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Germline Chromatin: Concepts Shared Between Germ Cells and ES Cells

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We are investigating the chromatin structure of the germline in humans and also in the zebrafish, focusing initially on sperm. In humans, nucleosomes are widely replaced by the small basic DNA packaging protein protamine in mature human sperm. Thus, epigenetic contributions of sperm chromatin to embryo development have been considered highly limited. However, we find the retained nucleosomes significantly enriched at loci of developmental importance including imprinted gene clusters, miRNA clusters, HOX gene clusters, and the promoters of stand-alone developmental transcription and signaling factors. Importantly, histone modifications localize to particular developmental loci. H3K4me2 is enriched at certain developmental promoters, whereas large blocks of H3K4me3 localize to a subset of developmental promoters, regions in HOX clusters, certain non-coding RNAs, and generally to paternally-expressed imprinted loci, but not paternally-repressed loci. Notably, H3K27me3 is significantly enriched at developmental promoters that are repressed in early embryos, including many bivalent (H3K4me3/H3K27me3) promoters in embryonic stem cells. Finally, developmental promoters are generally DNA hypomethylated in sperm, but acquire methylation during differentiation. Taken together, epigenetic marking in human sperm is extensive, and correlated with developmental regulators.

In zebrafish, we conducted a similar study, but find that the zebrafish genome is entirely packaged in histone. Interestingly, the concepts we observe in humans for the packaging of developmental regulators are largely conserved, including the lack of DNA methylation and the coincidence of positive and negative histone modifications. However, the strategy for packaging the remainder of the genome is different, and relies on a histone-based system for maximal chromatin condensation that includes specific histone modifications and the use of linker histones, which will be discussed.

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The ATAC Histone Acetyltransferase Complex Signals for the Inhibition of the MAP Kinase Cascade through its MoaE Subunit

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ATAC (Ada Two A Containing) is an essential metazoan acetyltransferase complex that contains several proteins implicated in transcriptional regulation including two distinct histone acetyltransferases. Drosophila ATAC also contains MoaE a subunit of the ancient molybdopterin synthase enzyme. Drosophila MoaE has been conscripted to also function as the MAPK- upstream protein kinase (MUK)-binding inhibitory protein (MBIP) and all metazoan MBIPs evolved from MoaE. Drosophila MoaE/MBIP functions to suppress the activation of the c-Jun-NH2-terminal kinase, JNK, by the MAP kinase signaling cascade. Suppression of JNK activation by MoaE requires the ATAC complex as depletion of ATAC subunits leads to hyperactivation of JNK in response to osmotic stress. Thus the ATAC histone acetyltransferase complex also functions to provide a feedback signal to suppress MAP kinase signals which activate gene expression programs in response to cellular stress.

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Copying and Reprogramming of Heterochromatin With RNAi

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Heterochromatin is composed of transposable elements (TE) and related repeats which silence genes located nearby, and play a major role in epigenetic regulation of the genome. Far from being inert, heterochromatin is transcribed and small interfering RNA corresponding to heterochromatic sequences can be detected in plants, animals and fission yeast. In plants, small interfering RNA (siRNA) corresponding to some classes of TE depends on DNA methyltransferase MET1, the SWI/SNF ATPase, DDM1, or both, but not on the histone deacetylase SIL1/HDA6. All three genes are required for silencing transposons in the absence of siRNA, and we are exploring the roles of these complementary mechanisms in the inheritance of epigenetic silencing from generation to generation, and in dividing cells during development. In Arabidopsis, down regulation of DDM1 and MET1 in pollen companion cells leads to heterochromatin reprogramming, and the translocation of siRNA into neighboring sperm, where promoting the silencing of transposons.

In fission yeast and in Arabidopsis, centromeric repeats are transcribed, but the transcripts are rapidly turned over by RNA interference, through the combined action of DNA dependent RNA polymerase, Argonaute and RNA dependent RNA polymerase, each of which is associated with heterochromatin. Histone H3 lysine-9 dimethylation (H3K9me2) depends on RNAi, mediated by the Rik1-Clr4 complex. We have found that heterochromatin is lost transiently during chromosomal replication, allowing heterochromatic transcripts to accumulate. Rapid processing of these transcripts into small RNA during S phase promotes restoration of heterochromatic modifications and the retention of cohesin in G2. These results explain how “silent” heterochromatin can be transcribed, and lead to a model for epigenetic inheritance during replication.

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Epigenetics of Major Psychosis

Identification of genetic and environmental causes of complex diseases, such as schizophrenia, diabetes, cancer, among numerous others, is a much more complicated task when compared to cloning the genes in simple Mendelian conditions. The slow progress in research of complex diseases could be due to limitations of the basic strategy. I will discuss the results of two recent experimental projects performed in our laboratory. The first one is dedicated to twin studies and is related to the molecular basis of heritability. Traditionally, phenomenological twin studies provided the basis for genetic and epidemiological studies in human complex diseases. As epigenetic factors can contribute to phenotypic outcomes, we performed a DNA methylation analysis in white blood cells (WBC) and buccal epithelial cells of nearly 100 sets of monozygotic (MZ) and dizygotic (DZ) twins using the 12K CpG island microarrays. An intraclass correlation (ICC)-based comparison of matched MZ and DZ twins revealed significantly higher epigenetic difference in buccal cells of DZ co-twins. While such higher epigenetic discordance in DZ twins can result from DNA sequence differences, our in silico SNP analyses and animal studies favour the hypothesis that this is due to epigenomic differences in the zygotes, suggesting that molecular mechanisms of heritability may not be limited to DNA sequence differences. The second group of experiments has been dedicated to elucidation of the molecular epigenetic basis of major psychosis, a prototypical human complex disease. Theoretically, we argue that in comparison to DNA sequence-based factors, epigenetic changes are more consistent with the non-Mendelian aspects of complex diseases. Experimentally, a CpG island microarray- based DNA methylation profiling revealed a number of epigenetic differences in the brains of individuals affected with major psychiatric disease vs. controls (N=98) that were verified using the bisulfite sequencing. The same principles and strategies can be applied to various other complex non-Mendelian diseases.

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Chromatin Biology, Inflammation and Lung Disease

The expression of inflammatory genes is under epigenetic control. Inflammatory lung diseases such as asthma, COPD and lung fibrosis represent a large cause of mortality and morbidity throughout the world and there is recent evidence for altered expression of chromatin modifying enzymes in these disease states. This may alter the ability of pro-inflammatory factors such as NF-κB and p38 MAPK to induce inflammatory and anti-microbial genes through the recruitment of distinct transcriptional co-factors or repressors with differing histone acetyltransferase/methylase activities. Cigarette smoke contains high levels of oxidants and oxidative stress is a key driver of severe chronic airways disease. Cigarette smoke is able to suppress the activity and enhance the proteasomal degradation of histone deacetylase (HDAC)2 and this may account for the reduced HDAC2 expression and activity seen in COPD lungs. In contrast, HAT activity is unaltered. Reduced HDAC2 activity may also affect corticosteroid responsiveness in these patients preventing effective treatment. Restoration of HDAC2 levels back to those seen in control cells enable effective corticosteroid suppression of inflammatory mediators. Clinical trials using an HDAC2 activator enable corticosteroids to improve lung function and inflammation in COPD patients.

Cystic fibrosis is another chronic lung inflammatory disease linked to mutations in CFTR in airway epithelial cells with a large oxidative stress inflammatory drive. Airway epithelial cells with reduced or absent CFTR function have decreased HDAC2 protein, resulting in hyperacetylation of the CXCL8/IL-8 promoter and increased CXCL8/IL-8 transcription. Importantly, reduced HDAC2 and HDAC2 activity, but not HDAC2 mRNA, is observed in CFTR-deficient cells.

In contrast, fibroblasts from patients with idiopathic pulmonary fibrosis (IPF) have reduced expression of the antifibrotic mediator prostaglandin E2 as a result of diminished cyclooxygenase 2 (COX-2) expression. Reduced HAT recruitment and enhanced HDAC recruitment to the COX-2 promoter resulted in the down-regulation of COX-2 transcription. This effect could be reversed by treatment with HDAC inhibitors or by overexpression of HATs.

Maternal and grand maternal cigarette smoking also affects susceptibility to asthma in young children. In animal models of asthma, dietary supplementation of methyl donors can affect the inflammatory response, histology and lung function across at least 3 generations. Recent evidence in man suggests that pre-natal exposure to cigarette smoke can affect global- and gene-specific DNA methylation patterns in young children. Further epidemiological studies are underway to confirm these data. More acutely, exposure to traffic pollution can affect lung function and inflammatory gene expression in adult asthmatics. This exposure has also been linked to changes in DNA methylation.

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Epigenetics and Metabolism: The Circadian Clock Connection

Circadian rhythms govern a number of fundamental physiological functions in almost all organisms, from prokaryotes to humans. The circadian clocks are intrinsic time-tracking systems with which organisms can anticipate environmental changes and adapt to the appropriate time of day. Disruption of these rhythms can have a profound influence to human health and has been linked to depression, insomnia, jet lag, coronary heart disease and a variety of neurodegenerative disorders. The circadian clock operates in most tissues via transcriptional feedback autoregulatory loops which involve the products of circadian clock genes. The complex program of gene expression that characterizes circadian physiology is possible through dynamic changes in chromatin transitions. These remodeling events are therefore of great importance to insure the proper timing and extent of circadian regulation. A central element of the clock machinery, the protein CLOCK, has HAT enzymatic properties (Doi et al. 2006). It directs acetylation of histone H3 and of the non-histonic protein BMAL1, its own dimerization partner, at K537, an event that is essential for circadian function (Hirayama et al. 2007). We show that the HDAC activity of the NAD^+-dependent SIRT1 enzyme is regulated in a circadian manner, correlating with rhythmic H3 K9/K14 at circadian gene promoters and BMAL1 acetylation. SIRT1 physically associates with CLOCK and is recruited to the CLOCK:BMAL1 chromatin complex at circadian gene promoters. Genetic ablation of the *Sirt1* gene or pharmacological inhibition of SIRT1 enzymatic activity leads to significant disturbances in the circadian cycle. Finally, using liver-specific SIRT1 mutant mice we show that SIRT1 contributes to circadian control *in vivo*. We propose that SIRT1 functions as an enzymatic rheostat of circadian function, transducing signals originated by cellular metabolites to the clock machinery (Nakahata et al. 2008). One question however arised: how acetylation can be oscillatory if the intracellular levels of CLOCK and SIRT1 do not cycle? We demonstrate that intracellular NAD^+ levels cycle with a 24 h rhythm, an oscillation driven by the circadian clock. CLOCK:BMAL1 regulate the circadian expression of *Nampt* (nicotinamide phosphoribosyltransferase), a rate limiting step enzyme in the NAD^+ salvage pathway. SIRT1 is recruited to the *Nampt* promoter and contributes to the circadian synthesis of its own coenzyme. Using the specific inhibitor FK866, we demonstrate that *Nampt* is required to modulate circadian gene expression (Nakahata et al. 2009). Our findings reveal an interlocked transcriptional-enzymatic feedback loop that governs the interplay between cellular metabolism and circadian rhythms.

Doi M., HirayamaJ., Sassone-Corsi P. (2006). Circadian regulator CLOCK is a histone acetyltransferase. *Cell 125*, 497-508.; Hirayama J., et al (2007). CLOCK-mediated acetylation of BMAL1 controls circadian function. *Nature 450*, 1086-90.; Nakahata Y., et al. (2008) The NAD+-dependent deacetylase SIRT1 modulates CLOCK-mediated chromatin remodeling and circadian control. *Cell* 134, 329-40.; Nakahata Y., et al. Circadian control of the NAD^+-salvage pathway by CLOCK-SIRT1. *Science* - Published online March 12, 2009.

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The Epigenome and the Transcriptional Control of Innate Immunity and Inflammation

The ability to efficiently cope with microbial infections and tissue damage is an essential requisite of complex life on earth. Indeed, prototypes of the innate immune system and rudimentary inflammatory responses already appeared in rather primitive multicellular eukaryotes, and extremely more complex and evolved versions of them are now found throughout not only the animal but also the plant kingdom. A large number of genes have been recruited and positively selected in evolution to participate in various phases of the inflammatory response. Because of the complexity of the response, the many phases in which it is deployed, and the many ‘flavors’ in which it appears (depending on quality and intensity of the stimulus, as well as the target organ), very elaborated mechanisms evolved to ensure that the expression of the induced genes is carefully and precisely regulated. In virtue of such mechanisms, each gene is expressed in response to specific stimuli and with kinetics and intensities that suit the peculiar function of its product(s). Data accumulated in the last years have strengthened the concept that chromatin and the epigenome are essential substrates at which multiple endogenous and environmental signals are integrated to regulate the usage of the underlying genome and promote a correctly choreographed expression of the genes involved in inflammatory transcriptional responses.

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Polycomb Targets and Alternative Chromatin States

Mapping of Polycomb Group (PcG) protein binding sites in the Drosophila, mouse and human genomes has shown that many hundreds of genes are targets of PcG mechanisms. These include the key effector genes for all differentiation pathways, suggesting that PcG repression is involved in controlling the trajectories from from stem cells to differentiated cells. PcG proteins assemble into two major complexes, PRC1 and PRC2, that are separately recruited to target genes, generally by a Polycomb Response Element or PRE. The PRC1 component PC contains a chromodomain while the PRC2 component E(Z) trimethylates histone H3K27. Together PRC1 and PRC2 create a broad methylation domain that marks the target region and serves as an epigenetic memory mark to facilitate the re-establishment of the chromatin state at each cell cycle. Evidence from different cell lines as well as during development shows that PcG states are in fact highly dynamic, depend on the relative concentrations of key PcG components, the antagonistic effects of Trithorax and related proteins, and on the specific activators and repressors that act on individual genes. Trithorax, a SET domain protein, binds to the PRE in both the repressed and the active state. When a PcG target gene becomes derepressed, TRX also binds to the promoter. In addition, the N-ter part of TRX, but not the C-ter part, binds to an extensive domain together with a second SET domain protein ASH1, setting chromatin marks antagonistic to PcG repression.

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Epigenetic Programming of Behavior and Physiology

Maternal care alters adaptive behavioral and endocrine responses to stress in the rat. The mechanisms for these 'maternal effects' involve stable changes in gene expression. Thus, the adult offspring of mothers that exhibit increased pup licking/grooming (LG) show increased hippocampal glucocorticoid receptor (GR) mRNA expression. The differences in GR expression associate with effects at the level of both negative feedback inhibition and HPA responses to stress.. Studies of the mechanisms for maternal effects on GR expression focus on DNA methylation within a brain-specific GR gene promoter. These studies reveal sustained effects of maternal behavior on the cytosine methylation of the consensus binding sequences for specific transcription factors that regulate GR gene expression. Pharmacological manipulations that reverse the maternal effect on cytosine methylation of the GR promoter also eliminate the effect at the level of both GR expression and HPA responses to stress. The maternal effect on DNA methylation involves an active demethylation at specific CpG dinucleotides targeted by intracellular signals driven by pup LG. Such processes reveal experience-dependent plasticity in the chemistry of the DNA and chromatin structure.

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Epigenetic Mechanisms Underlying Inflammatory Gene Expression Under Diabetic Conditions

Diabetes is associated with significantly accelerated rates of inflammation and multiple complications. Abnormal activation of vascular cells and circulating monocytes triggered by inflammatory genes has been implicated in these pathologies, but the underlying molecular mechanisms are not fully understood. We have investigated the regulation of key inflammatory chemokines and cytokines involved in the activation of vascular smooth muscle cells and monocytes under diabetic conditions in vitro and ex vivo from diabetic mice, as well as in cells from diabetic patients. In particular, we examined whether key nuclear transcriptomic and epigenetic chromatin remodeling mechanisms are involved in the expression of these genes.

We hypothesized that specific histone lysine methylation patterns can yield valuable information on the etiology of diabetes, metabolic memory and diabetic complications. Using chromatin immunoprecipitation (ChIP) assays, we observed that the dysregulation of specific epigenetic activating and silencing histone marks, and occupancy of the corresponding methyltransferases at gene promoters play key roles in the regulation of inflammatory genes and metabolic memory under diabetic conditions in cell and animal models. In order to identify diabetes-specific epigenetic signatures, we also used ChIP-chip methods and developed a systems biology approach to profile and compare genome-wide histone lysine methylation patterns in cells cultured under high glucose conditions versus normal glucose conditions, and also in blood cells obtained from patients with diabetes versus normal controls. Our epigenomic approaches provide evidence that the altered histone methylation of certain genes may be a key mechanism underlying metabolic memory and sustained vascular complications in the diabetic population.

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Epigenetic Link Between DNA Methylation and Histone Modifications

The methylation of mammalian DNA, primarily at CpG dinucleotides, has long been recognized to play a major role in controlling gene expression and in coordination with the parallel chromatin-marking system that operates at the level of histone modification. I will describe recent studies on, and discusses the resulting biochemical and structural insights into, the DNA nucleotide methyltransferases (Dnmts) and histone lysine methyltransferases (HKMTs) that modulate DNA methylation.

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TDRD3 is an Effector Molecule for CARM-Generated Methyl Motifs

The covalent marking of protein arginine residues by methyl groups can promote recognition by a binding partner or a modulation in biological activity. A small family of eukaryotic gene products (the PRMTs) that catalyze methylation reactions work in conjunction with a changing cast of associated subunits to recognize specific targets in the cell for modification. Physiological roles for protein arginine methylation have been established in signal transduction, mRNA splicing, transcriptional control, DNA repair, and protein translocation. Using different proteomic approaches, we have identified a number of PRMT substrates and methylarginine-dependent protein-protein interactions. One of the primary “readers” of methylarginine marks is TDRD3, a protein that harbors a Tudor and a UBA domain. Understanding the cellular functions of TDRD3 will helped us elucidate the molecular mechanisms behind the diverse biological roles of arginine methylation.

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Different Regulatory Mechanisms for Histone Acetyltransferases and Deacetylases

In the nucleus of eukaryotic cells, chromatin plays a key role in epigenetic regulation. As integral components of chromatin, histones are subject to different types of covalent modifications such as reversible acetylation of lysine residues. In addition to histones lysine acetylation have been found in hundreds of non-histone proteins, including transcription factors, cellular structural proteins, signaling regulators, and many others [1]. Histone acetyltransferases (HATs) promote this reaction, whereas histone deacetylase (HDAC) reverse this modification. Among the HAT superfamily are MOZ (monocytic leukemia zinc finger protein or MYST3) and MORF (MOZ-related factor, also known as MYST4), a pair of paralogs that have been directly involved in leukemia development [2]. We have recently found that MOZ and MORF are catalytic subunits of tetrameric complexes and the catalytic activity is regulated through intersubunit interaction [3,4].

In addition to this pair of HATs, we have also studied the function and regulation of HDACs. HDAC inhibitors have recently emerged as novel therapeutic agents for cancer and other human diseases. Compounds with such activities have been actively evaluated in clinical trials for treatment of leukemia, solid tumors, and other diseases. In 2006, the US FDA approved the first HDAC inhibitor Vorinostat for treatment of cutaneous T-cell lymphoma. My group has been characterizing one class of deacetylases, including HDAC4, -5, -7 and -9, which form a subgroup known as class IIa [5]. These four enzymes and their orthologs in metazoan model organisms function as signal-responsive transcriptional corepressors of DNA-binding transcription factors like MEF2 (myocyte enhancer factor 2), indicating that class IIa HDACs are novel signal transducers transmitting molecular information from cellular signaling networks to transcriptional regulation in the nucleus [6]. In addition to their deacetylase activity, these deacetylases stimulate phosphorylation-dependent sumoylation of MEF2, suggesting that multiple mechanisms are involved in mediating coreppressor function of class IIa HDACs. One key regulatory event is that 14-3-3 binds to these deacetylases and promotes their nuclear export in a signal-dependent manner. Different kinases pathways have been identified and characterized for controlling 14-3-3 association. These deacetylases occupy central positions at signaling networks in different cell types, and our recent data indicate that a tumor suppressor pathway is involved in the regulation.

1. Yang, X.J. and E. Seto (2008) *Mol Cell* **31**, 449.
2. Yang, X.J. and M. Ullah (2007) *Oncogene* **26**, 5408.
3. Doyon, Y. *et al.* (2006) *Mol Cell* **21**, 51.
4. Ullah, M. *et al.* (2008) *Mol Cell Biol* **28**, 6828.
5. Yang, X.J. and S. Grégoire (2005) *Mol Cell Biol* **25**, 2873.
6. Yang, X.J. and E. Seto (2008) *Nat Rev Mol Cell Biol* **9**, 206.

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Epigenomic Landscapes of Pluripotent and Lineage-Committed Human Cells

Human embryonic stem cells share identical genomic sequences with other lineage-committed cells yet possess the remarkable properties of self-renewal and pluripotency. It has been proposed that epigenetic regulatory mechanisms, involving DNA methylation and various chromatin modifications, are at least partly responsible for the distinct cellular properties between different cell types. Using next generation sequencing technologies, we have determined the profiles of a dozen chromatin modification marks and the state of DNA methylation at high resolution throughout the genome in the human embryonic stem cells and primary fetal lung fibroblasts. Analysis of the epigenomic profiles in these cells revealed a new set of relationships between chromatin modifications and DNA methylation. Additionally, we defined two types of chromatin domains: one that forms large blocks and the other that occupies small and punctuated regions. We found that epigenomic landscapes are drastically different between the ES cells and fibroblasts: over 40% of the human genome differs in their chromatin structure between the two cell types, and that chromatin dynamics at these sequences are frequently associated with change in DNA methylation. Our results provide new insights into epigenetic regulatory mechanisms underlying properties of pluripotency and cell fate commitment.

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Transgenerational Epigenetic Control of Early Mouse Development

Mammalian development starts by fusion of two highly differentiated germ cells, leading to the formation of the totipotent embryo. In contrast to other organisms, the mammalian germ line stems from a group of differentiating embryonic progenitor cells. As a result, developing germ cells revert their epigenetic settings to enable totipotency after conception. Genomes are also subjected to changes in their chromatin states during early pre-implantation development. The potential of such reprogramming in early embryos was illustrated by nuclear transfer experiments, in which the epigenome of a fully differentiated somatic cell is reprogrammed by the cytoplasm of an enucleated oocyte. The relative low success rate of reproductive cloning however suggests that resetting of epigenetic modifications on chromatin during natural germ cell development provides parental genomes the competence to effectively support early embryogenesis.

Recently we identified transgenerational transmission of methylated histones as a novel form of maternal contribution. Specifically, we showed that the transmission of histone H3 lysine 9 tri-methylation, established in oocytes by the Suv39h HMTs, is required for maintaining the canonical constitutive heterochromatic state at major satellite repeats of the maternal genome in mouse early embryos. In the absence of this germ-line signal, as observed at the paternal genome, an alternative repressive chromatin state is formed by Polycomb group proteins at major satellites in early embryos. These data underscore the importance of transgenerational transmission of histone methylation via the maternal lineage (Puschendorf et al., 2008). In contrast to their function at major satellites in early embryos, Polycomb group proteins are better known as general transcriptional repressors of genes that regulate development. I will discuss our recent findings on the function of Polycomb group proteins in inheritance of epigenetic information across generations.

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Regulation of H3K4 Demethylation Controls Neural Differentiation; A Molecular Investigation of Complex Neuro-Developmental Disorders

Autism spectrum disorder (ASD) affects approximately 1 out of every 150 children today. While the causes vary greatly for ASD, it has been linked to epigenetic dysregulation. It has become generally accepted that the loss/change in the activity of epigenetic factors is an important area of study to understand in order to gain further insight into possible treatments for ASD. A possible causal gene for ASD is KDM5c, a H3K4 demethylase, which is mutated in patients. This in conjunction to hypermethylation seen in ASD related disorders suggests that regulation of H3K4 demethylases may provide an entry point into understanding the role of epigenetic regulation in complex neurodevelopmental disorders. Here we show that the timing of the switch between KDM5c and its opposing family member KDM5b is instrumental to terminal differentiation. This is happens through regulation of the interaction between a TLE4 and the KDM5s. The binding of TLE4 to KDM5b or KDM5c is both necessary and sufficient for H3K4 nucleosomal demethylation activity. Using both endogenous and in vitro reconstituted KDM5 complexes we investigated TLE4's role in the biochemical activity. We then confirmed that this biochemical activity is important for neural development using the neurosphere assay. In vivo studies in neurospheres show that gain or loss of TLE4 leads to delays in neural differentiation and mis-expression of neural progenitor genes including genes linked to ASD. These studies suggest that the regulation of H3K4 methylation through where and when TLE4 interacts with KDM5 proteins is an important aspect of neural differentiation and possibly to understanding neurodevelopmental disorders.

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MLL5 Function in Hematopoeisis and Leukemia; Implications for Therapy

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Targeting Histone Deacetylases: Development of Vorinostat for the Treatment of Cancer

Histone deacetylase (HDAC) inhibitors represent a promising new class of cancer therapeutics. Vorinostat, a hydroxamic acid-based HDAC inhibitor, was originally identified as an inducer of differentiation of murine erythroleukemia (MEL) cells and has also been found to inhibit proliferation, induce apoptosis or autophagy of human cultured transformed cell lines derived from both hematologic and epithelial malignancies. Vorinostat inhibits the enzymatic activity of a subset of HDACs, including the Class I HDACs, (HDAC1, HDAC2, and HDAC3) and the Class II HDAC, HDAC6, at low nanomolar concentrations (IC50 < 86 nM). The antitumor actions of vorinostat are not fully understood however likely include both transcriptional and non-transcriptional mechanisms. Expression profiling studies show that vorinostat treatment leads to both activation and repression of gene expression and may involve both direct and indirect effects resulting from histone acetylation, or alternately, may involve the hyperacetylation of non-histone proteins, including transcription factors. Many nonhistone proteins, (e.g., tubulin, Hsp90 and p53) are known to be reversibly acetylated on lysine residues and undergo hyperacetylation following exposure to vorinostat. Acetylation of these proteins may also contribute to the antitumor activity of vorinostat. Based on the known activities of vorinostat, many rational combinations with other anticancer agents have been proposed and evaluated. These include the combination of vorinostat with radiation, kinase inhibitors, cytotoxic agents, and proteasome inhibitors and have demonstrated synergistic or additive activity in human transformed cell lines. Vorinostat is under evaluation in clinical trials for the treatment of cancer and is approved by the US FDA for the treatment of cutaneous manifestations in patients with cutaneous T-cell lymphoma (CTCL) who have progressive, persistent or recurrent disease on or following two systemic therapies. Additionally, anti-tumor activity has been observed in patients with acute myeloid leukemia, multiple myeloma, non-Hodgkin's lymphoma, glioblastoma multiforme, and metastatic mesothelioma, as well as breast, laryngeal, nasopharyngeal, papillary thyroid, ovarian, and non-small cell lung cancer. Continued investigation of vorinostat in phase II and III trials for patients with other hematologic malignancies and solid tumors as a single agent and in combination therapy is ongoing.

Stephen Frye
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Promoting Illiteracy in Epigenetics: Antagonists of the Readers of the Epigenetic Code

The malignant brain tumor (MBT) repeat is a structural domain of ca. 100 amino acids and occurs in at least 9 human proteins which recognize mono- and dimethyl-lysine modifications of histones. There are no known small molecule binders of MBT domains. This presentation will summarize our progress in assay development and the design and discovery of potent antagonists of methyl-lysine recognition by MBT domain containing proteins. The resulting chemical probes will permit exploration of the biological consequences of blocking this recognition in cell-based and in vivo models with relevance to normal and disease biology. Current understanding of the biological consequences of MBT domain antagonism would suggest that antagonists may be useful in de-differentiation, re-expression of silenced genes and cellular reprogramming.

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Synthetic Binding Proteins: New Tools for Epigenetics

Post-translational modifications of histone tails are major marks for transcription activation and silencing. Consequently, detecting and capturing post-translationally modified histones and the "readers" of these marks represent a critical step in epigenetic research. However, the current paucity of high-quality affinity reagents presents a major technological bottleneck. Polyclonal and monoclonal antibodies are the only widely available affinity reagents, but they have fundamental limitations in reproducibility, scalability, storage, production throughput and expenses. I will discuss our project that aims to generate high-quality recombinant affinity reagents for epigenetic targets by employing structure-based protein design and directed evolution.

Tom Heightman Ph,D
PI Chemical Biology & Epigenetics Project Manager
Structural Genomics Consortium, Oxford University

Discovering Chemical Probes for Bromodomains: 'Omeward Bound'

Our strategic Epigenetics Chemical Probes Consortium, a collaboration between SGC, the departments of Chemistry and Biochemistry of the University of Oxford, the NIH Chemical Genomics Center in Washington, and GlaxoSmithKline, has as its goal the generation of high quality open access chemical probes for writers, readers and erasers of histone lysine acetyl and methyl marks.

Working within a family of related proteins allows scientists to apply ligand design learnings from one member to accelerate identification of ligands for related targets. This approach, sometimes referred to as chemogenomics, has enhanced efficiency in a number of protein families including kinases, where the accumulation of protein structures and small molecule structure-activity relationships over more than a decade provides a platform for the generation of new leads and an understanding of selectivity determinants.

Many of the readers and writers of post-translational histone modifications are only recently identified, and so a body of protein structural and small molecule activity data has not yet accumulated. This presents an opportunity for high throughput protein production and crystallography techniques to be applied in concert with chemistry to rapidly and systematically explore new epigenetic protein families. In this talk I will review our progress on building a chemogenomic platform for bromodomains, including structural biology, development of biophysical and biochemical assays, and hit identification chemistry.