H3S28ph is responsive to inhibition of histone deacetylase and CBP. H3S28ph, but not H3S10ph, is associated with H3K27ac, providing evidence that H3K27ac guides MSK to phosphorylate H3 at S28. The recent results of our studies on the MSK-mediated nucleosomal response and gene programming will be presented. (This work was supported by grants from Research Manitoba, CancerCare Manitoba, Canadian Breast Cancer Foundation, and a Canada Research Chair to J.R.D.)

[28] Conserved Differentially Methylated Elements from One Generation to the Next: Inheritance versus Randomness

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Introduction: Analysis of treatment effects on epigenetic marks transmission, as DNA methylation, across multiple generations represent a complex design. High-throughput sequencing techniques enable genome-wide detection of differentially methylated elements (DME), commonly sites (DMS) or regions (DMR). Due to software design, the detection of DME is usually made on each generation separately. The common DME between generations due to randomness is not negligible when the number of DME detected in each generation is high. To judge the effect on DME that is inherited from a treatment in previous generation, the observed number of conserved DME needs to be compared to the randomly expected number.

Method: We present a permutation analysis that can successfully be used to test the hypothesis that the number of conserved DME, between several generations, is associated to an effect inherited from a treatment and that stochastic effect can be dismissed. The permutation analysis can be used on designs between two or multiple generations. To ensure valid results, convergence of the permutation analysis must be reached. A permutation analysis has been carried out on early-life organochlorines (OC) exposed Sprague-Dawley males and their descendants (project led by Dr. Janice Bailey). Methylation marks in DNA spermatozoa, on three generations, have been analyzed through reduced representation bisulfite sequencing (RRBS). DMS sites have been detected using methylKit software. Both intergenerational and transgenerational permutation analysis have been conducted.

Conclusion: Inferring relation between treatment and epigenetic effects from across generations is an important outcome. The relation between the resulting number of conserved DME from one generation to the next and a treatment can be efficiently inferred by permutation analysis. The intergenerational permutation analysis, done on OC-exposed Sprague-Dawley males and their descendants, confirms that the number of conserved DMS is significantly larger than the one expected by randomness. Unambiguously positive result was also obtained for the transgenerational permutation analysis. All results support methylation effects of OC exposure across generations. At last, the permutation analysis is also recommended for testing the resulting number of conserved DMR.

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[29] UTX/KDM6A demethylase activity is required for satellite cell-mediated muscle regeneration

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The X chromosome-encoded histone demethylase UTX (also known as KDM6A) mediates removal of repressive H3K27me3 to establish transcriptionally permissive chromatin. Loss of UTX in female mice is embryonic lethal. Unexpectedly, male UTX-null mice escape embryonic lethality due to expression of UTY, a paralog that lacks H3K27-demethylase activity, suggesting an enzyme-independent role for UTX in development and thereby challenging the need for active H3K27-demethylation in vivo. However, the requirement for active H3K27-demethylation in stem cell-mediated tissue regeneration remains untested. Here, we employed an inducible mouse knockout that specifically ablates *Utx* in satellite cells and demonstrated that active H3K27-demethylation is necessary for muscle regeneration. Loss of UTX in satellite cells blocked myofiber regeneration in both male and female mice. Furthermore, we demonstrated that UTX mediates muscle regeneration through its H3K27-demethylase activity, as loss of demethylase activity either by chemical inhibition or knock-in of demethylase-dead UTX resulted in defective muscle repair. Mechanistically, dissection of the muscle regenerative process revealed that the demethylase activity of UTX is required for expression of the transcription factor myogenin, which in turn drives differentiation of muscle progenitors. Thus, we have identified a critical role for the enzymatic activity of UTX in activating muscle-specific gene expression during myofiber regeneration and have revealed a physiological role for active H3K27-demethylation in vivo.

[30] Consumption of dietary *trans*-fat affects the miRNA profile of the HDL and the preferential localization of miRNAs in plasma

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Introduction: MicroRNAs (miRNA) are short (~22nucleotides) non-coding RNAs with important role in post-transcriptional regulation of their target genes (~60% of human transcripts). Recently, it has been shown that high-density lipoprotein (HDL) carries and delivers functional miRNAs to recipient cells. We hypothesize that HDL-carried miRNAs might be involved in the regulation of the HDL cardioprotective properties (e.g. reverse cholesterol transport or glucose homeostasis regulation), which might modulate the risk of cardiometabolic diseases.

Objectives: To assess whether consumption of *trans* fatty acids (TFAs) from various dietary sources modifies the HDL-carried miRNA concentration and contribution to the plasmatic miRNA pool (i.e., miRNAs preferential localization within HDL), and to identify the metabolic pathways enriched with these miRNAs.

Methods: In a double-blind, randomized crossover controlled study, 9 healthy men were fed each of 3 experimental isoenergetic 4-wk diets: 1- high in industrial TFA (iTFA); 2- high in TFA from ruminants (rTFA); 3- low in TFA (control). miRNAs were extracted from HDLs isolated by sequential ultracentrifugation and from plasma using the miRVana PARIS kit (Life Technologies). MiRNA were quantified relatively to exogenous control cel-miR-39-3p by RT-qPCR using the miScript miRNA PCR Array (Qiagen). The pathway analyses were performed using the mirPath v3.0 software (Diana tools).

Results: Among the 87 miRNAs tested, 5 miRNAs carried by HDLs were differentially concentrated among diets, including miR-486-5p (χ^2=12.667; p=0.002) and miR-100-5p (χ^2=9.556; p=0.008). These miRNAs were less concentrated in HDLs following the iTFA diet compared to the control diet (miR-486-5p: fold change (FC)=-2.06, p=0.008; miR-100-5p: FC=-2.81, p=0.008). The preferential localization of 15 miRNAs also differed among diets, including miR-375 (χ^2=16.222; p=0.0003) and miR-150-5p (χ^2=12.667; p=0.002). The miRNAs affected by the diet were enriched in lipid metabolism pathways.

Conclusions: Our results suggest that consumption of a diet rich in TFAs modulates the HDL-carried miRNA content and their preferential localization, potentially reflecting cellular miRNA response to the different dietary TFAs. These microtranscriptomic variants may mirror physiologically relevant changes in HDL functions and secondary signalling in response to the diet.

[32] ExpressionLncr: a pipeline for leveraging latent gene expression data in lncRNA studies

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Background: Investigating the functionality of lncRNA and other RNA in regulating the genome is an active and growing area of research. Databases of non-coding RNAs such as NONCODE and LNCipedia contain 141k and 119k human annotated lncRNAs to date, respectively—necessitating informatics tools to sift through and investigate functionality en masse. A wealth of functional genomics data is already deposited in NCBI GEO but, likewise, harnessing these 70 thousand experiments on 1.8 million samples in an effective manner can be challenging for those without informatics resources.

Hypothesis: Existing functional genomics data can be leveraged to predict functionality of lncRNAs.

Methods: We have created a program called ExpressionLncr(na) to harness this latent information. It is a pipeline to investigate the potential expression of lncRNAs by leveraging existing NCBI GEO gene expression information. The software sources lncRNAs from lncRNA databases such as NONCODE or LNCipedia and is not restricted to lncRNAs, allowing user-specified chromosome features. Features are restricted to reference organisms with expression probe array annotation information in Ensembl. The tool computes matches for positional overlap between Ensembl expression probes and lncRNAs. Summary results from GEO DataSets relevant to these overlapping features are used to calculate presence or absence of expression at each lncRNA. Positive links between probe expression data and lncRNA position may suggest possible functionality of the lncRNA worth further investigation. ExpressionLncr is written as a collection of command-line scripts with an optional graphical interface. The software will be available in two formats, both freely available: as a set of tools for the popular Galaxy bioinformatics web platform, and as an operating system independent desktop application. Future work is planned to extend the pipeline to RNA-seq information.

Summary: ExpressionLncr is a bioinformatics pipeline to investigate the functionality of lncRNAs and other chromosomal features by computing positional overlap between lncRNA databases and existing gene expression probe information in NCBI GEO. Exploiting this latent information should help investigators interested in non-coding RNAs in planning new studies as well as prioritising candidate non-coding RNAs for molecular biology experiments.

[33] Parent-of-origin effect in Asthma – GWAS Meta-analysis in three Canadian Cohorts

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Background: Asthma is a complex disease caused by a combination of genetic and environmental factors. Heritability is estimated to range between 0.48- 0.79. To date, 44 significantly associated SNPs have been identified by 23 asthma genome-wide association studies (GWAS) (at $p<10^{-6}$). The consensus is that the main genetic effects of these common SNPs (with modest effects) do not fully explain the heritability of asthma. Genomic imprinting is a potential mechanism which may explain some of the 'missing heritability'. Imprinting is an epigenetic phenomenon where the expression of genes depends on their parental origin (parent-of-origin effect). Imprinting effects have been reported in the development of many complex diseases.

Hypothesis: Imprinting is involved in the etiology of asthma.

Methods: To identify candidate genomic regions for imprinting we used GWAS data from three family-based studies (two parents and one offspring). These studies are: 1) the Canadian Asthma Primary Prevention Study (CAPPS), a high-risk asthma birth cohort and, 2) the Study of Asthma Genes and Environment (SAGE), a population-based asthma birth cohort 3) the Saguenay-Lac-Saint-Jean Québec Familial Collection (SLSJ), a founder population of French-Canadians. We used a likelihood-based variant of the Transmission Disequilibrium Test. Parent-of-origin effects in SLSJ as well as the combined CAPPS and SAGE were performed by including parental sex as a modifier in the analysis. Meta-analysis was conducted using the results of SLSJ and the joint analysis of CAPPS and SAGE weighted by the number of informative transmissions for each study.

Results: In SLSJ, 7 SNPs showed significant parent-of-origin effects with $p<10^{-5}$ (252 trios with asthmatic children). In the joint analysis of CAPPS and SAGE, 13 SNPs showed significant parent-of-origin effects with $p<10^{-5}$ (148 trios with asthmatic children). Notably, in the joint analysis of CAPPS and SAGE, we identified a parent-of-origin effect at a known imprinted gene, *CTNNA3*. This gene was previously identified in a GWAS study of occupational asthma. Of all significant results in those two analyses ($p<10^{-5}$), 12 out of 20 of the SNPs were in or near Long non-coding (lnc)RNA genes. LncRNAs are known to be involved in genomic imprinting and gene regulation. Meta-analysis resulted in two SNPs with significant parent-of-origin effects with $p<10^{-5}$ in the genes *SLC39A10* and *LNX2/POLR1D*.

[35] Placental lipoprotein lipase DNA methylation alterations are associated with body composition in childhood

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Background: Gestational diabetes mellitus (GDM) has been previously associated with childhood metabolic complications. This suggests that the consequences of *in utero* exposure to maternal hyperglycemia extend beyond the fetal development, possibly through epigenetic programming. We recently reported associations between maternal blood glucose concentrations and placental DNA methylation (DNA-me) levels within the *LPL* gene locus. The **aim** of this study was thus to assess whether these variations in placental DNA-me marks are associated with body composition at 5 years old.

Methods: Anthropometric and body composition (bioimpedance) were measured at age 5 for 66 children (24 from GDM mothers) from our initial birth cohort. *LPL* DNA-me levels were measured using bisDNA-pyrosequencing in placenta samples collected <30 minutes of delivery. Mann-Whitney and Spearman tests were used to assess associations between placental DNA-me levels and children body composition (weight, height, BMI, fat mass and lean mass) at birth and age 5. Weight, height and BMI z-scores were computed according to WHO growth chart (age-sex specific). The analyses were adjusted for confounding factors (pre-pregnancy age and BMI, gestational age, age and sex of the children.

Results: *LPL* DNA-me levels were correlated with newborns' (r=0.252; p=0.04) and children's (r=0.314; p=0.01) weight z-scores. *LPL* DNA-me levels were also correlated with mid-childhood BMI z-scores (r=0.212; p=0.09), lean mass (r=-0.260; p=0.04) and fat mass (r=0.230; p=0.07). After further adjustments for maternal 2h post-OGTT, these correlations remained statistically significant. We observed a strong negative correlation between *LPL* DNA-me and mRNA levels in placenta samples (r=-0.459; p<0.001), supporting the role of these LPL epivariations in transcriptional activity regulation.

Conclusions: Our results show that alterations in placental DNA-me levels at the *LPL* gene locus are associated with body composition of the offspring up to mid-childhood. These findings support the concept of fetal metabolic programming through epigenetic changes.

[36] Intestinal epithelial determination is driven by class I HDAC, notably Hdac1 and Hdac2

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BACKGROUND: Hdac1 and Hdac2 deacetylases regulate gene expression by deacetylating histones and a number of regulatory proteins. We have previously observed that defined thresholds of intestinal epithelial cell (IEC)-specific Hdac1 and Hdac2 expression led to intestinal inflammation in mice. Whether these homeostatic changes are IEC intrinsic or depend on subsequent environmental changes remain to be determined.

AIMS: Our objective was to determine the IEC-intrinsic effect of Hdac1 and Hdac2 in enteroid cultures, by using genetic and pharmacological approaches.

METHODS: As an inducible genetic model, jejunal *villin*Cre^{ER} *Hdac1* and *Hdac2* enteroid cultures were treated with hydroxytamoxifen to induce gene deletion. Pharmacological inhibition of class I Hdac was achieved with 5 µM of the HDAC inhibitor CI994 for 5 days in jejunal enteroid cultures. Enteroid structure was observed by microscopy, and enteroid growth by BrdU labeling. Expression of selected genes was assessed by qPCR. Protein expression was assessed by quantitative mass spectrometry approaches. Pathways were identified by bioinformatics methods.

RESULTS: Induced *Hdac1* and *Hdac2* deletion led to spheroidal structures, decreased enteroid growth, increased phospho-γH2AX staining, and determination defects, as assessed by decreased expression of lineage markers. CI994 treatment resulted in smaller enteroids, less crypt-budding formation, and altered differentiation. For example, antimicrobial Reg3β and Reg3γ mRNA expression, as well as Tgf-β, was increased. In contrast, goblet (Zg16) and endocrine (ChgA) marker expression was reduced. In response to CI994, proteins related to ATP and acetyl group production pathways (HK1 and PK) were increased while replication and cell cycle pathway proteins (MCMs group and Ki67) were decreased, as assessed by quantitative proteomic analysis.

CONCLUSIONS: Class I HDAC pharmacological or genetic inhibition disrupts enteroid growth, differentiation and development.

[37] Casein Kinase 2 regulates Spt6 histone chaperone function to assure transcriptional accuracy in *Saccharomyces cerevisiae.*

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Chromatin structure is highly dynamic during transcription, unfolded to get access to DNA and refolded back in the wake of RNA polymerase II (RNAP II). Multiple mechanisms act together to make this process highly efficient and allow cells to tightly regulate gene expression.

Ck2 is a protein kinase ubiquitously present among eukaryotes and implicated in various important cellular processes. In this study, we demonstrate that Ck2 is directly involved in chromatin structure modulation during transcription elongation. Using a Ck2 thermosensitive mutant (*ck2^{ts}*), we found that Ck2 depletion from yeast cells results in spurious transcription from cryptic promoters, and an increase of histone turnover in the coding region of transcribed genes. Futhermore, transcription seems to be generally affected in *ck2^{ts}* mutant as shown by an increased recruitment of an inactive form of RNAP II throughout *Saccharomyces cerevisiae* coding regions, thereby compromising mRNA optimal production.

Interestingly, we found that Ck2 interacts with Spt6 and phosphorylates it *in vivo* and *in vitro*. Spt6 is an essential histone chaperone that plays a major role in the wake of transcription. We mapped the Ck2 phosphorylation sites in Spt6 N-terminal region and found that the modification of all these potential phosphorylated residues results in altered Spt6 function and accumulation of cryptic transcripts. We generated phosphomimic mutants in all Ck2 potential phosphosites of Spt6 and found that they partially suppress cryptic transcript phenotype in *ck2^{ts}* mutant as well as histone turnover. Finally, we provide evidence that Ck2 phosphorylation regulates tightly Spt6 levels in the cell. We propose that this regulation plays a crucial role in the responses to environmental signals such as change in carbon source or a heat-shock.

Altogether, our data highlights Ck2 growth signaling pathway as a regulator of transcription accuracy by affecting the essential histone chaperone Spt6, and probably other factors directly involved in the transcriptional process. This mechanism is important to suppress cryptic transcription in steady state conditions but also seems to assure the fitness of cellular response to external stress signals.

[38] Global transcriptome analysis of CD34+ chronic-phase CML cells

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Chronic myeloid leukemia (CML) is a progressive multi-lineage leukemia characterized at diagnosis by the presence of a clone marked by a *BCR-ABL1* fusion oncogene. In the chronic phase (CP) of the disease, expression of this fusion gene in the myeloid-restricted CD34+ clonogenic CML cells deregulates their proliferative activity, but does not affect their ability to produce mature, functionally normal blood cells. This results in the establishment of a hierarchical differentiation structure within the CP CML clone in which the more primitive elements have a competitive advantage over the residual normal hematopoietic progenitor cells. As a consequence, the CP leukemic clone can become the dominant source of most of the circulating blood cells. The CD34+ *BCR-ABL1*+ compartment has been previously shown to possess both an abnormal autocrine mechanism of self-activation mediated by G-CSF and IL-3, and an insensitivity to several chemokines that exert stage-specific anti-proliferative effects on normal hematopoietic progenitors. Evidence of aberrant expression of other genes has also been reported, but the precise mechanisms that underpin the abnormal biology of CP CML cells remain poorly understood, possibly due to the reliance to date of deriving gene-expression differences mainly from microarray data that miss a large part of the transcriptome. Here, we present results of global gene expression profiles on primary samples of highly purified (>90% pure) CD34+ CP CML (n=7) and normal adult human bone marrow (n=3) or cord blood (n=1) cells generated from strand-specific RNA-Seq libraries. Sample relatedness was determined from Spearman distances and differentially expressed genes were identified using the DESeq tool and custom R scripts. Differentially regulated pathways were investigated with the Broad Institute's Gene Set Enrichment Analysis (GSEA) software. From these analyses, we found the majority of differentially expressed, protein-encoding genes had lower transcript levels in the CP CML cells as compared to their phenotypically normal counterparts isolated from adult bone marrow or cord blood. In both comparisons, affected genes included *IRF8*, *GAS2*, and *GLI2,* all of which have been previously associated with CML. GSEA also showed upregulated expression of genes targeted by MYC and downregulated expression of genes involved in KRAS signalling within CML CD34+ cells. Interestingly, comparison of the data for cord blood cells with either the CML or adult bone marrow data revealed differential expression of several let-7 target genes previously shown to distinguish primitive hematopoietic cells in fetal and adult mice, consistent with the likely preservation of this developmental program in humans. These findings set the stage for future analyses of the epigenomic mechanisms that mediate changes in gene expression within CML CD34+ stem and progenitor cells that determine their disease-specific properties.

[39] The DNA Methylome, Glucocorticoid Sensitivity and Clinical Heterogeneity in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS)

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Long-term changes in immune and endocrine stress responses are common in a number of complex diseases. Several reports have linked alterations in these systems with differences in the DNA methylome. Yet symptom heterogeneity constitutes a barrier to understanding disease etiology and treatment options. Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) is an idiopathic complex disease characterized by a persistent unexplained fatigue with some of the following symptoms: post-exertional malaise, headache, sore throat, lack of refreshing sleep, muscle or joint pain, tender cervical and axillary lymph nodes, and impaired memory and concentration. Several studies have reported differences in immune function and stress response, including sensitivity to glucocorticoids, in ME/CFS patients. We previously identified differences in the DNA methylome in a cohort of sudden onset ME/CFS patients, suggesting a role for this epigenetic modification in ME/CFS. To explore heterogeneity in ME/CFS, we examined the DNA methylome in immune cells using the Illumina 450K BeadChip array, and investigated the associations of the DNA methylome with the cellular response to glucocorticoids and clinical symptoms. We obtained peripheral blood mononuclear cells (PBMCs) from 49 ME/CFS and 26 healthy females who did not consume medications with known epigenetic or immunomodulatory effects. Self-reported quality of life health scores according to the RAND-36 inventory were collected from each participant. Glucocorticoid sensitivity was assessed by quantifying cell proliferation after stimulation with phytohaemagglutinin, a T-cell mitogen, and suppression with dexamethasone, a synthetic glucocorticoid. DNA methylome data were corrected for batch, age, BMI, and cellular admixture. Differentially methylated loci with ≥ 5% mean difference were identified using a statistical cutoff $p \leq 0.05$, DNA pyrosequencing, and permutation analyses.

We observed 12,608 significant differentially methylated loci in our ME/CFS cohort compared to healthy controls. ME/CFS patients showed increased mean sensitivity to dexamethasone in ME/CFS overall. In addition, two response subgroups emerged among the ME/CFS patients. We identified 13 differentially methylated loci potentially related to glucocorticoid sensitivity, and found significant relationships between methylation, glucocorticoid sensitivity, and quality of life reports germane to ME/CFS. The results indicate a potential role of DNA methylation modifications in ME/CFS pathology and in glucocorticoid hypersensitivity observed in some ME/CFS patients. The glucocorticoid response subgroups within the ME/CFS cohort highlight the heterogeneity of the disease and the need for clinical subtyping. The differentially methylated loci observed in this study could be used in future ME/CFS for reliable biomarkers, accurate clinical subtyping, and understanding the biological basis of ME/CFS as well as related complex disease states.

[40] Quantitative comparison of genome wide distribution of histone marks in patient-derived glioblastoma cell lines using ChIP-Rx method

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Glioblastoma is a grade IV malignant brain tumor with poor prognosis and rapid disease progression. Somatic mutations in histone 3 (H3) genes have been identified in pediatric glioblastomas. The acquired mutation K27M in H3.1 and H3.3 impairs lysine methylation on wild-type H3 at K27, while H3.3 G34R/V possibly affects methylation at the K36 position. However, how these histone mutations drive tumor development remains poorly understood. Previous studies have shown that both K27M and K36M mutations in H3 dramatically change global levels of specific post-translational modifications in the H3 tail. The current method for profiling genome wide distribution of histone marks, chromatin immunoprecipitation combined with next generation sequencing (ChIP-seq), is not quantitative, a significant caveat in glioblastoma epigenomic research, especially when facing genetic alterations that drastically change global levels of specific histone marks. We aim to understand the effects of H3 mutations on the epigenome by quantitatively profiling patient-derived cell lines for a number of key histone H3 modifications.

We have assembled a collection of cell lines derived from the glioblastoma patients, carrying different mutations (*H3F3A*-K27M, *H3F3A*-G34R, *H3F3A*-G34V, *HIST1H3B*-K27M). Genome-wide distribution of six histone marks (H3K4me1, H3K4me3, H3K27ac, H3K27me3, H3K36me2, H3K36me3) has been profiled in these cell lines using a modified ChIP-seq protocol. This modification of ChIP-seq protocols, ChIP-Rx, allows quantitation of histone marks by adding reference drosophila chromatin in the ChIP reaction and using the reference normalization of peak intensity. Using ChIP-Rx, we observe significantly lower levels of H3K27me3 mark and higher levels of H3K27ac mark in K27M mutant cell lines. However, despite very low total levels of H3K27me3 mark, K27M mutant cells display enrichment of the mark in certain regions, at comparable levels to wild-type cell lines. These changes induced by K27M mutations also appear to be specific to the cell of origin and/or the tissue they occur in and may help better understand the effect they have in reshaping the epigenome and promoting oncogenesis.

[41] Supervised machine learning using visual labels for training and testing ChIP-seq peak detectors

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Motivation:
Many peak detection algorithms have been proposed for ChIP-seq data analysis, but it is not obvious which method and what parameters are optimal for any given data set. In contrast, regions with and without peaks can be easily labeled by visual inspection of profile data on a genome browser.

Results:
We propose a supervised machine learning approach to ChIP-seq data analysis, using labels that encode an expert's qualitative judgments about which genomic regions contain or do not contain peaks. The main idea is to manually label a small subset of the genome, and then learn a model that makes consistent predictions on the rest of the genome. We show how our method can be used to quantitatively train and test peak detection algorithms on specific data sets. We created 7 labeled data sets consisting of two histone marks, and analyzed 3 existing transcription factor data sets. Of the 11 peak detectors we tested, the supervised PeakSeg method was the most accurate.

Availability:
An R package for computing the number of incorrect labels is on https://github.com/tdhock/PeakError, and our benchmark labeled histone data sets can be downloaded from http://cbio.ensmp.fr/~thocking/chip-seq-chunk-db/.

[42] Topoisomerase II beta interacts with cohesin and CTCF at topological domain borders

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Background
Type II DNA topoisomerases (TOP2) regulate DNA topology by generating transient double stranded breaks during replication and transcription. Topoisomerase II beta (TOP2B) facilitates rapid gene expression and functions at the later stages of development and differentiation. To gain new insight into the genome biology of TOP2B, we used proteomics (BioID), chromatin immunoprecipitation, and high-throughput chromosome conformation capture (Hi-C) to identify novel proximal TOP2B protein interactions and characterize the genomic landscape of TOP2B binding at base pair resolution.

Results
Our human TOP2B proximal protein interaction network included members of the cohesin complex and nucleolar proteins associated with rDNA biology. TOP2B associates with DNase I hypersensitivity sites, allele-specific transcription factor binding and evolutionarily conserved transcription factor binding sites on the mouse genome. Approximately half of all CTCF/cohesin bound regions coincided with TOP2B binding. Base pair resolution ChIP-exo mapping of TOP2B, CTCF, and cohesin sites revealed a striking structural ordering of these proteins along the genome relative to the CTCF motif. These ordered TOP2B-CTCF-cohesin sites flank the boundaries of topologically associating domains with TOP2B positioned externally and cohesin internally to the domain loop.

Conclusions
TOP2B is positioned to solve topological problems at diverse cis-regulatory elements and its occupancy is a highly ordered and prevalent feature of CTCF/cohesin binding sites that flank topologically associating domains.

[43] Molecular characterization of the role of RUNX1 in Notch signaling in T-cell Acute Lymphoblastic Leukemia (T-ALL)

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T-cell acute lymphoblastic leukemia (T-ALL) is a hematopoietic malignancy driven by oncogenic activation of Notch signaling. T-ALL accounts for 15% of pediatric and 25% of adult acute lymphoblastic leukemia cases (Pui et al, NEJM, 2004;350:1535–48). Runt-related transcription factor 1 (RUNX1) is hypothesized to participate in supporting Notch signaling, which is well characterized as an oncogenic pathway in T-cell leukemia. In support of this hypothesis we find that human T-ALL patient-derived samples and cell lines (e.g., KOPT-K1) are sensitive to RUNX1 depletion mediated by lentiviral shRNAs and pharmacologic Notch pathway inhibition. In order to dissect the molecular mechanism(s) underlying these phenotypes, we have performed mRNA-seq and ChIP-seq against a panel of histone modifications (H3K4me1, H3K4me3, H3K27ac, H3K36me3, H3K27me3 and H3K9me3) for samples that have either been depleted of RUNX1 (shRNA) or NOTCH1 (pharmacologic inhibition). Gene expression analysis revealed that 14 genes (e.g., CR2, DTX1, EGR1, FGR, HES4, MYC, NOTCH3 etc.) with NOTCH1 and RUNX1 binding site at proximal region (+-5Kb of TSS) are down regulated in absence of either NOTCH1 or, RUNX1. We found NOTCH1 and RUNX1 sites localized to H3K27ac and H3K4me3 enriched promoters of those 14 genes, whereas these regions lose enrichment when NOTCH1 or, RUNX1 is turned off. These NOTCH1 and RUNX1 co-regulated sites also intersect with other co-activators e.g., P300, ETS1, BRD4, MED1, GABPA, RBPJ (data source: Hongfang Wang et. al. PNAS, 2014;111(2):705-10). Kitabayashi I. et al, The EMBO Journal, 1998,17, 2994-3004, showed that RUNX1 interacts with p300 and overexpression of p300 stimulates RUNX1-dependent transcription to induce myeloid cell differentiation. We observed a global loss of H3K27ac upon RUNX1 silencing which is possibly the absence of RUNX1 halts the recruitment acetylation through P300. This result might suggest the role of RUNX1 in recruitment of histone acetyl transferases in T-ALL. Simultaneously, there is gain of H3K27me3 in proximal regions and ~30% active enhancers become poised in absence of RUNX1. The loss of H3K27ac and gain of H3K27me3 indicates a broad chromatin re-structuring in absence of RUNX1. KEGG pathway enrichment analysis shows RUNX1 target genes are involved in cell cycle, receptor sinaling and metabolic pathways. Consequently, we observed a good proportion of cells stop replication and block cell cycle in BrdU incorporation assay. Together these results we suggest an essential role for RUNX1 in regulating the expression of genes involved in maintaining T-ALL through recruiting H3K27ac.

[44] A Novel Neurodevelopmental Disorder caused by Recessive Mutations in ACTL6B

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In Canada, one in twelve individuals are affected by a rare disorder, of which 80% have a genetic origin. We identified four families with mutations in *ACTL6B*, in which children with homozygous recessive mutations exhibit developmental delays, seizures, hypotonia and spasticity. *ACTL6B* codes for BAF53B, a component of the BAF complex, a protein complex involved in neuronal differentiation. To determine the molecular mechanism of mutations in *ACTL6B*, expression constructs carrying the genetic from affected individuals were cloned into HEK cells. Sucrose gradient centrifugation of these cell lysates revealed that the mutated BAF53Bs are incapable of incorporating into the BAF complex, indicating that the BAF complex dysfunction in these patients is due to improper association of BAF53B with the complex. Fibroblasts from one patient and two controls were converted into induced pluripotent stem cells, and subsequently differentiated into neural progenitor cells (NPCs). We performed RNAseq on pre- and post-mitotic NPCs in patient and control cells and found that the mutation in *ACTL6B* led to a profound reduction in BAF53B mRNA and protein, confirming a loss of function mutation in patient derived neuronal cells. Since the BAF complex is a transcriptional regulator, we also performed ChIPSeq using a non-mutated protein in the BAF complex, and cross referenced differential BAF binding to Gene expression differences in patient and control pre- and post-mitotic NPCs. These data support the notion that mutations in *ACTL6B* affect binding of the BAF complex to DNA and alter gene expression patterns. We have identified a novel syndrome caused by mutations in ACTL6B, on the same BAFopathy spectrum as Coffin-Siris and Nicolaides-Barisiter Syndromes.

[45] Functional crosstalk between histone methyltransferases EHMT1 and KMT2C involved in Intellectual Disability and Autism

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Dynamic regulation of gene expression is essential for brain development and function. To date, more than 55 genes encoding chromatin regulating proteins have been implicated in the etiology of ID. This suggests that chromatin-mediated gene regulation is an important biological process in neurodevelopmental disorders, and leaves the open question as to whether disruption of gene regulatory networks through mutations in different genes causes an overlapping clinical spectrum that could be viewed as a common disorder. Here we provide evidence at the clinical and molecular level that this may be true, at least for a subset of chromatin modifiers. At the clinical level we describe 5 patients with *de novo* heterozygous loss of function mutations in *KMT2C*, and demonstrate a striking clinical overlap in comparison with Kleefstra syndrome, an ID disorder defined by heterozygous mutations in *EHMT1*. KMT2C and EHMT1 are both histone methyltransferases that are involved in methylation of H3K4 and H3K9, respectively. In *Drosophila*, we demonstrate that the highly conserved KMT2C ortholog, trr, is required for memory and binds to the promoter of many genes that are involved in neuronal development and function. These findings are consistent with our previous data regarding the *Drosophila* EHMT1 ortholog, G9a (Kramer et al., 2011, PLoS Biol.), and further support a potential functional relationship between these two proteins. At the genomic level, we demonstrate a high level of convergence in target genes and biological pathways that are under the control of trr and G9a in fly heads, and identify 5 candidate genes that many be critical common targets involved in the regulation of neuronal function and metabolism. The observed clinical and molecular convergence between these two proteins suggests that they are important players in an overlapping gene regulatory network that is critical for neuronal function in both healthy and disease states.

[46] Identifying DNA methylation differences that contribute to an age-dependent increase in bovine innate immunity

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Reduced representation bisulfite sequencing (RRBS) was used to identify DNA methylation differences in paired dermal fibroblast cultures taken from six heifers at 5 and 16 months of age. Prior to RRBS, we determined that the older fibroblast cultures responded with greater ($P<0.05$) interleukin-8 (IL8) and interleukin-6 (IL6) protein secretion and gene expression following a 36 h LPS treatment. From the same fibroblast cultures, DNA was analyzed for CpG methylation by RRBS (Methyl-MiniSeq™; Zymo Research). A paired t-test comparing methylation ratios revealed 14,094 differentially methylated CpGs ($P<0.05$) with ≥5X coverage. To identify sites with greatest biological relevance, we refined our analysis to include only CpGs with ≥25% methylation ratio difference and ≥7X coverage leaving 1,063 sites. The majority (833, 80%) of these sites were hyper-methylated in young cultures, including 77 out of the 90 sites found within gene promoter regions (-2.5 kb to +1.0 kb from the transcription start site). Transcription factor analysis was performed (TRANSFAC v8.2) on genes containing differentially methylated sites ($P<0.05$, ≥25% meth diff, ≥7X coverage) between young and old cultures. In agreement with their lower LPS response, genes hyper-methylated in cultures from young animals showed an association ($P<0.05$) with NF-κB regulated genes and genes controlled by MAZR, which regulates expression of the acute phase proteins serum amyloid A (SAA1-3). To determine if differential methylation was associated with gene expression, a panel of 6 differentially methylated ($P<0.05$, ≥25% meth diff, ≥7X coverage) immune-related genes (*TNFSF13, FES, PIK3R1, RORA, NFATC1* and *TCF7*) were selected for analysis by RT-qPCR on RNA obtained 0, 2, and 8 hours post-LPS treatment. With the exception of *TCF7*, young cultures with greater methylation in all 6 genes also had lower levels ($P<0.05$) of gene expression. This indicates that DNA methylation may have affected expression of these genes and may contribute to age-dependent differences in the dermal fibroblast response to LPS. However, a caveat to RRBS is the potential to miss regions of the genome lacking a sufficient number of CCGG *MSP*I cut sites. One such gene, *TLR4*, the main receptor for LPS signaling, was found to be significantly ($P<0.05$) more expressed in 16-month cultures at 0, 2 and 8 hours post LPS treatment with no induction in response to LPS. Furthermore, measuring *TLR4* gene expression in ear notch tissue from a cohort of adult (3-6 y) dairy cows revealed a ~1,000-fold difference in expression between highest and lowest animals. Expression of *TLR4* was then re-measured in the 16 high and low animals (n=8 per group) and was found to be repeatable in both ear notch ($P<0.05$, $R^2=0.84$) and PBMC ($P<0.05$, $R^2=0.72$) samples. Fibroblast cultures from the same group of high and low animals also reflected a significant ($P<0.05$) difference in *TLR4* expression. Bisulfite sequencing analysis of the *TLR4* promoter is underway to determine whether expression of *TLR4* is regulated by DNA methylation. If methylation differences are identified, the current study has the potential to shed some light onto causes of between-animal variation in innate response and differential susceptibility to economically important diseases, such as bovine mastitis.

[47] Genome-wide methylation and gene expression according to HPV status in penile carcinomas is consistent with two distinct mechanisms of carcinogenesis

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Penile carcinoma (PeCa), a rare disease in developed countries, is often associated with HPV infection, particularly HPV-16 and HPV-18. The impact of HPV infection on genetic and epigenetic alterations is poorly understood in PeCa. In this study, genome wide methylation and gene expression profiles of PeCa samples were compared according to HPV status. Gene expression analysis (4x44K Whole Human Genome platform, Agilent Technologies, Santa Clara, CA) was performed in 33 usual PeCa samples. Twenty-five of these cases were also evaluated using a 244K Human DNA Methylation Microarray platform (Agilent Technologies, Santa Clara, CA). Our large scale transcriptome analysis showed 386 differentially expressed genes according to HPV positivity. The gene expression profile was able to discriminate two groups according to HPV infection. A functional *in silico* analysis of these genes indicates that HPV infection promotes inflammation, and disrupts cell cycle control and DNA repair pathways. Seventeen independent samples evaluated by HTA Array 2.0 (Affymetrix) confirmed the gene expression signature showing a 60% of overlap between the two platforms. *PCNA, MSH6* and *RFC4* (genes involved in the MMR pathway*)* overexpression in HPV positive cases was confirmed by RT-qPCR. Methylation analysis revealed 298 hypermethylated and 470 hypomethylated probes in HPV positive cases. Integrative analysis of DNA methylation and gene expression (Agilent) revealed 12 genes downregulated/hypermethylated and three genes upregulated/hypomethylated in HPV positive cases. DNA methylation analysis revealed that pathways related to RB suppressor gene, infection and immune response are altered in HPV positive cases.

In the case of HPV negative cases we found pathways involved with development and regulation of stem cells. Six of these genes (*ONECUT1, CDX2, OTX2, NKX2.2, SOX3* and *SOX14)* were evaluated by pyrosequencing confirming decreased methylation levels in HPV negative tumors. These data provide new evidences for the distinct pathways associated with penile carcinomas and can be useful to identify different therapeutic strategies according to HPV status.

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[48] DNA methyltransferase 1 controls unmethylated transcription start sites in the genome in *trans* by a DNA methylation-dependent mechanism

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It is expected that DNA methylation enzymes downregulate transcription initiation through promoter methylation. We used ChIP-sequencing with an antibody to RNApolII-Ser5 (RNAPII-Ser5) to map the genomic landscape of transcription initiation positions that are unraveled by either pharmacological inhibition of DNA methyltransferase (DNMT1) with 5-aza-2'-deoxycytidine (5-azaCdR) or knockdown with shRNA. We show that in sharp contrast to the commonly held notion, inhibition of DNMT1 dramatically expands the number of transcription initiation positions in the genome not only of methylated regions as expected but also surprisingly of many unmethylated regions. Our data suggest multi-tiered and multi-faceted regulation of the transcription landscape by DNA methylation and DNMT1. These data change our understanding of the role of DNA methylation in controlling genomic-programs in development, physiology and pathology and our assessment of the potential outcomes of the clinical use of DNA methylation inhibitors

[51] Hypoxia and Nodal: Epigenetic Modulators in the Development of Cancer Cell Plasticity

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Nodal, an embryonic morphogen, and hypoxia contribute to pluripotency in Embryonic Stem Cells (ESCs), but also support cancer progression when its signalling pathways are dysregulated. Both of these factors have been shown to epigenetically alter gene expression, and are crucial in the development of Cancer Stem Cells (CSCs). CSCs are a subpopulation of cancer cells characterized by plasticity and self-renewal, which afford CSCs with the ability to metastasize and resist therapies, leading to reduced survival in patients.

Here we propose to address how the epigenome of CSCs respond to micro-environmental factors such as hypoxia and Nodal by examining the alterations in histone modifications in concert with the resulting transcriptional response. Cell lines that up-regulate stem cell sustaining genes such as Nodal in response to hypoxia was used, namely breast cancer cell lines, T47D and MDA-MB-231. To determine epigenetic changes, Chromatin Immuno-Precipitation (ChIP) with high throughput sequencing (ChIP-seq) was conducted using antibodies to repressive (H3K27Me3) and active (H3K4Me3) histone marks, which were chosen for their association with hypoxia, Nodal, and regulation of the stem cell phenotype. Additionally, RNA sequencing was performed to match gene expression changes and PCR will be incorporated to validate ChIP-seq and RNA-seq results. Future work will further incorporate H9 hESC for comparison of the cancer epigenome with the primed epigenetic state present in ESCs.

Elucidating the role of histone modifications in the transcriptional response to hypoxia and Nodal, as well as the role of Nodal in hypoxia associated alterations, will better our understanding of how the microenvironment regulates CSCs and ESCs, leading to the discovery of potential therapeutic targets.

[52] Association between *DMPK* DNA methylation and muscle strength in myotonic dystrophy type 1

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Myotonic dystrophy type 1 (DM1) is an autosomal dominant disorder caused by a CTG repeat expansion within the 3'-untranslated region (exon 15) of the *DMPK (dystrophia myotonica protein kinase)* gene, which is on chromosome 19. The repeat is part of a CpG island and harbours one CTCF binding site on each side. Clinical manifestations of DM1 include muscular weakness and myotonia but other symptoms such as cataracts, cardiac arrhythmia, hypersomnolence, respiratory insufficiency, cognitive and endocrine problems can be seen in the affected patients. Large variability exists between individuals with DM1 in term of severity of symptoms, which only partially correlates with the CTG repeat length. Our group and others have hypotheses that epigenetics could explain remaining phenotypic variability. **Objective:** To assess the impacts of DNA methylation profile at *DMPK* gene locus on phenotypic variability in DM1. **Methods:** Pyrosequencing of bisulfite treated DNA (from 115 patients with late (25) and adult (90) onset DM1) was used to quantify DNA methylation levels at *DMPK* gene locus. *SIX5* and *DMPK* mRNA levels were quantified using real-time PCR. Clinical data were collected using standard procedures. Associations were tested using Spearman rank coefficient correlations on residuals, corrected for age, sex and rs635299 genotypes. **Results:** Close to the CTCF-binding site just downstream the CTG repeats, we first observed lower DNA methylation levels in participants with late-onset as compared to adult phenotypes (ex: 3.4% vs 5.6% $p<0.001$). Among participants with the adult phenotype, some of these CpGs were also associated with pinch strength ($r_s=0.220$; $p=0.040$) and CTG repeat length ($r_s=0.251$ and 0.253; $p=0.018$ and 0.019). Finally, *DMPK* mRNA levels were correlated with handgrip strength ($r_s=-0.255$; $p=0.019$) and CTG repeat length ($r_s=0.445$; $p=<0.001$). **Conclusion:** DNA methylation at *DMPK* gene locus is associated with DM1 phenotype, muscle strength and CTG repeat length. Epivariations at this locus could thus explain part of the phenotypic variability observed in DM1 and might help predict the progression and prognostic of the disease, but further studies are needed.

[53] Early Embryonic Induction of Fetal Alcohol Spectrum Disorders Leads To Heterogeneity In Mouse Brain DNA Methylation Perturbations

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Prenatal alcohol exposure (PAE) is known to altered epigenetic profiles in cells during brain development. These alterations are believed to be part of the molecular basis underpinning Fetal Alcohol Spectrum Disorders (FASD) etiology. However, the consequences of a PAE during early embryonic life –a period when women are still unaware about their pregnancy– on the future epigenetic landscape of the brain remain unknown. **Our research hypothesis is that a PAE during pre-implantation will initiate DNA methylation dysregulation**. Consequently, we believe that these original epigenetic alterations will be perpetuated and amplified in the developing brain, leading to abnormal brain functions associated to FASD.

To test this, we instigated FASD in mouse 8-cell embryos by injecting (2x) ethanol [2.5g/kg] at 2.5 days of pregnancy (E2.5). We collected FASD (ethanol) and control (saline) E10.5 embryos/placentas and performed morphological analyses. Genome-wide quantitative DNA methylation profiles of E10.5 forebrains and placenta were established by Reduced Representation Bisulfite Sequencing (RRBS). Compared to controls, FASD embryos had more brain and placental abnormalities, however most FASD embryos/placentas were normal. As suspected, we also observe heterogeneity in DNA methylation profiles between each FASD sample (n=6) vs all-controls samples (n=4), reflecting the variety of symptoms and phenotypes present in FASD children. For each FASD samples, regions with differentially methylated tiles (100bp tile, 2 CpG min, 15x reads in all samples, ±≥20% differences) ranged from 382 to 8200. However, despite this heterogeneity, gene ontology analysis linked DMTs with processes implicated in nervous system processes and in organ morphogenesis. When we compared all-FASD vs all-control forebrains, we found a total of 830 common DMTs. Similar analyses were performed for placenta samples and we observed a total of 1757 shared DMTs. Interestingly, we exposed 18 specific regions abnormally methylated in both FASD forebrain and placenta samples.

Our study establishes that early embryonic PAE can cause epigenetic dysregulations that leads to permanent alteration in the future epigenetic program of brain cells. The epigenetic dysregulations also observed in FASD placental tissues allow us to believe that the placenta could be used for epigenetic FASD screening at birth. Altogether, our results allow us to have a better understanding of how epigenetic perturbations can alter the normal function of the brain and lead to neurodevelopmental disorders present in children with FASD.

[54] Murine diet/tissue and human brain tumorigenesis alter *Mthfr/MTHFR* 5'-end methylation

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Polymorphisms and decreased activity of methylenetetrahydrofolate reductase (*MTHFR*) are linked to disease, including cancer. However, epigenetic regulation has not been thoroughly studied. Our goal was to generate DNA methylation profiles of murine/human *MTHFR* gene regions and examine methylation in brain and liver tumors. Pyrosequencing in four murine tissues revealed minimal DNA methylation in the CpG island. Higher methylation was seen in liver or intestine in the CpG island shore 5' to the upstream translational start site or in another region 3' to the downstream start site. In the latter region, there was negative correlation between expression and methylation.

Three orthologous regions were investigated in human *MTHFR*, as well as a fourth region between the two translation start sites. We found significantly increased methylation in three regions (not the CpG island) in pediatric astrocytomas compared with control brain, with decreased expression in tumors. Methylation in hepatic carcinomas was also increased in the three regions compared with normal liver, but the difference was significant for only one CpG. This work, the first overview of the *Mthfr/MTHFR* epigenetic landscape, suggests regulation through methylation in some regions, demonstrates increased methylation/decreased expression in pediatric astrocytomas, and should serve as a resource for future epigenetic studies.

[55] Loss of the H3K36 methyltransferase Setd2 leads to increased myogenic differentiation

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Histone modifying enzymes regulate both cell lineage specification during early embryogenesis and the differentiation of specialized cell types. The covalent modifications of histone residues catalyzed by these enzymes often generate docking sites for additional proteins on chromatin that play a more direct role in modulating transcription process. Genome-wide mapping of histone modifications has allowed for correlations to be established with particular transcriptional states (active or repressed). The H3K4me2/3 marks deposited by Trithorax group protein MLL1/2 and Set1A/B are enriched on promoter proximal regions of actively transcribed genes, while H3K27me3 mark modified by Polycomb group proteins covers the promoter and gene-body of genes within facultative heterochromatin. Similar to H3K4me3, the H3K36me3 mark has been shown to strongly correlate with actively transcribed genes, where the modification can be observed at histones across the gene body. While the histone marks H3K4me3 and H3K27me3 have been well characterized, we currently lack a good understanding of the function of H3K36me3 mark in mammalian cells. Here we set out to explore the role for H3K36me3 by studying the H3K36 methyltransferase Setd2/Hypb in mammalian cell differentiation after cell lineage specification. For this purpose, myogenesis serves as an ideal model for studying the activation of cell-specific gene expression by histone modifying enzymes. When cultured in high-serum conditions myoblasts proliferate extensively, while a switch to low-serum conditions induces efficient differentiation and fusion of myoblasts to form multi-nucleated myotubes. As H3K36me3 is associated with actively transcribed genes, we hypothesized that loss of Setd2 would disturb the activation of muscle gene expression program and lead to a block in muscle differentiation. In growth conditions, we found that the removal of Setd2 by lentivirus-mediated shRNAs has minimal effects on the survival of C2C12, an immortalized rodent myoblast cell line. Surprisingly, upon differentiation, we found that knocking-down Setd2 protein significantly improved the fusion of C2C12 myoblasts into myotubes. Characterization of the Setd2 knock-down cells showed a global loss of H3K36me3 without affecting global H3K36me1 and H3K36me2 levels. This is consistent with previous reports suggesting that Setd2 is the major tri-methyltransferase responsible for H3K36me3. Interestingly, qPCR and Western blot did not reveal changes in expression of myogenic genes in the absence of Setd2, even though N-ChIP assays revealed a marked decrease of H3K36me3 mark within the body of these myogenic genes. This suggests that H3K36me3 modification of histones is not required for high levels of gene expression in mammalian cells. We note that in yeast, H3K36me3 mark is required to ensure efficient transcription initiation at the TSS instead of cryptic TSSs within gene body. It remains to be seen if the H3K36me3 mark is playing a similar role in mammalian cells. Our data discussed here challenge the necessity of the H3K36me3 modifier for myogenesis, and further investigation needs to be focused directly on H3K36me3 mark to decipher its biochemical function during transcription process.

[57] Nucleosome density ChIP-seq identifies distinct chromatin modification signatures associated with MNase accessibility

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Nucleosome position and the posttranslational modification of their histone subunits act in concert to regulate local transcriptional states. Here, we present a modified native ChIP-seq method combined with a novel analytical frame work that integrates nucleosome density with histone modification profiles and apply it to 10,000 primary human cord blood progenitor cells and pluripotent human embryonic stem cells. Correlating immunoprecipitated fragment size, determined by paired-end read boundaries, with ChromHMM-derived chromatin states derived from matching International Human Epigenome Consortium datasets suggest that these states are refined by the inclusion of local nucleosome densities. Using Histone 3 lysine 4 trimethylation (H3K4me3) and H3K27me3 immunoprecipitated fragment size distributions at MACS2 identified enriched regions and a gaussian mixture distribution algorithm, we separate gene promoters into single- or two-nucleosomes dominated for each chromatin modification. We further classified the promoters based on nucleosome density (single- or two-nucleosomes) harboring H3K4me3, H3K27me3, or both into 4 distinct states. Correlation with matching RNA expression and DNA methylation datasets revealed that each state was associated with a unique transcriptional and methylation profile. The resulting classes effectively divided bivalent marked promoters into heterogeneously marked promoters and true bivalent promoters in the populations studied. In addition, our analysis highlighted changes in chromatin structure with prevalent H3K27me3 marking of nucleosomes at CpG-rich regions in the earliest stages of human embryo development, represented by ESCs, with later acquisition of a more compacted chromatin structure at CpG-rich H3K27me3-marked promoters despite their retention of their original DNA methylation status. In summary, we provide a method that integrates nucleosome density with histone modification levels and apply it to a rare population of primary human progenitor cells and pluripotent cell types to reveal novel insights into the combinatorial nature of epigenetic regulation

[58] MTF2 regulates leukemogenic networks in human hematopoietic stem and progenitor cells

Harinad Maganti

Polycomb Repressive Complex 2 (PRC2) proteins have been implicated in various cancers. We have recently shown that Pcl2/Mtf2 is a member of the PRC2 and is required for cell fate regulation in embryonic stem cells and hematopoietic stem and progenitor cells (HSPCs). Our analysis of MTF2 expression levels in AML patients determined that patients with abnormal expression of MTF2 have a poorer prognosis. These results were also supported by analysis of publicly available AML datasets and were found to be independent of the cytogenetics. To dissect the functional role of MTF2 in AML, we transduced human hematopoietic stem and progenitor cells (HSPCs) with lentiviruses to over- and under-express MTF2. Abnormal expression in HSPCs resulted in a pre-leukemic phenotype. HSPCs expressing abnormal MTF2 were more proliferative and exhibited a differentiation bias towards the myeloid lineage in vitro and in vivo. RNA-seq and ChIP-seq analyses were performed and a MTF2 gene regulatory network (GRN) in human HSPCs was drafted. Next, we integrated the MTF2 GRN with publicly available AML gene expression and survival data and identified a leukemogenic module consisting of MTF2 targets that regulate various molecular networks including those controlling proliferation, differentiation and DNA damage response. Functional validation of the nodes within this MTF2 specific leukemogenic module revealed that they phenocopy and rescue the MTF2 pre-leukemic and chemo-resistance phenotypes and therefore could be potential therapeutic targets to treat AML.

[59] Effect of P-TEFb inhibition on the development of cardiac hypertrophy in neonatal rat cardiomyocytes

Ryan Martin

Cardiac hypertrophy initially develops as a compensatory response in heart disease, but a prolonged hypertrophic response is frequently associated with poor cardiac function and eventual heart failure. This pathological response, characterized by an increase in cardiomyocyte surface area, is elicited in the heart in response to neurohormones such as endothelin-1 and norepinephrine. Stimulation of their cognate G protein-coupled receptors activates a signalling cascade leading to the reactivation of a fetal gene program and stimulation of global RNA transcription. One mechanism implicated in the altered gene program involves Cdk9, the catalytic subunit of positive transcription elongation factor b (P-TEFb). P-TEFb releases RNA polymerase II (RNAPII) from a promoter-proximal paused state into productive elongation via phosphorylation of the C-terminal domain of RNAPII, DRB sensitivity inducing factor (DSIF) and negative elongation factor (NELF). Hypertrophic signals cause an increase in P-TEFb activity in cardiomyocytes via disassembly of the inhibitory complex. In contrast, preventing recruitment of P-TEFb to chromatin via inhibition of Brd4, decreases the hypertrophic response and increases RNAPII pausing. Phosphorylation of RNAPII and the Spt5 subunit of DSIF creates a scaffold to recruit co-transcriptional factors necessary for mRNA processing and deposition of histone modifications. The objective of our study is to investigate the role of factors upstream and downstream of P-TEFb in the development of cardiac hypertrophy. Primary neonatal rat cardiomyocytes isolated from 1-3 day old Sprague-Dawley pups were used to model the hypertrophic response in vitro. Endothelin-1 or phenylephrine treatment for 24h stimulated a significant increase in cell surface area of cardiomyocytes as analyzed by high-content microscopy. Co-treatment with iCdk9, a specific P-TEFb inhibitor, blunted the hypertrophic response to either ligand. 5-ethynyl uridine incorporation was measured to assess changes in nascent RNA transcription in response to either agonist and iCdk9. Treatment with endothelin-1 or phenylephrine led to an increase in P-TEFb mediated phosphorylation of RNAPII and Spt5 within 15min, returning to basal levels by 24h. Co-treatment of iCdk9 prevented this increase and caused a significant decrease in basal phosphorylation. Histone modifications associated with actively transcribed genes exhibited small changes in response to either agonist or iCdk9, with only monoubiquitinated histone H2B decreasing in response to iCdk9 treatment. The hypertrophic response was further characterized by analysis of gene expression changes using RT-qPCR. Overall, our study will provide mechanistic insight into to the potentially distinct gene expression patterns induced by different pro-hypertrophic ligands. Uncovering the mechanisms regulating gene expression in cardiac hypertrophy will assist with the development of specific therapies for cardiovascular disease.

[60] Characterization of the transcription and chromatin regulator Prf1/RTF1: novel functions of the Plus3 domain

Jean Mbogning

Transcription elongation by RNA polymerase II (RNAPII) is facilitated by numerous factors including the highly conserved Polymerase-Associated Factor 1 complex (PAF1C). PAF1C regulates gene expression through several mechanisms such as the control of RNAPII promoter-proximal pausing [1,2]. PAF1C plays important roles in co-transcriptional histone modifications [3,4]. PAF1C acts downstream of P-TEFb/Cdk9 to promote histone H2B monoubiquitylation which is a prerequisite for histone H3 methylation at lysine 4 and lysine79. In budding yeast PAF1C is composed of five subunits (Cdc73, Ctr9, Leo1, Paf1 and Rtf1), but in fission yeast and higher eukaryotes, Rtf1 (known as Prf1 in fission yeast) is not stably associated with the remaining core components of PAF1C [5,6]. Previous studies have shown that Prf1/Rtf1 and PAF1C have some distinct functions and get recruited to chromatin through distinct mechanisms [5,6,7]. The significance of the functional relationship between Rtf1 and PAF1C has yet to be elucidated.

Prf1, PAF1C, Spt5 and H2B monoubiquitylation machinery cooperate to regulate RNAPII elongation but a detailed interactions network between these factors is not completely known. Using the fission yeast *S.pombe* as a model system, we could show by in vitro studies that Prf1 contacts PAF1C via its +3 and C-terminal domains. We found that the ubiquitin E3 ligase Brl2 but not the E2 conjugating enzyme Rhp6 also interact with Prf1 but solely through its +3 domain. Interestingly we uncovered that Prf1 can bind simultaneously to Spt5-P, PAF1C and Brl2, suggesting that upon binding to Spt5-P on chromatin, Prf1 could serve as a scaffold for the recruitment of PAF1C and H2Bub1 machinery. Furthermore, point mutational studies revealed a separate binding sites of PAF1C and Spt5-P on Prf1. We found that *R262E* and *R429A* point mutations respectively on the +3 domain and the C-terminal fragment of Prf1 strongly reduced the interaction with PAF1C whereas Spt5-P binding was unaffected. conversely the well characterised *R227A* point mutation on Prf1 which disrupts its interaction with Spt5-P had little effect on PAF1C interaction. In vivo, ChIP experiments argue that the Prf1-PAF1C interaction is important for PAF1C recruitment to a subset of genes, and for Prf1 recruitment in general. These data reveal novel functions for the Prf1/Rtf1 +3 domain and suggest that the Prf1-PAF1C interaction may have gene-specific functions.

1. Fei Xavier Chen, Ashley R. Woodfin, Alessandro Gardini, Ryan A. Rickels, Stacy A. Marshall,Edwin R. Smith, Ramin Shiekhattar, and Ali Shilatifard (2015). PAF1, a Molecular Regulator of Promoter-Proximal Pausing by RNA Polymerase II. Cell 162, 1003–1015
2. Ming Yu, Wenjing Yang, Ting Ni, Zhanyun Tang, Tomoyoshi Nakadai, Jun Zhu, Robert G. Roeder (2015). RNA polymerase II–associated factor 1 regulates the release and phosphorylation of paused RNA polymerase II. Science 350, 6266
3. Wood A, Schneider J, Dover J, Johnston M, Shilatifard A (2003). The Paf1 complex is essential for histone monoubiquitination by the Rad6-Bre1 complex, which signals for histone methylation by COMPASS and Dot1p. J Biol Chem 278:34739-34742
4. Chu Y, Simic R, Warner MH, Arndt KM, Prelich G (2007). Regulation of histone modification and cryptic transcription by the Bur1 and Paf1 complexes. EMBO J 26:4646-4656
5. Manasi K. Mayekar, Richard G. Gardner, Karen M. Arndt (2013). The recruitment of the Saccharomyces cerevisiae Paf1 complex to active genes requires a domain of Rtf1 that directly interacts with the Spt4-Spt5 complex.Mol. Cell. Biol. doi:10.1128/MCB.00270-13
6. Jean Mbogning , Stephen Nagy, Viviane Page , Beate Schwer , Stewart Shuman , Robert P. Fisher4 , Jason C. Tanny (2013). The PAF Complex and Prf1/Rtf1 Delineate Distinct Cdk9-Dependent Pathways Regulating Transcription Elongation in Fission Yeast. PLoS Genet 9: e1004029.
7. Qing-Fu Cao, Junichi Yamamoto, Tomoyasu Isobe, Shumpei Tateno, Yuki Murase, Yexi Chen, Hiroshi Handa, Yuki Yamaguchi (2015). Characterization of the Human Transcription Elongation Factor Rtf1: Evidence for Non- overlapping Functions of Rtf1 and the Paf1 Complex. Mol. Cell. Biol. doi:10.1128/MCB.00601-15

[61] Vitamin C induced epigenomic remodeling in HOXA9-immortalized IDH1 R132H bone marrow cells

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The genomes of myeloid malignancies are characterized by epigenomic abnormalities. Heterozygous, inactivating TET2 mutations and neomorphic IDH mutations are recurrent and mutually exclusive in acute myeloid leukemia (AML) genomes. Ascorbic Acid (vitamin C) has been shown to stimulate the catalytic activity of TET2 *in vitro* and thus we sought to explore its effect in a leukemic model expressing $IDH1^{R132H}$. Vitamin C treatment induced an $IDH1^{R132H}$ dependent reduction in cell proliferation and an increase in expression of genes involved in leukocyte differentiation. Genome-wide assessment of 5mC and 5hmC revealed a set of vitamin C differentially methylated regions (DMRs), many of which displayed demethylation myeloid enhancers enriched for binding elements for the hematopoietic transcription factors RUNX1 and PU.1. A majority of DMRs associated with binding sites for the pioneering transcription factor PU.1 and we observed a significant loss of PU.1 DNA binding upon vitamin C treatment. Conversely, vitamin C treatment increased the binding of RUNX1 and corresponded with de-methylation at its binding sites in enhancer elements but not promoters. Genome-wide profiling of histone modifications revealed a negative correlation between H3K4 methylation and DNA methylation at vitamin C induced DMRs. Additionally, vitamin C induced an increase in H3K27ac flanking sites bound by RUNX1 in vitamin C treated cells, exclusively. Taken together our results show that vitamin C is able to stimulate epigenetic remodelling of transcription factor binding sites and drive differentiation in a leukemic model.

[63] Exploring epigenome impact of rare variants

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Understanding the regulatory impact of SNPs is an on-going challenge in disease genomics. Genome-wide association studies (GWAS) of complex traits revealed that the majority of the associated SNPs lie in non-coding regulatory regions. A popular method to dissect the regulatory role of SNPs, is to correlate genetic and functional variation in population samples (eQTLs or allele specific expression (ASE)), DNA methylation (mQTLs), histones marks (hQTLs) or allele specific chromatin immunoprecipitation (AS-ChIP)). These studies showed an enrichment of GWAS SNPs among the molecular QTLs. Even though GWAS have identified thousands of associated SNPs in hundreds of complex diseases, this method only effectively assess the impact of common SNPs and low allelic frequency variation are less well covered. The role of rare variation in disease discoverable by whole genome sequencing (WGS) is now pursued. Utility of WGS depends on ability to functionally classify discovered variation. In this project we explore the impact of rare variation on layers of gene regulation including gene expression, DNA methylation and histone modifications.

We accurately identified rare coding and non-coding variation using a targeted capture panel that includes exome regions and non-coding regulatory regions selected based on genome-wide mapping of immune cell regulatory elements (Immunoseq). The selective DNA capture was performed on 410 individuals followed by next generation sequencing. We assessed the functional impact of rare variants on RNA sequencing data from CD3+ and CD4+ cells in 210 samples, methylation levels at 5M CpGs of naïve T-cells in 98 samples and multiple layers of regulation (Illumina 450K, chromatin, expression) in 3 immune cell types from up to 200 blood donors.

Using Immunoseq, we identified a large number of rare and novel variants that appear to be functional. Relying on allele specific expression (ASE) measurements, we observed that rare and novel variants are enriched around allelically differentially expressed genes, where the effect is stronger when the top associated SNP is homozygous in the sample. We observed nearly two-fold enrichment (P<1E- 4) of rare variants in immediate vicinity of TSS for genes showing tissue independent extreme ASE (>99%th percentile). We also observed that singleton variation could have a strong impact on DNA methylation levels of a CpG located <500bp from it. We detected higher proportion of significant Allele-specific methylation (ASM) and higher evolutionary constraint (GERP++) of the targeted CpG.

We demonstrated that rare or novel variants have a potential functional role in immune cells. We also showed that there is possibility that these variations explain part of the difference in DNA methylation or gene expression that is usually linked to common variants. Our current work explores role of inter-individual loss of regulatory elements due to rare variants in chromatin/methylation data.

[64] Involvement of the histone variant H2A.Z C-terminal docking domain in genomic localization and transcriptional activation

Hannah Neumann

The eukaryotic genome is organized as chromatin that consists of repeated nucleosomes, and which represents a barrier to gene transcription. Therefore, nucleosomes have to be remodeled at specific sites to allow transcriptional activation. The incorporation of histone variants alters the chromatin structure and creates specialized chromatin regions, which can be permissive to transcription. The highly conserved H2A.Z, a histone variant of H2A, has thoroughly been studied. In the budding yeast *S. cerevisiae*, H2A.Z has shown to be involved in positive and negative regulation of transcriptional activation. Initially, experiments carried out in *D. melanogaster* reported that the H2A.Z special function in gene induction resides in its C-terminal region, the most divergent region between the structures of H2A and H2A.Z histones. The H2A.Z-containing nucleosomes present an extended acidic patch formed by the amino acids around the H2A.Z αC helix in the docking domain and an amino acid of histone H2B. This acidic patch is thought to provide a binding platform for nuclear proteins and thereby modulate nucleosome remodeling activities.

In this study, we aim to investigate the functional role of H2A.Z C-terminal docking domain in gene induction and genome-wide localization of the histone variant. In order to study the function of the H2A.Z C-terminal region, we used a transcriptional activation domain that can mimic the key interactions with the H2A.Z acidic patch leading to transcriptional activation. We use a chimeric protein named ZA-rII' in which the H2A.Z C-terminal region is replaced by the corresponding region of H2A and the Gal4 acidic transcriptional activating region rII' is added to the C-terminus. First, we confirmed that the H2A.Z special function in gene induction resides in its C-terminal region because the ZA-rII' fusion could efficiently restore full activation of the *DAL5* gene in *S. cerevisiae* compared with *htz1*Δ mutant cells. Moreover, the transcriptional activating region can confer the special function of H2A.Z in resistance to genotoxic stress, because the ZA-rII' is able to complement *htz1*Δ-associated growth defects on rich media containing Hydroxyurea (HU), caffeine or methyl methanesulfonate (MMS). Interestingly, the control fusion ZA could not restore full activation of the *DAL5* gene and resistance to genotoxic stress suggesting that those special functions of H2A.Z are specific to its C-terminal region. Unlike recent studies, we show that specific incorporation of H2A.Z at promoters is not dependent on its C-terminal region since the control fusion ZA displays a similar localization pattern to H2A.Z and ZA-rII' over the *DAL5* promoter. Furthermore, in a *swr1*Δ mutant, the enrichment of H2A.Z, ZA-rII' and ZA are lost over the *DAL5* promoter and the new distributions are rather uniform and similar to that of H2A.

Taken together, these results suggest that some H2A.Z functions, including gene induction and resistance to genotoxic stress, are dependent on its C-terminal region. Moreover, we propose a model for H2A.Z role in transcriptional activation where the C-terminal region of H2A.Z is not essential for proper localization at promoters, but absolutely essential once H2A.Z is incorporated into chromatin to recruit appropriate interacting partners leading to transcriptional activation.

[65] Deregulated Expression and Localization of the Epigenetic Factor MeCP2 is Associated with Altered Levels of Important Functional Regulators of the Brain Cells in Rett Syndrome Patients Compared to Normal Human Brains

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Rett Syndrome (RTT) is a severe neurological disorder that is caused by mutations in the methyl CpG-binding protein-2 (*MECP2)* gene. MeCP2 has an important role in brain function and is widely expressed in mammals. Alternative splicing of the *MECP2* gene produces MeCP2E1 and MeCP2E2 isoforms that bind to DNA at methyl-CpG dinucleotides and influence gene expression as an epigenetic regulator of transcriptional activity. Although widely expressed, it is the loss of MeCP2 protein function in the central nervous system (CNS) that is associated with a delayed onset of RTT. However, it is not yet understood how the impaired brain function in RTT patients is associated with changes at the cellular level caused by *MECP2* mutations.

Studies to date on the underlying mechanisms of RTT have utilized different mouse models, focusing on null male animal models of disease. However, RTT is an X-linked disease and patients are heterozygote females. Despite the valuable contribution made by studying animal models towards our understanding of MeCP2 function, a concentrated effort must be made to further our understanding of the underlying involvement of MeCP2 in RTT in humans. We hypothesize that MeCP2 protein expression and localization may not be exactly conserved between male mouse and female humans, and that sex-related differences may occur. Our aim is to elucidate the functional role of MeCP2 protein in the male and female human brain, with complementary molecular biology techniques, including immunohistochemistry, Western blot, and real time PCR. We herein report our findings for regional expression and localization of MeCP2 protein in post-mortem RTT brain and compare our findings with normal CNS. We show regional protein detection differences between mouse and human brain. Further, we describe the association of MeCP2 with cell type-specific markers and important transcriptional regulators. We conducted these studies under approval by the Research Ethics Board of the University of Manitoba. The results of our studies will contribute towards a better understanding of the role of MeCP2 in CNS function and in Rett Syndrome.

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[68] S-Adenosyl methionine (SAMe) as an epigenetic based therapeutic agent in breast cancer

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Metastasis is the leading cause of breast cancer-associated morbidity and mortality. Pioneering works done at our labs have shown that pre-treatment of various (breast, prostate, osteosarcoma) cancer cell lines with a methylating agent S-adenosyl methionine (SAMe) followed by inoculation into immunocompromised mice can block tumor growth and metastasis to non-skeletal and skeletal sites. However, the anti-metastatic effect of SAMe has never been examined in a therapeutic setting. In a series of studies, we have examined the plausibility for dietary supplementation of SAMe for the prevention and treatment of breast cancer development, growth and metastasis in various *in vitro* assays and in *vivo* using xenograft and transgenic mice models of breast cancer. Treatment of highly invasive, triple negative human breast cancer cells MDA-MB-231 expressing green fluorescent protein (MDA-MB-231-GFP) with SAMe resulted in a significant decrease in tumor cell proliferation, invasion and increased apoptosis *in vitro*. Affymetrix gene expression array showed that SAMe treatment caused down regulation of a number of genes implicated in key intracellular signaling in cancer progression. Real time PCR (qPCR) validated the SAMe effect on selected genes implicated in tumor metastasis (*uPA, MMP2*) and epithelial-mesenchymal transition (*SPARC, HAS3*). In *in vivo* studies, MDA-MB-231-GFP cells were inoculated via mammary fat pad into female nude mice. From day three post tumor cell inoculation, animals were treated with SAMe (0.8-1.6 mg/day) or S-adenosyl homocysteine (SAH) as control via daily oral gavage and tumor volume was determined at weekly intervals for 10 weeks. SAMe caused a significant dose dependent decrease in tumor volume and metastasis to lungs, liver and spleen. Analysis of primary tumors by qPCR showed the ability of SAMe to cause a marked decrease in the expression of metastatic (*uPA, FABP7)* and EMT pathway (*SPARC, HAS3*) genes.

In follow up studies in MMTV-PyMT transgenic mouse model of breast cancer, SAMe treatment (3.2 mg/day) from week 4 after birth until week 12 caused a significant delay in the development of mammary tumors which were of significantly smaller number and volume. Following sacrifice, the number and areas of lung metastasis was significantly smaller in experimental PyMT mice treated with SAMe. Mass-spectrometry based bioavailability assay from the serum of control and experimental animals showed highest levels of SAMe at 30-60 minutes post treatment and SAMe levels went down to the basal state after 4 hours of treatment. Further studies examining the effects of SAMe on bone metastasis, gene expression as well as any potential side effects including animal behavior and toxicity will be presented and discussed.

Taken together results from these studies provide compelling rationale for the initiation of clinical trials with SAMe in patients with breast cancer as monotherapy or in combination setting with current chemopreventive and therapeutic agents to reduce breast cancer associated morbidity and mortality.

[69] Role of folic acid supplementation in the prevention of epigenetic and birth defects associated with the use of assisted reproduction

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Infertility affects approximately 10% of couples and is an important factor contributing to the relatively high proportion of children being conceived using assisted reproductive technologies (ART). Adverse outcomes such as decreased birth weight, an increased incidence of birth defects and preeclampsia have been associated with the use of ART. These procedures have been shown to alter epigenetic (DNA methylation) programming events during germ cell and early embryo development. For proper establishment of DNA methylation profiles during these stages of development, the availability of methyl donors such as dietary folate is essential. The objective of my research is to determine whether folic acid supplementation can prevent birth defects and epigenetic abnormalities associated with ART. Female mice are exposed to control (2 mg folic acid/kg diet), low dose (8 mg folic acid/kg diet) and high dose (20 mg folic acid/kg diet) folic acid-supplemented diets six weeks before in vitro fertilization or natural mating; diets are continued throughout gestation. Mouse ART consists of treatments used in human ART: a combination of superovulation, in vitro fertilization, embryo culture to blastocyst and embryo transfer. Hypomethylation at imprint control regions of certain imprinted genes has been previously reported in IVF-conceived placentas. We examined imprinted gene methylation in placentas collected at midgestation by bisulfite pyrosequencing. Compared to the control group, the low dose folic acid-supplemented group demonstrated a trend of increased methylation at *Snrpn* DMR. Although less pronounced, this trend was also observed for the high dose folic acid-supplemented group. These results suggest that folic acid supplementation is affecting imprinted gene methylation in a dose-dependent manner. This study will help determine whether folic acid supplementation in the ranges used clinically is beneficial or deleterious in pregnancies resulting from ART. (Supported by CIHR).

[70] Loss of C-Myc And Chromatin Acetylation Induce Epigenetic Reprogramming In Acute Lymphoblastic Leukemia

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Acute lymphoblastic leukemia (ALL) represents 25% of all pediatric cancers. Since 20% of patients do not respond to the treatment, relapse ALL is the main cause of death by disease in children, underlying the importance of new therapeutic options. Cancer cell epigenome is altered leading to silencing of tumor suppressors and activation of oncogenes. These epigenetic modifications have profound impact on cell phenotype, causing loss of differentiation and unlimited cell proliferation. Epigenetic aberrations at the levels of DNA methylation and chromatin modifications are actionable therapeutic targets. Epigenetic drugs reset cancer cell epigenome leading to cancer cell differentiation and apoptosis. In a drug screening initiative, we recently reported a series of FDA-approved drugs with unsuspected epigenetic and anticancer activities. Here, we tested in a secondary screen the activity of these drugs in a panel of ALL cell lines (MOLT-4, NALM-6, REH). We found that proscillaridin A, a cardiac glycoside used for heart failure treatment, was the most active with IC_{50} values in the low nanomolar range, suggesting drug repositioning potential. To characterize its mechanism of action, we performed RNA sequencing and gene set enrichment analysis in ALL cells treated with proscillaridin A at 5 nM for 48h. We observed that several thousand genes where up- or down-regulated. Most significant up-regulated gene sets were associated with cell differentiation and apoptosis pathways whereas down-regulated gene sets were associated with C-MYC target genes. C-MYC is a master oncogenic driver in ALL and one of the most common drivers in human cancer that amplifies the expression of cell proliferation genes marked by histone acetylation. In only 8 hours after treatment, C-MYC protein levels were rapidly decreased, explaining why its gene targets were down-regulated. Notably, C-MYC rapid down-regulation was associated with a 75% reduction of its lysine acetylation whose loss is known to precede MYC proteosomal degradation. We identified that loss of acetylation was associated with rapid down-regulation of lysine acetyltransferases CBP, P300 and Tip60. Consequently, we also detected significant decreases in histone 3 acetylation levels (H3K14ac, H3K9ac, and H3K27ac), while histone 4 acetylation was not affected. Altogether, our findings show that targeting lysine acetyltransferase expression simultaneously induces loss of C-MYC and H3 acetylation leading to epigenetic reprogramming in ALL cells. This therapeutic strategy, using drugs such as proscillaridin A, has the potential to reprogram cancer cells that are driven by MYC overexpression or hyperactivation.

[71] The Role for Negative Elongation Factor in Regulating Muscle Regeneration

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Genome-wide studies of RNA Polymerase II (RNA Pol II) binding in various cell types has revealed the importance of promoter proximal pausing as a means to regulate the expression of developmentally important genes. In this context, pausing of RNA Pol II immediately upstream of the transcription start site allows the maintenance of active transcriptional machinery on 'stand-by' for subsequent quick and efficient gene expression upon specific signaling cues.

Using *Drosophila* as a model organism, the ability of RNA Pol II to become paused in the promoter-proximal region was suppressed by knocking down Negative Elongation Factor (NELF), a key protein complex required to establish the paused polymerase during transcriptional regulation. Subsequent gene expression meta- analysis showed that only a small subset of genes had altered expression upon the NELF knockdown. Further analysis with *Drosophila* showed that heat shock induction of *hsp70* was also under the control of NELF-mediated promoter-proximal pausing, effectively showing that NELF-mediated promoter proximal pausing could be used to regulate gene expression in light of environmental factors. Collectively, this work suggests that NELF-mediated promoter-proximal pausing is not a general transcriptional mechanism, but suggests instead that it acts as a checkpoint to effectively suppress expression of specific genes until a proper temporal or environmental signal becomes available to allow transcription to proceed.

Given the speed and coordination with which cellular fates of myogenic progenitor cells change during muscle regeneration, we hypothesize that *NELF maintains key muscle differentiation genes involved in a paused state, awaiting cues to induce transcriptional elongation. This would allow myoblasts to proliferate and produce a sufficient population of cells needed for proper muscle repair, at which point NELF repression would be lifted to allow expression of genes required for differentiation*. To explore my hypothesis, I am using a combination of *in vitro (aim 1)* and *in vivo (aim 2) approaches:*

Aim 1: Determine the contribution of NELF to the regulation myogenic cell fate in vitro. Using knockdown and overexpression lines, I plan to examine the proliferation and differentiation of cultured myoblasts. ChIP-sequencing and comparative analysis of RNA-sequencing will be used to identify genes directly regulated by NELF. Gro-Seq will also be used to examine the extent to which RNA Pol II stalling is altered in myoblasts in the absence of NELF-B.

Aim 2: Determine the contribution of NELF to muscle regeneration in vivo. Using a satellite cell-specific conditional knockout NELF-B in mice, I will characterize the ability of muscle stem cells to regenerate injured muscle in the absence of NELF complex. Defects observed *in vivo* will be further characterized *ex vivo* using isolated myofibers to determine the stages of regeneration that require NELF activity. Progress on these aims will be presented. From this work, we expect to establish whether the regulation of proximal promoter pausing offers potential therapeutic targets for improving muscle regeneration in patients with muscle wasting disorders.

[72] *Salmonella* mediated alteration of host DNA methylation patterns during infection

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Salmonella enterica serovar Typhimurium (*S.* Typhimurium) is responsible for tens of millions of gastrointestinal illnesses globally each year and antibiotic resistance is increasing. As an intracellular pathogen, *S.* Typhimurium is adept at controlling host cells and creating a favorable environment for replication. Pathogens are known to alter host regulatory networks to create an appropriate niche for replication, including by modifying the host chromosome and the structural and regulatory proteins of chromatin, however this has not been shown for *Salmonella*.

Upon entry into a human host, *Salmonella* survives and replicates inside a variety of host cell types including intestinal epithelial cells and macrophages. To create a favorable replication environment, *Salmonella* disrupts the normal endocytic pathway of host cells, resulting in a vacuole containing the replicating *Salmonella*. *Salmonella* also translocate protein effectors into the host using two Type III Secretion Systems (T3SS), encoded in *Salmonella* Pathogenicity Islands (SPI-1 and SPI-2). These effectors have a variety of functions during invasion, survival and replication. Generally, SPI-1 secreted effectors are required for the invasion of epithelial cells and during early cellular infection, while SPI-2 secreted effectors are required later during infection. The identity and functions of all the *Salmonella* effectors are not yet known.

Recently it has been shown that pathogens, both bacterial and viral, induce changes in host cell gene regulation at the chromatin level. These regulatory alterations are evidenced by changes in the host cell methylation pattern on the chromosome and by changes in the modifications of histone tails. Some of these epigenetic changes are the result of the host acting upon itself in response to infection, while others are the direct result of bacterial proteins that act both inside and outside the host nucleus. To determine whether and how *Salmonella* infection alters the host epigenome, we have infected the human macrophage-like cell line, THP-1, with both live and killed *Salmonella* Typhimurium, as well as a strain lacking SPI-2 effector secretion. By comparing the changes in the epigenetic code during these different model infections, we hope to distinguish changes that are the result of the host acting on itself versus those that are effected by *Salmonella*. These data will provide insight into a new mechanism of *Salmonella* control of host cells, and may be more broadly applicable to intracellular bacterial pathogens. We will further add to the expanding field of patho-epigenomics.

[73] Establishment of an *in vitro* model to study epigenetic reprogramming in rat fetal male germ cells.

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Epigenetic reprogramming is a critical step in male germ cells development that occurs during fetal life. It is characterized by the remodeling of different epigenetic marks such as DNA methylation (5mC) and post-translational modifications of histone H3. This process is important for erasure of potential epimutations. Epigenetics marks acquired in the fetal germ cells (gonocytes) during that phase may have a determinant role in the future of subsequent germ cells and eventually on the quality of spermatozoon. It was suggested that exposure to some environmental pollutants affect male fertility especially if it occurs during the phase of epigenetic reprogramming. However, the mechanisms involved are not yet elucidated. The organ culture is a powerful *in vitro* system that reproduces the *in vivo* kinetics of testicular development. This model was used to show the negative effects of estrogen-mimicking endocrine disruptors on fetal testis development. We hypothesize that organ culture can reproduce the epigenetic reprogramming and therefore would be a good model to study the impact of pollutants on testis development. Here, we aim to test if this model can reproduce the epigenetic reprogramming in gonocytes by quantifying the *ex vivo* dynamic of epigenetic marks that are known to vary during that time *in vivo*.

As the chronology of epigenetic reprogramming has mostly been established in mice, we first established the *in vivo* kinetic of six histone modifications and 5mC in rat fetal germ cells using immunofluorescence. To do so, we explanted testis at four different stages of development: 16, 18, 20 days post-coïtum (dpc) and 3 days post-partum (dpp). Our results show that H2BK20ac does not vary in gonocytes during that time, whereas H3K27me3, H3K9me2, H3K4me2, H3K4me3, H2AK119Ub, and 5mC show dynamic profiles. Second, we explanted testis at 16 dpc and cultured them in different conditions: two supports (filter or insert) and with/without FBS. On insert and without FBS, the immunofluorescence quantification results done after 48h or 96h of culture, show that DNA methylation (5mC) and the two histone marks tested (H3K4me2, H3K4me3) follow similar patterns as the one observed *in vivo*.

Our results suggest that the dynamic of epigenetic marks in male germ cells during fetal development is conserved between rat and mouse with a slight difference in timing. Furthermore, we showed that the dynamic of three marks of the epigenetic reprogramming (H3K4me2, H3K4me3, 5mC) was reproduced *ex vivo* without addition of serum. These results suggest that the organ culture can reproduce the process of epigenetic reprogramming. Thus, it would be a good model to elucidate the mechanism by which environmental pollutants could affect the establishment of epigenetic marks in fetal male germ cells.

[74] Epigenetic regulation of the adenovirus genome and identification of cellular epigenetic modifiers as potential therapeutic targets in adenovirus infection

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The human adenovirus (Ad) causes minor, self-limiting respiratory illnesses in most patients, but can lead to severe disease and death in pediatric and geriatric populations, and in immunocompromised individuals. No approved antiviral therapy currently exists for the treatment of severe Ad-induced diseases.

Within the virion, Ad-encoded protein VII (pVII) tightly condenses the viral genome into a structure that is refractory to transcription and replication. Within the first few hours of infection, the Ad DNA dissociates from pVII, associates with cellular histones, and adopts a nucleoprotein structure similar to the host DNA. Assembly of the viral genome into this repeating nucleosome-like structure is crucial for efficient expression of virus-encoded genes, and it allows viral gene expression to be regulated by the same epigenetic mechanisms as the host. Consequently, one approach to treating Ad-induced disease may be to prevent the viral DNA from transitioning to this chromatinized, transcriptionally active state. Thus, we are investigating the epigenetic regulation of Ad gene expression to identify cellular epigenetic modifiers that can serve as novel therapeutic targets in Ad infections.

We have produced a wildtype-like Ad construct encoding the red fluorescent protein (RFP) gene under “late” transcription regulation (Ad-late/RFP). RFP from the Ad-late/RFP is only expressed following viral DNA replication, and therefore, can be used to effectively monitor Ad gene expression and replication. Using the Ad-late/RFP, we then designed and validated an efficient method to screen small molecules that modulate the activities of epigenetic modifiers. A preliminary screen has identified the pan-histone deacetylase (HDAC) inhibitor vorinostat as a compound that significantly delays the onset of viral gene expression and replication. These effects were found to be mediated through the inhibition of HDAC2 activity. We are currently working to elucidate the molecular mechanism underlying these results and conducting *in vivo* studies with vorinostat, as well as validating additional compounds identified in our screen.

The costs associated with Ad-induced disease are significant in terms of medical expenses, lost work hours and loss of life in some populations. Identification of epigenetic modifiers as therapeutic targets to combat Ad infections will not only lead to decreased disease pathogenesis, but also provide novel insights into virus-cell interactions in Ad infections.

[75] Expansion of BioID toolbox for study of protein interactome in primary cells

Payman Samavarchi-Tehrani, Anne-Claude Gingras

The genesis of the hundreds of different cell types in the human body is the result of a highly orchestrated transcriptional program, which establishes a unique gene expression signature in each cell. This signature is a result of combinatorial binding of transcription factors (TF) to specific genomic loci and recruitment of epigenetic readers, writers and remodelers. These in turn result in the establishment of a cell type specific chromatin landscape with distinct loci of transcriptional activity or suppression. The application of genomic techniques is providing insight into overall chromatin architecture and TF genomic occupancy patterns. However, due to challenges in biochemical purification of intact chromatin associated complexes, there is limited understanding of the full gamut of proteins and complexes implicated in gene regulation.

A recently developed technique that can offer advancement in our study of transcriptional proteome is the proximity biotinylation approach, BioID. In this technique, a promiscuous biotin ligase (BirA*) fused to a bait protein can carry out in vivo biotinylation of the closely associated proteins. In the context of chromatin biology, this enables capturing interactions that occur in the nucleoplasm and on chromatin (an accompanying abstract by Lambert et al. demonstrates the application of BioID to defining complexes involved in acetyl lysine recognition). Yet, while BioID overcomes a number of technical hurdles associated with traditional biochemical purification techniques, it has so far been primarily used in Flp-In T-REx cell lines or immortalized cancer cell lines. However, there are various biological questions in which use of other cell types, or primary cells, will provide a unique opportunity to study specific biological processes. To facilitate our specific study of TF interactome in primary cells, we set out to expand the BioID toolbox to allow for its implementation in the diverse cell types of interest.

To enable these studies, we have added to the BioID toolbox a set of lentiviral vectors that will broaden the application of BioID to other cell models. We demonstrated that these viral BioID vectors are efficient in determining the proximity proteome across different primary cells and immortalized cell lines. We sought to benchmark the approach using a number of well-studied bait proteins such as LMNA, TUBB, nucleosomal protein H2B, and basal transcription factor TBP. We demonstrate that when used in the same cellular contexts as standard BioID vectors, they recover similar sets of proximity interactors, highlighting the utility of the powerful BioID approach to a wide range of new biological questions. This approach now allows us to address our specific biological questions regarding TF interactome implicated in cellular reprogramming and lineage specification.

[76] Characterization of the KDM4A histone demethylase in pediatric acute myeloid leukemia

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In human cancers, genes coding for epigenetic regulators are frequently mutated, deleted or amplified, making them promising targets for the development of novel therapies. The Jumonji (JmjC) domain–containing family of demethylases are overexpressed in a number of different cancers, including acute myeloid leukemia (AML) associated with rearrangements of the mixed lineage leukemia 1 (MLL) gene. We aim to characterize the role of the histone lysine demethylase JMJD2A/KDM4A, which mediates the removal of methyl groups from di- or tri-methylated lysines 9 and 36 of histone H3 (H3K9 and H3K36). KDM4A also binds the di- and tri-methylated lysines 4 of H3 (H3K4) and lysine 20 of histone H4 (H4K20). The role of this epigenetic regulator in the progression of hematopoietic malignancies remains largely unexplored to date. Using methods to specifically control gene expression in MLL-AF9/N-Ras expressing leukemic cells, we demonstrate that the depletion of KDM4A triggers DNA damage, cell death and senescence of hematopoietic cancer cells. In a parallel experiment, we chemically inhibited the activity of KDM4A using a small molecule (ML324), which attenuated the proliferative potential of AML cells. The results of our study indicate that KDM4A is specifically required for the proliferation of AML cells and represents a promising therapeutic target for pediatric leukemia, a disease still plagued with dismal survival rates.

[77] Whole Genome Bisulfite Sequencing Quality Control Pipeline

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Epigenetic deregulation is a hallmark of cancer and recent large-scale cancer genome sequencing efforts have revealed recurrent gain and loss of function mutations to proteins involved in maintaining normal DNA methylation homeostasis - notably in leukemia and brain tumor subtypes. Whole genome bisulfite sequencing (WGBS) involves bisulfite-mediated deamination of cytosine followed by PCR amplification and massively parallel sequencing. Methylated cytosines are protected from deamination providing a methodology for single base pair quantitative genome wide methylation profiling. As part of the Canadian Epigenetics, Environment and Health Research Consortium (CEEHRC), we are currently employing WGBS to a wide collection of normal and transformed primary human tissue types to understand its role in normal differentiation and transformation. However WGBS is an emerging and complex technique that poses considerable molecular and computational challenges and requires quality control (QC) metrics prior to the extraction of methylation signatures and biological interpretation of the data. Here we report on the development of a WGBS database and computational pipeline (QCB) that outputs multiple quality metrics (n=24) such as bisulfite conversion rate, alignment metrics, coverage statistics, GC bias, duplication rate along with sample metadata, files and data processing information, allowing for fast assessment of the overall quality of WGBS dataset. In addition to bisulfite sequencing, our pipeline can handle a different upper protocol called Post Bisulfite Adapter Ligation (PBAL), which can be applied for single cells. Our database contains metadata for 171 samples (for 2 organisms, human and mouse), QC information for 280 libraries (composed of 755 lanes including 315 bisulfite sequencing, 150 bulk PBAL, 290 single cell PBAL) from 12 different projects including the Centre for Epigenome Mapping Technologies project and the Roadmap Epigenomics Mapping Consortium project. QCB accepts alignment files (bam) and generates genome wide coverage and fractional methylation calls in the form of an integrated genome browser track for further analysis and visualization. Implemented in Java, Perl, Unix shell and MySQL, QCB can be run on a single CPU and completes a human genome WGBS dataset (1 billion aligned reads) in less than 3 hours. Our system and data collection was, so far, used to define WGBS quality control core metrics and associated thresholds, which were submitted to the International Human Epigenome Consortium (IHEC), and currently leverage the development of new Quality Control metrics.

[79] Optimizing experimental and bioinformatic approaches for the analysis of histone methylation in sperm from mice and men

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The phenomenon of epigenetic inheritance has been observed in models ranging from worms to mice, suggesting that life experiences may be transmitted through the sperm epigenome to affect the health and development of future generations. However, the underlying molecular mechanisms driving epigenetic inheritance remain unknown. Potential routes implicated in the encoding of environmental exposures in the sperm epigenome include histone methylation, non-coding RNAs and DNA methylation. Recently we showed that disruption of histone methylation in developing sperm leads to reduced survivability and abnormal development of offspring. Remarkably these effects persisted transgenerationally (Siklenka et al., 2015, *Science*).

Sperm chromatin is unique due to the high degree of chromatin compaction achieved after replacement of most histones by protamines. Between 1% and 15% of histones are retained in sperm of mice and men, respectively. Initial studies of the sperm epigenome determined that nucleosomes were preferentially enriched at gene regulatory regions (Brykczynska et al., 2010, *Nat. Struct. Mol. Biol*; Hammoud et al., 2009, *Nature*). In contrast, using different technical and analytical approaches, Carone et al., 2014 (*Dev. Cell*) and Samans et al., 2014 (*Dev. Cell*) observed genome-wide nucleosome retention and reduced representation at gene-regulatory CpG dense regions.

Our **objective** was to use a sperm customized approach for ChIP-seq, in combination with pooled samples from men (n=30) and mice (n=3x3) and to generate high coverage, robust data sets for determination of H3K4me3 localization in the sperm genome. We also compared bioinformatic approaches to optimize histone peak detection in sperm. **Methods:** We prepared native chromatin made highly accessible using treatments of DTT, detergent and MNase followed by immunoprecipitation by H3K4me3, and sequencing on an Illumina HiSeq platform. Comparative analysis was performed testing various parameters in Homer (v4.8), MACS, MACS2, and R/Bioconductor. **Results:** DNA was sequenced at a depth of over 160 million reads in sperm from men and at a depth of 30 million reads in sperm from mice. Our analysis confirms that H3K4me3 is enriched at transcriptional start sites high in CpG density. Intriguingly, although nucleosomes were not enriched at intergenic sequences we observed a sizable portion of positive H3K4me3 peaks that may associate with distal gene regulatory regions.

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[80] Reducing epigenetic heterogeneity in autism spectrum disorder by substratifying based on genetic variants

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Autism spectrum disorder (ASD), the most common paediatric neurodevelopmental disorder (NDD), demonstrates a significant amount of etiologic and phenotypic heterogeneity. Over 200 ASD-risk genes have been identified, but genetics alone can only account for ~25% of cases. Epigenetic dysregulation is likely to also contribute to ASD etiology given the number of ASD-risk genes encoding proteins that function as epigenetic regulators. Previous studies have identified alterations in DNA methylation (DNAm), the most commonly assessed epigenetic mark, in ASD patients. However, the impact of these findings is limited by small sample sizes and inconsistent results across studies, likely due to DNAm variance across tissue types, different analytical methods and the heterogeneity of the ASD groups examined. To investigate the role of epigenetic dysregulation in ASD, genome-wide DNAm was measured using the Illumina Infinium HumanMethylation450 BeadChip array in DNA extracted from whole blood. First, we compared DNAm in a heterogeneous ASD group (n=52) with age- and sex-matched neurotypical controls (n=30). Second, we sought to improve the discovery of DNAm differences by examining more homogeneous groups of individuals substratified based on known ASD-associated genomic variants; DNAm was also assessed in blood from patients with 16p11.2 deletions at the 600kb risk locus (16p11.2del; n=9) and heterozygous loss-of-function mutations in chromodomain helicase DNA binding protein 8 (*CHD8*$^{+/-}$; n=7), a chromatin modifier. These groups were compared with age- and sex-matched controls (n=23 and n=21, respectively). No ASD-specific DNAm patterns fully distinguished the heterogeneous ASD cases from controls. However, unique DNAm patterns were identified for the 16p11.2del and *CHD8*$^{+/-}$ groups when compared to controls. These DNAm patterns consist of specific sets of differentially methylated sites (adjusted $p<0.05$, absolute difference ≥5%) comprising novel DNAm signatures. These signatures classified individuals with 16p11.2del and *CHD8*$^{+/-}$ mutations distinctly from each other, as well as from the heterogeneous ASD group and controls. Our DNAm signatures also properly classified additional 16p11.2 copy number variants, *CHD8*$^{+/-}$ sequence variants, and independent controls, indicating potential for clinical application. Examining the genes in each signature reveal biological pathways that could be important to the pathophysiology of ASD, overlapping with known ASD-risk genes. Furthermore, differentially methylated genes in the *CHD8*$^{+/-}$ signature were found to overlap with an independent study that identified differentially expressed genes in *CHD8*$^{+/-}$ (CRISPR/Cas9) human induced pluripotent stem cell-derived neurons, demonstrating cross-tissue functional relevance. Combined, these data demonstrate an improved method of molecular classification of a heterogeneous NDD such as ASD and will help to enhance our understanding of the underlying biological mechanisms of this disorder.

[81] Motif Variation Specifies the Affinity Landscape and Epigenetic State of the MyoD Regulome

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The *cis*-regulatory code is the cellular lexicon by which the transcriptional machinery converts sequence information within *cis*-regulatory modules (CRMs) into gene expression output. Tissue-specific transcription factors such as MyoD orchestrate gene expression programs by binding to short DNA motifs called E-boxes within their target CRMs to modulate chromatin state and to fine-tune gene expression output. Despite extensive research, the mechanism of how the relatively short and ubiquitously distributed E-box motifs contribute to binding specificity, assembly, and the functional output of the transcriptional machinery remains poorly understood. We have carried out an integrative analysis of the relationship between MyoD occupancy, nucleosome positioning, CRMs-associated histone marks and motif sequences within the myogenic CRMs in differentiating muscle cells. Our data suggest that variant sequences within MyoD-binding motifs and their numbers within the CRMs, as well as the spatial arrangement of the motifs, in combination confer binding affinity of MyoD, nucleosome occupancy and the epigenetic state of the myogenic CRMs. Our comparative genomic analysis of single nucleotide polymorphism (SNPs) across publically available data from 17 strains of laboratory mice suggests that variant sequences within MyoD-bound motifs, but not their genome-wide counterparts, are under selection. Taken together, this data suggests that variant sequences within MyoD-binding motifs are retained and have function to confer a dynamic range of affinities, enabling MyoD to establish a broad spectrum of activity across myogenic CRMs.

[82] The SWI/SNF chromatin remodeling complex is essential in the regulation of *Drosophila* short-term memory

Max Stone

The SWI/SNF ATP-dependent chromatin remodeling complex has been identified through gene ontology analysis as the most enriched protein complex amongst all genes involved in the dominant genetic forms of intellectual disability (ID). Despite these implications, the majority of studies on this 15-subunit epigenetic regulatory complex have focused on its role in differentiation and tumour suppression. In this study, I systematically investigated the role of this highly conserved protein complex in memory, using *Drosophila melanogaster* as a model system. *Drosophila* is an excellent model due to the transgenic techniques and behavioural assays available. The UAS-GAL4 transgenic system and RNAi technology was used to perform cell-type-specific knockdown of SWI/SNF genes in the mushroom body, which is the learning and memory centre of the fly brain. Knockdown flies were tested for short-term memory using an established learning and memory assay called courtship conditioning. In this assay, male flies are subjected to pre-mated females that are not receptive to mating attempts. Wild type flies learn to suppress their courtship behaviour when re-introduced to a different pre-mated female, but flies with defective memory fail to learn to suppress this behaviour. From these experiments, six of the 15 SWI/SNF complex genes were identified as essential genes in the formation of short-term courtship memory, and the gene encoding the ATP-binding subunit of this complex, *brm*, was identified as an essential gene in the regulation of baseline *Drosophila* courtship behaviour. This is the first systematic analysis of the SWI/SNF complex in the context of learning and memory. The results demonstrate an critical role for the SWI/SNF complex in the regulation of neurological processes and have identified genes and phenotypes that can be further studied in order to determine the specific role of SWI/SNF-mediated gene regulation in complex brain function. Due to the high level of conservation between flies and humans, these results provide the essential first steps in understanding the role of genes involved in SWI/SNF-associated forms of ID.

[83] A comparative epigenomics approach to identify functional non-coding elements in primate induced pluripotent stem cells

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Much of the DNA sequence found between human and closely related primate species is conserved, but the differences in protein-coding sequences alone do not fully explain the phenotype differences observed between these species. Epigenomic changes due to mutations in non-coding DNA will also help explain some of the phenotypic differences. To explore the evolution of chromatin in induced pluripotent stem cells (iPSCs) and to identify potentially functional regulatory elements, we generated ChIP-seq data for 6 different histone modifications collected from 4 primate species (human, chimpanzee, gorilla, macaque). Next, we used the program ChromHMM to divide genomes into different chromatin states (CS) and the UCSC tool LiftOver to find homologous genomic locations between species. We analyzed the epigenomes of human and of the non-human primates (NHPs) while looking deeper into general conservation trends and tried to identify sites in human with CS not seen in NHPs while looking at different repetitive elements that contribute to these differences. We showed that about 85% of chromatin in the promoter state was conserved and that this was higher than for enhancers and transcribed regions at 60% and repressors at 40%. We also noted that transposable elements (TEs) contributed more than expected by chance to certain classes of CS and certain classes of conserved CS (e.g. LTR elements were over-represented in enhancers and also about 10% more conserved than non-LTR enhancers) and that the overlap of different chromatin states with TEs correlated with the age of their insertion. Together our results confirmed some previously studied trends and pave the way to refine the identification of human specific functional sites.

[84] The dynamic mucosal environment can influence the regulation of innate immunity at the female genital tract, possibly via affecting the epigenetic regulatory mechanisms.

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Female genital tract (FGT) represents an important site for the dissemination of sexually transmitted Infections (STIs). Innate immunity at the lower FGT already does a remarkable job in protecting against STI transmission (e.g., only 1–2 cases per 1000 coital acts). However, factors influencing the FGT immune activation can compromise the mucosal defense and greatly facilitate STIs. *Yet, little is known of how the FGT innate immune activation is regulated and/or affected by the dynamic mucosal environment.* Epigenetic molecular mechanisms can be influenced by environmental factors, such as microbial products and pH. Data supporting the involvement of epigenetic mechanisms in "training" innate immune responses is mounting, but our knowledge of their role at regulating mucosal innate function remains limited. *This study examines whether there is a relationship between the mucosal pH, H_2O_2 levels and nitric oxide (NO) concentrations in the lower FGT and enzymatic activities (HDAC, HAT) in the cervical cells (CMC), and whether adjustment of these parameters in culture affects gene expression.*

Interestingly, the mucosal H_2O_2 level negatively associated with the HDAC activity (r^2=-0.79, p<0.001, n=32), but not HAT, in the matching CMC samples, suggesting that H_2O_2 may inhibit HDAC but not HAT activity. Yet, lower cervical pH corresponded with elevated cellular HDAC activity (r^2=-0.63, p<0.001, n=32) and trending reduction of HAT activity (r^2=0.42, p=0.06, n=32). Treatment of CMC with HDAC-inhibitor (trichostatin A, or sodium citrate), reduced expression of interferon effector genes (IFN-γ, CXCL-10, MX-1, IRF-1, IL-12p35) and decreased the transactivation of HIV-1 genes (gag, p24) by >90% (p<0.001) and hence, HIV-1 acquisition. We also showed that levels of H_2O_2 and NO in the cervical lavage were associated with cytokine and chemokine secretion in the cervical lavage.

Significance: This study provides direct evidence to suggest that the dynamic mucosal environment in the lower FGT may play a role in the regulation of innate immune function, and influence the susceptibility to STIs. Our data further suggest that the exogenous factors, such as low pH, H_2O_2, and NO levels, and possibly the citric acid produced by commensal microbes can affect the expression of immunologic genes via at least in part, altering the enzymatic activity of cellular epigenetic modifiers. *Further investigation in how epigenetic mechanisms may participate in "shaping" mucosal innate immunity* will greatly enhance our understanding of the host-environment interactions at the mucosal site and shed lights into the mechanisms underlying mucosal susceptibility to STIs.

[85] Global regulation of antisense transcription by Cdk9 and histone H2B monoubiquitylation

Jason Tanny

Monoubiquitylation of histone H2B (H2Bub1) is a universal marker of RNAPII transcription. H2Bub1 is a potential tumour suppressing histone modification: Loss of H2Bub1 is associated with uncontrolled growth and impaired differentiation in cell culture models, as well as tumorigenesis in vivo. The gene regulatory mechanisms impacted by H2Bub1 remain elusive. H2Bub1 formation is coupled to transcription through the activity of CDK9, a key regulator of RNAPII elongation. We have uncovered a novel mechanism of transcriptional regulation in the model eukaryote fission yeast (*Schizosaccharomyces pombe*) that involves coordinate action of Cdk9 and H2Bub1. Combined inhibition of Cdk9 and genetic ablation of H2Bub1 caused gene-specific effects on protein-coding genes but widespread de-repression of antisense transcripts (>50% of fission yeast genes). Genetic and bioinformatics analyses implicated a conserved histone deacetylase (HDAC) complex as a potential downstream mediator of these effects, and elevated histone acetylation correlated with increased antisense transcripts at select genes. Interestingly, histone acetylation and sense gene expression was decreased in the absence of H2Bub1 at subtelomeric genes, which were also repressed by HDACs. Our data suggest that antisense regulation could be a major function of epigenetic regulators in cancer cells and illuminate novel connections between histone modifications.

[86] Functional significance of DNA methylation and histone modification patterns at gene regulatory elements in mouse adult liver

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Transcription factors (TFs) are key components of transcription regulation and their binding to *cis* regulatory regions, known as enhancers, can establish the transcriptional status of genes, leading to specification of cell fate. Epigenetic modifications, such as DNA methylation and histone modifications, regulate the binding and occupancy of TFs to these regulatory elements. It has been well documented that DNA methylation affects the binding of TFs *in vitro;* however, the effects on binding *in vivo* are not clear. Some transcription factors have been shown to bind to DNA and cause local hypomethylation or demethylation. Using ChIP-Seq, we previously identified thousands of enhancers bound by FOXA2 and HNF4A in mouse embryonic liver cells (hepatoblasts) and mouse adult liver. We have also obtained ChIP-seq data to identify the localization of histone modifications as compared to DNA methylation status. In the adult liver, we found that FOXA2 can bind to closed, methylated DNA regions while almost all HNF4A bound regions are hypomethylated. Future work is needed to confirm if HNF4A can interact with TET proteins to hydroxymethylate these regions and regulate gene expression. These findings indicate that regulation of gene expression may be impacted by the relationship between DNA methylation, FOXA2 and HNF4A binding during liver differentiation.

[87] Transcriptomic variations in the developing rat hippocampus are associated with genomic region–specific changes in histone variant H2A.Z occupancy

Anita A. Thambirajah, Nicholas O'Toole, Mei Lyn Ong, Josie Diorio, Neerja Karnani, Michael J. Meaney

The integrated development of the various brain structures relies upon precisely coordinated molecular and cellular events to create the functional neural infrastructure. Central to these events are transcriptional cascades that control the spatiotemporal expression of neurotransmitters, architectural proteins, receptors and abundant molecular components that give rise to the specialized cellular heterogeneity of the brain. A prevailing query is how are these transcriptional cascades exactingly controlled?

Comparison of transcriptomic changes in the developing male rat hippocampus between postnatal day 4 (P4) and P21 revealed regulatory shifts using RNA-sequencing. At P4, gene expression is related to neurogenesis, synaptogenesis, ion transport and signal transduction and concurs with the established perinatal neurophysiology. At P21, transcription predominantly associates with cell cycle control and DNA damage repair mechanisms, which may be essential to maintaining the differentiated cell phenotype. Parallel ChIP-sequencing of the histone variant H2A.Z was done to assess whether genomic variations in H2A.Z deposition are associated with transcriptional changes. H2A.Z is well characterized for its control of gene expression and maintenance of chromosomal integrity. H2A.Z association within the genome dynamically changes during postnatal development and most notably at the transcription start site (TSS) in relation to transcription. At either age, genes that are highly transcribed display enhanced H2A.Z incorporation within the -1 and +1 peaks that flank the TSS. Moreover, the region between these two peaks, the nucleosome depleted region (NDR), shows diminished H2A.Z occupancy. As transcription levels decrease, H2A.Z occupancy within the -1 and +1 peaks is reduced, while the NDR has an enhanced occupancy of H2A.Z. Genes that are developmentally regulated show shifts in H2A.Z occupancy. Genes that are highly expressed at P21 display an enhanced occupancy of H2A.Z in the -1 and +1 peaks and depletion of the NDR compared to P4. Development-dependent ontological analyses of genes having both differential transcription and associated H2A.Z occupancy mirrored the RNA-Seq results. At P4, key networks regulated involve neurogenesis, synaptogenesis, nerve impulse transmission and ion transport. The formation of synaptic contacts, cell cycle control, DNA damage responses and neurogenesis were major networks at P21. This work provides the first description of development-dependent, *in situ* variations of a histone variant. These naturally occurring variations strongly support the active remodeling of H2A.Z within the genome having associated transcriptional consequences that are pivotal for hippocampal development.

[88] Transcriptome Sequence Analysis of Depot-specific Adipose Tissue Identifies Differentially-Expressed Signatures Linked to Obesity Traits

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Obesity is one of the prevalent problems around the world and a major contributor to the increased burden of type 2 diabetes (T2D) and cardiovascular disease (CVD). Excess intra-abdominal visceral white adipose tissue (WAT) is considered to increase risk of both T2D and CVD. On the other hand, predominant subcutaneous WAT for a given total adiposity level is considered protective. We performed Transcriptome Sequencing (RNA-Seq) on subcutaneous and visceral WAT as well as on isolated adipocytes derived from up to 20 obese individuals undergoing bariatric surgery (~55M reads/sample). We also utilized the publicly available GTEx (http://www.gtexportal.org) resource where RNA-Seq data from 155 WAT samples are available (68 M reads/sample). Differential Gene Expression (DGE) analysis was done using DESeq2 where log2 of the fold change (log2FC) reflects expression status in visceral WAT. We found *WT1* – a marker of the intermediate mesoderm during development – to be only expressed in adult visceral WAT (log2FC: 11.4, p=1E-150) confirming the different origins of the two WAT depots. We further noted striking differences in brown adipose tissue (BAT) or novel WAT browning signatures across fat depots with visceral WAT expression signatures being enriched for brown adipocyte markers. For instance, the classical BAT marker *UCP1* was significantly up regulated in visceral WAT (log2FC: 5.8, p=6E-63) whereas the WAT marker *HOXC9* (log2FC: -3.2, p=5E-156) was down regulated. Interestingly, many of the differentially expressed genes related to BAT or browning of WAT overlap genetic loci from large meta-analysis of body fat distribution including *HOXC8*, *HOXC10*, *TBX15*, and *CYP1A2*. We also found that *IGFB1* gene, which is a predictor of diabetes development, is expressed only in visceral adipose tissue (log2FC: 3.5, p=2.015E-18). We are currently incorporating phenotypic information (T2D and CVD traits) as well as open chromatin (assessed by ATAC-Seq) information from fat depot-specific adipocytes that will help to provide insight regarding epigenetic mechanisms underlying metabolic disease risk in a depot-specific manner.

[89] Protein dynamics and the pericentromere: identifying signatures of heterochromatin dysfunction in cancer

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Chromatin alterations are fundamental to the molecular and histopathological classification of cancer, and are key drivers of oncogenesis. Therapeutic targeting of these alterations has therefore become a rapidly growing area for drug development and clinical trials. Chromatin exhibits considerable specialization beyond 'open' euchromatin or 'closed' heterochromatin. For instance, the pericentromere and inner centromere make up the centromere, which has critical roles in mitosis and safeguarding genome content during cell division. Although both centromeric compartments are categorized as constitutive heterochromatin, they differ extensively with respect to histone variants, histone post-translational modifications, non-coding RNAs, and associated proteins. Notably, pericentromeric heterochromatin is defined by repressive marks involving trimethylation of histone H3-lysine 9 (H3K9me3) and histone H4-lysine 20 (H4K20me3). Loss of the latter has emerged as a consistent feature of cancer cells, yet the underlying basis remains undefined. The goal of our research has been to characterize the dynamics of these epigenetic marks and their associated protein machinery as a function of malignant progression in a breast cancer model. Using a series of mouse and human breast cancer cell lines, patient samples, and *in vivo* tumor models, we have found that H4K20me3 levels are consistently attenuated. Moreover, analysis of quantitative proteomic data indicates this occurs together with global changes in protein composition of the pericentromere. In this context, the lysine methyltransferases SUV39H1/2 (H3K9me3) and SUV420H1/2 (H4K20me3), together with the chromobox protein CBX5 (aka HP1α), have critical roles in the stability and propagation of pericentromeric heterochromatin. To investigate the basis of these roles, we have evaluated the localization and mobility of these proteins with a combination of fluorescence recovery after photobleaching (FRAP) and time lapse microscopy. This has revealed fundamental differences in the dynamics of the SUV39H1/2, SUV420H1/2 and CBX5 proteins that provide novel insight to the maintenance of pericentromeric heterochromatin. Together, these data provide an important framework for understanding heterochromatin dysfunction in cancer.

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[90] Transcriptional regulation by HNF4a, FOXA2, and the Hippo pathway in the liver.

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The Hippo signaling pathway is implicated in control of cell proliferation, organ size, and tumorigenesis by regulating gene expression through the transcription factors TEADs, and the co-factor YAP. Transgenic mice with liver-specific expression of YAP display increased liver size, hyperplasia, and eventually hepatocellular carcinoma (HCC). Our previous work has shown that the Hippo pathway may regulate the transcription of genes associated with liver identity by regulating DNA-binding patterns of the the liver master regulatory transcription factors, HNF4a and FOXA2, to hepatoblast-specific or adult-specific enhancers. RNA-seq analysis of YAP over-expressing adult hepatocytes revealed upregulation of hepatoblast genes that are also frequently re-expressed in HCC, including GPC3. In addition, transcriptional factors that control cholangiocyte commitment were re-expressed. Analysis of HNF4a and FOXA2 hepatoblast and hepatocyte ChIP-seq data indicated that a subset of these differentially expressed genes appears to be potential HNF4a and FOXA2 target genes. HNF4a and FOXA2 peaks were found upstream of GPC3 in hepatoblasts, but not in hepatocytes. These regions overlapped H3K27ac in the embryonic liver but not in the adult liver, suggesting they may be embryonic-specific enhancers. Importantly, these enhancers contain TEAD motifs, suggesting that TEAD may influence HNF4a and FOXA2 occupancy at these enhancers. Furthermore, ENCODE ChIP-seq analysis showed that HNF4a, FOXA2, and TEAD4 share a significant amount of binding sites in the human HCC cell line HepG2. These results indicate the Hippo pathway exerts its effects on liver differentiation through multiple transcriptional pathways.

[91] An oncogenic long non-coding RNA in human colon cancer

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Background
Colon cancer is the second leading cause of cancer-related death worldwide. However, curative treatment of metastatic colon cancer remains an unmet health need. Long non-coding RNAs (LncRNAs) are a family of transcripts that do not encode proteins but are involved in regulating gene expression through interacting with DNA, RNAs, and/or proteins. There is increasing evidence showing that lncRNAs play important roles in the pathogenesis of cancer. Although a large number of human lncRNAs have been already identified, only less than 1% has been experimentally characterized.

Aims
To profile dysregulated lncRNAs and to define the functional significance of the identified lncRNAs in colon cancer cells.

Methods
Paired colon cancer and adjacent noncancerous colon epithelial tissues were subjected to transcriptome microarray. QPCR was used to confirm the changes in the expression of identified lncRNAs. ShRNA knockdown by lentiviral transduction was performed to inhibit the expression of selected lncRNAs. Colonogenic and MTS assays were used to reveal the impact of the lncRNAs on colon cancer cell proliferation and survival.

Results
One of the lncRNAs that were differentially expressed between colon cancer and normal colon epithelial tissues was markedly increased. Knockdown of this lncRNA significantly inhibited colon cancer cell proliferation. We are currently using the capture hybridization analysis of RNA targets (CHART) assay to identify its DNA and/or protein targets. The mechanism responsible for regulation of colon cancer proliferation will also be investigated.

Conclusion
We have found in this study that an array of lncRNAs are dysregulated in colon cancer cells, and have identified a lncRNA that plays important oncogenic roles in colon cancer cells.

[92] Visual Analysis and Interactive Exploration of Epigenomics Data using the VisRseq framework

Hamid Younesy, Torsten Möller, Matthew C. Lorincz, Mohammad M. Karimi, Steven J. M. Jones.

I will present VisRseq[1] (visrseq.github.io), a visual analysis framework for interactive analysis of epigenomics data sets including ChIP-seq, methylation data as well as RNA-seq data. I will demonstrate the features while walking through some typical epigenomics analysis use cases.

VisRseq provides a computationally rich and accessible framework for biologists without requiring programming expertise. This is achieved through apps, which offer a semi-auto generated and unified graphical user interface for computational packages in R and repositories such as the Bioconductor. The framework provides a simple API to allow developers to extend the functionality by creating apps from virtually any R code.
In addition, to address the interactivity limitation inherent in R libraries, the framework includes several native apps that provide exploration and gating operations as well as an integrated genome browser. The apps can be linked together to create more powerful analysis work flows.

VisRseq has built-in support for sequencing data file formats (BAM and WIG) and is integrated with IGV genome browser, makes it particularly suitable for analysis of next generation sequencing data. But it can also be used for analysis of large data (several millions of rows) presented in a tabular format.

References:
[1] VisRseq: R-based visual framework for analysis of sequencing data. Hamid Younesy, Torsten Möller, Matthew C. Lorincz, Mohammad M. Karimi, Steven J. M. Jones. *BMC bioinformatics 16.Suppl 11 (2015): S2*

[94] An epigenomic roadmap to PAX3 target gene networks in melanoma

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Melanoma is one of a few cancers that continue to increase in incidence and mortality. Although representing only 8% of skin cancers, it accounts for 70% of skin cancer-associated mortality. The developmental origin of melanocytes is thought to be a driver of this aggressive behavior. In this context, melanocyte identity is determined during embryogenesis by the hierarchical action of transcription factors, exemplified by the MITF, SOX10 and PAX3 proteins. Each of these factors has key roles in melanoma, reflecting their capacity to control pathogenic gene expression. Within this scheme, PAX3 acts as a gatekeeper to control cell division and differentiation, yet we do not have a comprehensive view of how it regulates these processes. PAX3 alters gene expression through the recognition of specific target sequences in the genome. To this end, PAX3 contains two DNA-binding domains, the homeodomain and the paired domain, which is comprised of two subdomains. The inherent modularity of this architecture permits the recognition of an array of DNA-binding patterns based on the combination of the 3 domains used. *We hypothesize that PAX3 utilizes multiple modes of DNA recognition contributing to melanoma progression through an altered target gene network.* We have repurposed data derived from cyclic amplification and selection of targets to statistically model DNA-binding specificities for human and mouse PAX3 proteins. Significantly, these binding profiles represent the first set of optimal motifs described for full-length PAX3. The robustness of this library was validated *in situ* by calculating its enrichment across published ChIP-seq datasets for PAX3 and the PAX3-FOXO1 pathogenic variant. This provided a foundation for predicting PAX3 occupancy across putative cell-specific regulatory regions defined using epigenomic signatures. Notably, PAX3 motifs were significantly enriched in human melanocyte enhancer regions derived from the Roadmap Epigenomics Project, as well as mouse melanocyte enhancers characterized by H3K4me1-flanked EP300 binding. To identify potential downstream targets of PAX3, we used RNA-sequencing to profile differential gene expression following PAX3 attenuation across syngeneic mouse melanocyte and melanoma cell lines. These data were integrated with predicted PAX3 occupancy to subsequently connect distinct DNA-binding profiles to specific transcriptional pathways in normal and pathogenic cell types. Collectively, these analyses provide novel insight into the discrete target gene networks associated with the differential use of PAX3 DNA-binding modules and how these programs may be altered as melanoma progresses from melanocyte to metastatic disease.

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