

Distinct and overlapping roles of STAG and PDS5 proteins in cohesin localization and gene expression in embryonic stem cells

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The three-dimensional organization of the genome in the nucleus plays an integral role in many biological processes including gene expression. The genome is folded into DNA loops that bring together distal regulatory elements and genes. Cohesin, a ring-shaped protein complex, plays an important role in forming DNA loops between pairs of CTCF-occupied sites. The core cohesin complex consists of subunits SMC1A, SMC3, and RAD21. HEAT-repeat proteins can associate with this core complex, but it is unclear how they may regulate cohesin localization and function in DNA looping and gene expression. Here, we investigate the HEAT-repeat proteins, otherwise known as accessory proteins, STAG1, STAG2, PDS5A, and PDS5B. We hypothesize that the differential incorporation of these four proteins into the cohesin ring may yield distinct complexes that have functionally different roles in genome architecture and gene expression. We report both distinct and overlapping roles for STAG and PDS5 proteins in cohesin localization and gene expression. STAG1 and STAG2 localize to the same sites across the genome, yet they exist in separate cohesin ring complexes that do not co-immunoprecipitate. While PDS5A and PDS5B also localize to the same sites, they can be detected in the same complex. The individual loss of each accessory protein does not cause a redistribution of cohesin across the genome. Despite localizing to the majority of the same binding sites and not dictating cohesin distribution, loss of the STAG and PDS5 proteins reveals strong and partially distinct effects on gene expression. Overall, these accessory proteins show somewhat distinct effects on genes that are contained within DNA loop domains, with the STAGs mostly affecting Super-enhancer domains and the PDS5s affecting Polycomb domains. Knowledge of cohesin biology is critical for unraveling the mechanisms underlying human diseases and developmental disorders. Together, these results provide insight into the contributions of STAG1, STAG2, PDS5A, and PDS5B in cohesin-mediated genome structure and function.

Role of chromatin remodeler INO80 in mouse spermatogenesis

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INO80 is the catalytic subunit of the multi-subunit INO80-chromatin remodeling complex and it has been implicated in DNA replication, repair and transcription regulation. *Ino80* deficiency in murine spermatocytes results in pachytene arrest of spermatocytes due to incomplete synapsis and aberrant DNA double strand break repair (DSBR). We explored the mechanism by which INO80 mediated both DSBR and meiotic progression.

INO80 is highly expressed and shows nuclear localization in the wild type spermatocytes (*Ino80*WT). INO80-null (*Ino80*^{Δfl}, *Stra8-Cre*) spermatocytes (*Ino80*cKO) at P18 lacked characteristic ATR immunostaining, suggesting perturbation of DNA damage repair pathway in these cells. Differential expression between *Ino80*WT and *Ino80*cKO spermatocytes corroborated this observation, where pathway enrichment analysis showed both spermatogenic pathways and DSBR pathways to be the most affected, suggesting an aberrant transcription program in *Ino80*cKO spermatocytes. In *Ino80*WT, genome-wide INO80 binding analysis showed INO80-binding sites were mostly promoter proximal and across all cohorts of genes regulating meiosis. However, overall INO80-enrichment in the up-regulated genes in *Ino80*cKO compared to *Ino80*WT is higher than that in the down-regulated genes. Further, among *Ino80*-regulated differentially expressed genes in *Ino80*cKO, the majority of meiotic genes were downregulated and most of the premeiotic genes were upregulated.

To elucidate the role of INO80 in spermatogenic gene expression, analysis of activating (H3K4me3) and repressing (H3K27me3) histone marks around INO80-bound genes indicated that almost half of the INO80-binding sites are bivalent in nature, while INO80-repressed genes were mostly enriched with H3K27me3 compared to INO80-promoted genes. Analysis of global enrichment of H3K27me3 among *Ino80*WT and *Ino80*cKO spermatocytes revealed an overall decrease in the enrichment of H3K27me3, which was mostly observed around Ino80 binding sites at the upregulated genes upon Ino80 deletion. Additionally, among the cohorts of genes that are temporally regulated during meiosis, a major decrease in H3K27me3 enrichment was observed in premeiotic genes, while meiotic and constant groups did not show any major change.

Mass spectrometry analysis of co-immunoprecipitated proteins with INO80 from *Ino80*WT spermatocytes revealed that SUZ12 and EED, members of PRC2 complex that is responsible for H3K27 trimethylation, interacts with INO80. To determine if INO80 regulates SUZ12 binding, analysis of genome-wide SUZ12 binding revealed an overall reduction of SUZ12 enrichment in *Ino80*cKO spermatocytes compared to the *Ino80*WT spermatocytes at the INO80-repressed genes, suggesting an involvement of INO80 in proper localization of SUZ12 in *Ino80*WT spermatocytes. Taken together, these data suggest INO80 is a major regulator of meiosis by regulating the PRC2 activity and thereby maintaining H3K27me3 mark in the poised chromatin in developing spermatocytes.

Histone Methyltransferase SETD2 Regulates Nuclear Lamina Integrity Through Its Intrinsically Disordered Amino Terminus.

SETD2 is a tumor suppressor that is frequently mutated in multiple cancers, especially clear cell renal cell carcinomas (ccRCC), which harbor a heterozygous deletion in SETD2 in >90% of cases. It is the sole methyltransferase responsible for H3K36me3 deposition. H3K36me3 occurs co-transcriptionally and has been implicated, mainly through recruitment of its reader proteins, in splicing, DNA damage repair, HR and mRNA methylation. While all the functions of SETD2 thus far have been attributed to its catalytic activity, little is known about the role of its large intrinsically disordered amino terminus, which makes up more than half the protein. Here we uncover an unexpected and novel role for the N terminus in regulating nuclear lamina integrity thereby maintaining genome stability. In a proteomic screen to map SETD2 interactome, we discovered that SETD2 associates with structural proteins that constitute the nuclear lamina such as Lamin A, B1, B2 and Emerin, in addition to its canonical partners associated with transcription. These interactions were validated by co-immunoprecipitation in kidney cell extracts, indicating that SETD2 physically interacts with nuclear lamina proteins. Moreover, we found that SETD2 colocalized with Lamin A/C specifically during mitosis but not interphase, suggesting SETD2-lamin interaction occurs only during mitosis. Importantly, we found that SETD2 depletion causes gross nuclear morphology defects, including blebbing and micronuclei, which are a hallmark of defective nuclear lamina. Re-expression of full length wild type SETD2 but not a truncated form (tSETD2) lacking the N terminus, was able to rescue the nuclear morphology defects, suggesting a structural role for the N terminus in regulating nuclear lamina integrity. Furthermore, we found that SETD2 depletion lowers overall Lamin A/C phosphorylation, which is critical for lamin depolymerization and nuclear envelope breakdown during mitosis. Lamin A/C phosphorylation levels were restored by expression of full-length SETD2 but not tSETD2. Interestingly, SETD2 regulates Lamin A/C phosphorylation in a catalysis independent manner, as the expression of full-length catalytically inactive enzyme also restored Lamin A/C phosphorylation. Taken together, these results demonstrate that SETD2's intrinsically disordered N terminus, perhaps through phase separation, is critical for Lamin A/C phosphorylation, thereby maintaining nuclear lamina integrity and preventing genomic instability. Our studies, for the first time, have identified a novel and exciting function for SETD2, outside of its canonical histone methyltransferase activity.

Metazoans require accurate and timely replication of genetic information for proper development and maintenance of homeostasis. This is achieved by high-fidelity DNA replication during S phase of the cell cycle, but complete genome replication also requires the propagation of epigenetic information to each daughter cell. Furthermore, the chromatin environment determines the timing of “firing” at origins of DNA replication: euchromatin generally displays early replication, while heterochromatin displays late replication. Failure in this coordinated process is associated with a predisposition to cancer and disease. While the relationship between DNA replication and the chromatin environment is essential, the mechanisms governing the timing and location of replication initiation within the metazoan genome remains poorly understood. To explore this relationship, we employed genomics techniques and our histone gene replacement platform in *Drosophila melanogaster*. We found that loss of transcriptional activating H4K16 acetylation prevents transcription and early DNA replication. Perturbation of heterochromatin structure via a H3K9R mutation surprisingly resulted in small rather than global changes in replication timing. However, an examination of endo-replicating tissue revealed that this mutation leads to over-replication of heterochromatin. To explore other mechanisms of replication timing control, we examined the trans-acting factor Rif1 and found that it coordinates replication timing in a tissue-specific manner. Together these data suggest that several mechanisms coordinate to control replication timing in metazoan genomes. To further elucidate these mechanisms and explore the consequences of loss of chromatin states or factors, we are developing a new genomics tool to more precisely assay the location and number of origins of replication. By assaying the genomic location of the ORC-MCM pre-replication complex in a variety of epigenetic contexts, we will enhance our understanding of metazoan DNA replication dynamics.

Development of Covalent Antagonists of Methyl-Lysine Reader MPP8 as a Therapeutic Target in Triple-Negative Breast Cancer

Jarod Waybright

Abstract:

Triple-negative breast Cancer (TNBC) is an aggressive form of breast cancer with a poor prognosis and limited therapeutic options. In collaboration with the Westbrook lab at Baylor College of Medicine, we performed a series of genome-wide shRNA screens in Human Mammary Epithelial Cells (HMECS) with oncogene hyper-activation to mimic the hyper-activation seen in TNBC and normal HMECs. We Utilized an shRNA library targeting chromatin factors, kinases and phosphatases, and ubiquitin ligases to identify genes that are required for survival of the oncogene hyper-activated HMECS as potential targets in TNBC. Some of the most potent dependencies identified were with shRNAs targeting several members of the human silencing hub (HuSH) complex including MPP8, a methyl-lysine reader protein of the chromodomain family. This was further validated in TNBC xenograft models. A single point (W80A) chromodomain mutant of MPP8, which abolishes the chromatin association, recapitulated these results suggesting ligands targeting the chromodomain could serve of therapeutic relevance in TNBC. Unfortunately, there are currently no available chemical tools to validate the role of MPP8 in cancer and assess the potential for therapeutic intervention. I will describe the development of UNC7713, a covalent antagonist of MPP8, which demonstrates a cellular TE_{50} (the concentration at which 50% of the target is engaged) of 180 nM and selectivity across the chromodomain family of proteins. Treatment with UNC7713 leads to selective cell death of oncogene driven TNBC cell lines making it a powerful tool to confirm the therapeutic relevance of MPP8 in cells and *in vivo*.

Mitochondrial RNA polymerase pausing is mediated by guanine rich sequences

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Abstract

This project identifies and characterizes the pausing of the mitochondrial RNA polymerase (mtRNAP). RNA polymerase pausing is a key regulatory step in the transcription of many nuclear-encoded genes; in contrast, only one promoter-proximal pause for the mtRNAP has been reported. The circular mitochondrial genome has two polycistronic strands, a guanine (G)-rich light strand and a G-poor heavy strand. In the nucleus, RNA polymerase II pauses most often in gene promoters, but in the mitochondria, with two polycistronic genes, questions arise as to whether mtRNAP pauses beyond the promoters and how pausing could affect mitochondrial gene expression. With the precision nuclear run-on assay (PRO-seq) in human primary fibroblasts from five individuals, we mapped the locations of transcriptionally active mtRNAP. The results show that there are hundreds of sites where the mtRNAP accumulates, and this includes the one known mtRNAP pause site. In total, with cells from different individuals, we found that as the polymerase transcribes the mitochondrial genome, it pauses over 400 times at consistent locations. These brief stops occur most often after the polymerase has just added a cytosine to the nascent RNA, where the RNA-DNA duplex is thermodynamically stable, and after transcribing through guanine-rich regions. We found there are more than twice as many pause sites on the G-rich strand compared to the G-poor strand. To determine how these G-rich sequences contribute to pausing, we found that they form G-quadruplexes. Stabilization of G-quadruplexes impaired mtRNAP transcription elongation, leading to decreased expression of genes transcribed by mtRNAP, and reduced mitochondrial function. In this presentation, I will discuss how dynamic tuning of nucleic acid secondary structure can regulate transcription and gene expression.

Investigating the Role of Histone H3 Lysine 4 in Developmental Gene Regulation.

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Histone H3 lysine 4 (H3K4) methylation is an evolutionarily conserved modification of chromatin closely linked to gene activation. The three methylation states of H3K4, monomethylation (me1), dimethylation (me2), and trimethylation (me3), are often used to annotate functional elements of eukaryotic genomes: H3K4me1 marks poised and active enhancers, H3K4me2 is enriched at tissue-specific transcription factor binding sites, enhancers, and promoter edges, and H3K4me3 marks active transcriptional initiation at promoters. These methylation states are mediated by the human methyltransferases MLL3/4, MLL1/2, and SET1A/B, which perform conserved roles in human gene regulation and are commonly mutated in cancers. While these histone-modifying enzymes have been found to be major regulators of development, differentiation, and disease, their enzymatic activity is not limited to histones and they also have non-enzymatic functions. Moreover, the functional study of H3K4 has been difficult because the canonical histone genes in many organisms, including humans, are found at multiple chromosomal locations. *Drosophila* is an ideal system to study the role of H3K4 in development because its canonical histone genes are clustered at a single chromosomal locus. H3K4 methylation in *Drosophila* is mediated by the methyltransferase enzymes Trr, Trx, and dSet1 (the human homologs are MLL3/4, MLL1/2, and SET1A/B, respectively). The organization of the canonical histone genes allows for their precise deletion and replacement with transgenic versions that have been mutated to encode for a non-modifiable residue (H3K4R). We previously showed that the wild-type histone replacement transgene supports the propagation of a stable stock lacking all endogenous histone genes (1). By contrast, here we find that the H3K4R replacement transgene is predominantly embryonic lethal, and although H3K4R embryos survive until the end of embryogenesis at expected Mendelian frequencies, they fail to make the transition into larvae. The few H3K4R embryos that hatch have very low survivability and remain stuck in early development. We also find that H3K4R mutant cells survive and appear to proliferate normally with diminished H3K4me1. These data demonstrate a requirement for H3K4 in development, however interpretation is complicated by the fact that H3K4 is subject to multiple methylation states. In future work, we will determine the effects of H3K4 mutation on gene expression through RNA-seq analysis, as well as its impact on the histone post-translational modification landscape through top-down mass spectrometry. Together, this work will extend our understanding of the mechanistic role that histone modifications contribute to developmental gene regulation.

Reference: (1) McKay, Daniel J., et al. "Interrogating the function of metazoan histones using engineered gene clusters." *Developmental Cell* 32.3 (2015): 373-386.

The C-terminus of VRK1 governs interactions with nucleosomes to influence histone H3 threonine 3 (H3T3) phosphorylation

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Vaccinia-related kinase 1 (VRK1) is an understudied nuclear kinase that is known to phosphorylate the histone H3 tail at threonine 3 (H3T3) during mitosis. This modification, also placed by the haspin kinase, is part of a series of events that ensures that daughter cells receive an appropriate amount of DNA following cell division. VRK1 overexpression is correlated with poor survival in breast cancer and glioma, suggesting that the kinase plays a key role in supporting pro-tumorigenic processes. Despite the importance of VRK1 to cell cycle regulation, little is known about the biochemical basis of its activity on nucleosomes. To date, no potent and selective inhibitors for VRK1 exist, highlighting a need to understand how this kinase functions on nucleosomes and may be regulated in the absence of direct inhibitors.

Our work reveals that VRK1 directly and stably interacts with nucleosomes with a tight K_d of ~100 nM. VRK1 binds nucleosomes via a C-terminal alternating arginine motif, mutations or truncations of which have been found in human disease. We find that mutation or truncation of this C-terminal arginine motif leads to a 4-50 fold reduction in nucleosome affinity that tracks with mutation severity. Importantly, mutation of the arginine motif results in dissociation of VRK1 from chromatin during mitosis in living cells. VRK1 shows a nucleosome substrate preference and is capable of phosphorylating mononucleosomes *in vitro*, but not individual histones or histone tetramers bearing the H3 tail. Furthermore, C-terminal arginine mutants are defective in histone H3T3 phosphorylation, suggesting that stable interaction with nucleosomes is required for nucleosome-directed kinase activity at H3T3. The nucleosome acidic patch, a negatively charged region at the dimer interface of H2A/H2B, appears to play some role in binding VRK1 in direct interaction assays, but its presence is negatively correlated with nucleosome-directed kinase activity compared to acidic-patch-neutralized nucleosomes. In contrast, extranucleosomal linker DNA promotes both nucleosome binding and phosphorylation of H3T3. Taken together, this evidence suggests that VRK1 may adopt at least two different binding modes on the nucleosome (acidic patch-governed vs. linker DNA-governed) that either promote or inhibit nucleosome-directed kinase activity based on the position and distance of VRK1 from the H3 tail. Future work will provide further evidence delineating these binding modes and their influence on VRK1 nucleosome-directed kinase activity.

Deciphering the histone interactions and reader functions of ASH1L and the PHD-BRD reader domain family in biology and cancer

Mutation or dysregulation of the proteins that read, write, and/or erase histone post-translational modifications are thought to underlie ~50% of all human cancers. One such family of proteins that are frequently altered in cancer is those containing paired histone reading plant homeodomains (PHD) and bromodomains (BRD). ASH1L is a histone H3 lysine-36 (H3K36) dimethylase which is altered in ~7% of cancers and contains a poorly understood triple histone reader PHD-BRD-BAH domain module. *Although ASH1L and other PHD-BRD proteins contain commonly studied reader domains and have been linked to cancer, how they regulate normal and cancer development is still not well understood.* Thus, there is a critical need to systematically characterize the histone reading interactions and mechanistic processes for ASH1L and the rest of the PHD-BRD family, especially with respect to how these domains in combination can form multivalent interactions with nucleosomes. To address how ASH1L and PHD-BRD readers function, I will employ a suite of innovative histone-binding assays, structural methodologies, and cellular-based analyses. Specifically, I will use modernized histone peptide and nucleosome screening assays, X-ray crystallography and Cryo-EM in parallel, and a knockdown/complementation system of ASH1L in normal and breast cancer cell lines. I hypothesize that ASH1L targets specific acetylated and methylated histone tails via its BRD-PHD-BAH module to regulate homeotic gene expression, but that aberrant expression of ASH1L promotes breast cancer through increased expression of pro-growth and survival genes. Through this examination, this work aims to further decipher the “Histone Code” and provide new insights into cancer biology

Title: A cohesin cancer mutation reveals a role for the hinge domain in genome organization and gene expression

The cohesin complex functions in the spatial organization of the genome into DNA loop structures. While cohesin is known to occupy enhancers, promoters and CTCF sites, the molecular mechanisms through which cohesin is loaded onto chromatin, localizes to these specific genomic sites, then forms and maintains loops are not fully understood. In yeast, targeted mutagenesis and suppressor screens identified the hinge domains of Smc1 and Smc3 as being required for cohesin loading onto chromatin and the topological entrapment of sister chromatids during DNA replication. It is not known whether the cohesin hinge also regulates these functions in mammalian cells or participates in metazoan-specific functions in interphase chromatin organization. Investigation of cancer sequencing databases revealed a hotspot mutation in the SMC1A hinge at amino acid residue arginine (R) 586 in leukemia. We used CRISPR/Cas9 genome editing to generate murine embryonic stem cell (ESC) lines with an endogenous tryptophan (W) mutation at amino acid 586, as seen in cancers. Transcriptomic analysis of SMC1A-R586W ESCs reveals global dysregulation of the transcriptional programs, with reduced expression of pluripotency-controlling TFs, activation of differentiation programs, and misexpression of oncogenes. Strikingly, RAD21 ChIP-seq indicates that SMC1A-R586W reduces cohesin enrichment at promoters and enhancers, but not at CTCF sites. Hi-C analysis reveals altered genome organization, including a reduction in short-range, intra-domain DNA contacts. This work indicates that the cohesin hinge participates in the cohesin loading reaction at promoters and enhancers, and/or promotes the localization of cohesin to these elements. These findings further suggest that cohesin mutations can contribute to oncogenesis through selective loss of cohesin at transcriptional regulatory elements.

Characterization of the effects of a dual HDAC/LSD1 inhibitor on chromatin accessibility in Ewing sarcoma cells

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Ewing Sarcoma is a pediatric tumor of bone and soft tissue that is driven by the fusion oncoprotein, EWS-FLI1. EWS-FLI1 binds to GGAA microsatellite repeats, which results in aberrantly accessible chromatin at these sites. Our lab developed and performed a high throughput screen for chromatin accessibility. Hit compounds included inhibitors of histone deacetylase (HDAC) proteins. Treatment of Ewing sarcoma cells with HDAC inhibitors such as entinostat, vorinostat, and panobinostat resulted in loss of EWS-FLI1 transcription and closing of aberrantly open chromatin. In studies performed by other labs, inhibitors of the lysine demethylase protein, LSD1, were shown to reverse the transcriptional signature associated with EWS-FLI1 expression. These inhibitors are currently in a clinical trial for treatment of Ewing sarcoma. Both LSD1 and HDAC1/2 are components of the CoREST corepressor complex. The Cole lab recently developed a dual HDAC and LSD1 inhibitor called corin that targets the CoREST complex. We hypothesize that corin could serve as a potent inhibitor of both the aberrant chromatin accessibility and gene expression patterns associated with EWS-FLI1 expression. We are currently using both the Assay for Transposase Accessible Chromatin (ATAC) and Formaldehyde Assisted Isolation of Regulatory Elements (FAIRE) to interrogate chromatin accessibility states in Ewing sarcoma cells treated with corin, followed by quantitative polymerase chain reaction (qPCR). These assays will determine if corin reduces the aberrant chromatin accessibility associated with EWS-FLI1 expression.

EZH2 as a Transcriptional Activator of NF- κ B Target Genes in TNBC

Breast cancer (BC) is diagnosed in hundreds of thousands and leads to the death of tens of thousands of patients annually in the US. BC is a heterogeneous disease and can be categorized by hormone status, which includes Triple Negative BC (TNBC), or by molecular subtyping, which includes Basal-Like BC (BLBC). Cases categorized as TNBC and BLBC largely overlap and are more aggressive, present a worse prognosis, and lag behind other subtypes in treatment advances. Thus, identifying therapeutically targetable facets of TNBC/BLBC is critical.

EZH2, a core subunit of Polycomb Repressive Complex 2 (PRC2), is a histone methyltransferase that catalyzes the trimethylation of histone 3 lysine 27 (H3K27me₃), ultimately contributing to epigenetic gene silencing. This activity is essential in embryonic development, but EZH2 is overexpressed in TNBC/BLBC and is considered an oncogene. Multiple attempts are underway to clinically target EZH2, but 'epidrugs' often face primary resistance in solid tumors.

Interestingly, our lab and others have demonstrated that EZH2 is involved in paradoxical *activation* in TNBC/BLBC cells. Specifically, we have shown that EZH2 activates transcription of NF- κ B factors and target genes and may contribute to Cancer Stem Cell growth and expansion. We propose that EZH2 forms non-PRC2 protein interactions that mediate non-canonical activities, meaning the current therapeutic strategies considering canonical EZH2-PRC2 functions may not be effective and susceptible to resistance.

To demonstrate that EZH2 functions as a transcriptional activator of NF- κ B genes in TNBC/BLBC cells, we utilize high-throughput sequencing and qPCR under EZH2 knockdown, overexpression, and inhibited conditions. We additionally use affinity purification mass spectrometry and immunoprecipitation to investigate non-PRC2 protein interactions of EZH2 that mediate its non-canonical activity. Finally, we utilize a proteolysis-targeting chimera (PROTAC) to demonstrate the PRC2-independence of transcriptional activation by EZH2.

Our findings contribute to the fundamental understanding of epigenetic and inflammatory dysregulation in TNBC/BLBC, and our ongoing characterization of EZH2-NF- κ B crosstalk and contribution to cancer cell growth will provide insights into TNBC/BLBC disease mechanisms and highlight potential novel targets for therapeutic investigation.

One of the hallmarks of cancer is uncontrolled cell growth, which requires the cell to overcome pathways intended to promote senescence. Telomere length is a key component for cell growth, as each cell division results in telomere shortening. Once telomeres become too short, the cell is signaled to stop dividing and go into senescence, because it can no longer safely divide without damage. Cancer cells are able to overcome this growth-limiting process via telomere maintenance mechanisms (TMMs). The majority of cancers upregulate telomerase to extend telomeres, while 10-15% of cancers utilize the alternative lengthening of telomeres pathway (ALT). The underlying mechanisms of ALT are not as well understood as the telomerase pathway, however, targeting the ALT pathway may have therapeutic potential. If the ALT pathway is successfully inhibited, it could promote senescence specifically in cancer cells, which would make an ALT inhibitor a valuable cancer therapeutic. In order to develop chemical probes to study the ALT pathway and assess the potential of targeting ALT for cancer therapy, we sought to develop a cell-based high throughput screen for determining anti-ALT activity using C-circle level as a readout. C-circles are partially single stranded extrachromosomal circular DNA structures that accumulate specifically in ALT cells. The goal of this project was to miniaturize an existing assay that measures C-circle levels to create a high-throughput screen that could be used to screen large compound libraries for inhibitors of the ALT phenotype. Hit compounds would be used to better understand the mechanisms of the ALT pathway as well as to assess the plausibility of using ALT as a target for anti-cancer therapies.

Epigenetic control of ACE2 by the chromatin remodeler, SMCHD1

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After gaining entry into human cells via the ACE2 receptor, SARS-Cov-2 causes a dramatic decrease in ACE2 cell surface expression, thereby wreaking havoc on the many organs that rely on a finely tuned balance between angiotensin II and angiotensin 1-7 activities. Thus, it is critical to understand how ACE2 expression is modulated by SARS-Cov-2. Recent studies in mouse heart cells demonstrated mechanical stress-induced recruitment of Brg1, a component of the SWI/SNF chromatin remodeling complex, to the *Ace2* promoter, where it repressed *Ace2* expression (*Yang '16 PNAS*). Following ablation of another chromatin remodeler, SMCHD1, we observed 15-fold upregulation of ACE2 mRNA and increased ACE2 protein in human cranial placode cells, which are precursors of olfactory neurons and their support cells. Importantly, olfactory support cell death is the cause of viral-induced anosmia and dysgeusia (loss of smell and taste) in humans. We have also demonstrated increased ACE2 protein abundance in SMCHD1 KO HepG2 (liver), HEK293 (kidney), and A549 (lung alveolar) cell lines. Of note, the primate-specific isoform dACE2 was minimally expressed in these WT and KO cells. Taken together, these data demonstrate that ACE2 is an SMCHD1 epigenetic target and raise the intriguing possibility that SARS-Cov-2 represses ACE2 expression in target tissues by hijacking the host epigenetic machinery. Hi-C studies are now in progress to investigate how loss of SMCHD1 activity may lead to changes in 3D chromosomal architecture that de-repress ACE2 in human cells. We are also leveraging our structural biology program to design small molecule SMCHD1 inhibitors as a potential therapeutic strategy to restore ACE2 levels in disease-relevant organs.

Title: Nanodroplets enable extraction of high-quality chromatin from archival tissue

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Abstract:

Chromatin states that influence gene expression and other nuclear processes can be altered in disease. The assembly of nucleosomes, transcription factors and chromatin regulatory complexes in cells results in characteristic patterns of chromatin accessibility. Studying these patterns in tissue has been limited since existing chromatin accessibility assays are ineffective for formalin-fixed, paraffin embedded (FFPE) tissues. We have developed a method to efficiently extract intact chromatin from archival tissue via enhanced cavitation with a nanodroplet reagent consisting of a lipid shell with a liquid perfluorocarbon core. The addition of nanodroplets to the chromatin accessibility assay FAIRE (formaldehyde-assisted isolation of regulatory elements) increases the yield of accessible and nucleosome-bound chromatin and does not affect the chromatin signal. The inclusion of nanodroplets on FFPE rodent tumor xenografts enabled us to identify tumor-relevant regions of accessible and inaccessible chromatin. These regions were proven to match with the cancer cell lines used in the formation of these rodent tumor xenografts, proving that FFPE xenografts are an appropriate model for investigating chromatin signatures associated with primary human tumors. Another variable considered for optimizing chromatin extraction from FFPE tissue was formaldehyde fixation time, specifically on MCF-7 rodent tumor xenografts. Our data indicates that formaldehyde fixation time does affect chromatin extraction yields, but not chromatin accessibility patterns. We will now apply our chromatin extraction technique to FFPE human tumor xenografts to establish a catalogue of accessible and inaccessible chromatin patterns specific to different cancers.

Title: Acetylation-mediated histone tail accessibility governs the read-write mechanism of H3K4

Abstract:

The highly positively charged nature of histone tails along with the PTMs that decorate them govern their equilibrium between free/accessible states and collapsed/DNA-bound states in nucleosomes. This dynamic structural feature of histone tails affects how reader proteins recognize and bind to particular PTMs. Specifically, acetylation of the H3 N-terminal tail has been linked to increased recognition by the PHD finger from BPTF to H3K4me3. To test whether these structural dynamics go beyond protein-protein recognition and influence histone writers, we have tested the effect of downstream H3 N-terminal tail acetylation on the catalytic activity of the MLL1 complex. Our results demonstrate that mononucleosome acetylation on H3 K9, 14, and 18 significantly increases MLL1 activity and binding affinity for the H3K4 residue as a substrate. Furthermore, mass spectrometric analysis of MCF-7 cells treated with butyrate, an HDAC inhibitor, revealed a significant positive correlation between rising levels of histone acetylation and H3K4 methylation. Together, these observations show that the dynamic structural environments around nucleosomes broadly impact how protein complexes engage with, modify, and eventually regulate chromatin structure and gene expression.

3C Symposium Abstract

The multi-subunit protein complex Cohesin dynamically structures the genome during DNA replication, DNA repair, gene expression, and three-dimensional chromatin interactions. There are many open questions about how cohesin functions in such varied biological processes and how cohesin loads, translocates, and unloads at specific genomic locations. Recent work from our lab revealed that in mouse embryonic stem cells (mESCs), loss of the zinc finger protein WIZ (Widely Interspaced Zinc Fingers Protein) led to altered Cohesin localization in two distinct ways. First, 20,000 new cohesin binding sites were observed in the absence of WIZ. In addition to these ectopic binding sites, Cohesin levels at retained binding sites were increased, as measured by quantitative ChIP-seq. In order to understand the mechanisms that regulate Cohesin localization across the genome, we set out to investigate how Cohesin is recruited to ectopic peaks in the absence of WIZ. Deletion of WIZ does not genocopy loss of the known Cohesin unloader WAPL, suggesting that the observed increase in Cohesin occupancy in the absence of WIZ is not due to a defect in canonical Cohesin unloading from chromatin. Since WIZ regulates heterochromatin formation in WT cells, we examined the chromatin landscape at proto-ectopic Cohesin sites in WT mESCs. Surprisingly, proto-ectopic Cohesin sites displayed hallmarks of open chromatin but did not show an enrichment of specific transcription factor motifs. Furthermore, despite exhibiting a prolonged S-phase, cells lacking WIZ display a reduced interaction between Cohesin and PCNA, suggesting that Cohesin's increased occupancy is not due to a greater association with active replication forks. Together, these studies shed light on how Cohesin is positioned throughout the genome.

Abstract

Jeanne-Marie McPherson

Genomes encode a vast amount of information in DNA. To control access to this information, eukaryotes organize their genome into chromatin. The structure of chromatin regulates all DNA-dependent processes including DNA replication, DNA damage repair, and transcription. The basic building block of chromatin is a nucleosome composed of 147 bp of DNA wrapped around an octamer of histone proteins. Cells contain two types of histones: replication-coupled histones (e.g. RC H3.2) that are expressed during DNA replication, and replication-independent histones (e.g. RI H3.3) that are expressed throughout the cell cycle and in post-mitotic cells. *Drosophila melanogaster* has a single RC histone locus, which contains 100 copies of each RC histone gene, including H3.2. RI H3.3 is expressed from two loci—*His3.3A* and *His3.3B*—which produce identical H3.3 proteins. We refer to the coordinated control of RC H3.2 and RI H3.3 abundance as histone homeostasis, a process critical for regulating access to information within the genome, but whose underlying mechanism is unknown. Here we combined histone gene engineering and classical genetics to investigate how manipulating RC H3.2 and RI H3.3 gene copy number affects histone protein expression, histone deposition into chromatin, and viability in *Drosophila*. We used the *Drosophila* histone replacement platform to manipulate RC H3.2 copy number by replacing the endogenous histone locus with a transgene expressing 12 RC histone gene copies (12xWT). These data indicate that RI H3.3, but not *His3.3A* specifically, is essential for 12xWT viability. We hypothesize that (1) RI H3.3 is required to express RC histone genes and/or the RC 12xWT histone array or, (2) H3.3 protein levels are up-regulated in 12xWT animals to compensate for loss of RC H3.2. We are testing the mechanism by quantifying RC H3.2 and RI H3.3 mRNA and protein levels in multiple tissues. The histone replacement platform is the only animal experimental system in which precise numbers of RC histone gene copies can be generated and provides a unique opportunity to directly examine the coordination between RC and RI histones.

Mammalian SWI/SNF chromatin remodeler is essential for reductional meiosis in males

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Abstract (193 words):

Meiosis is critical for gamete generation and therefore sexual reproduction. We previously reported an essential role for the mammalian SWI/SNF (SWItch/Sucrose Non-Fermenting) ATP dependent chromatin remodeling complex in mouse spermatogenesis. Specifically, BRG1, a SWI/SNF catalytic subunit was shown to promote meiotic progression by regulating germ line transcription. BRG1 associates with several other subunits, to form biochemically distinct SWI/SNF subcomplexes, whose functions in meiosis remain unknown. Here, we identify a role for the SWI/SNF subcomplex, PBAF (Polybromo_- Brg1 Associated _Factor) in the regulation of meiotic cell division. The germ cell-specific depletion of PBAF DNA binding subunit, ARID2 resulted in a metaphase-I arrest. Mutant metaphase-I spermatocytes displayed defects in chromosome organization, spindle assembly and were devoid of centromeric Polo-like kinase1 (PLK1), a known regulator of the spindle assembly checkpoint (SAC). Interestingly, histone modifications such as Histone H3 threonine3 phosphorylation (H3T3P) and Histone H2A threonine120 phosphorylation (H2AT120P) that are normally limited to centromeric regions were detected chromosome-wide in the absence of ARID2. Such abnormal localization of both H3T3P and H2AT120P coincided with a defect in chromosome passenger complex (CPC) recruitment. We therefore propose that ARID2 facilitates metaphase-I exit by regulating spindle assembly and centromeric chromatin.

Impact of cancer-associated cohesin variants on gene expression and cellular identity

Cohesin is a ring-shaped protein complex that controls dynamic chromosome structure. Cohesin activity is important for a variety of biological processes, including formation of DNA loops that regulate gene expression. The precise mechanisms by which cohesin shapes local chromosome structure and gene expression are not fully understood. Recurrent mutations in cohesin complex members have been reported in various cancers, though it is not clear whether many cohesin sequence variants have phenotypes and contribute to disease. Here, we utilized CRISPR/Cas9 genome editing to introduce a variety of cohesin sequence variants into murine embryonic stem cells and investigate their molecular and cellular consequences. Some of the cohesin variants tested caused changes to transcription, including altered expression of gene encoding lineage-specifying developmental regulators. Altered gene expression was also observed at insulated neighborhoods, where cohesin-mediated DNA loops constrain potential interactions between genes and enhancers. Furthermore, some cohesin variants altered the proliferation rate and differentiation potential of murine embryonic stem cells. This study provides a functional comparison of cohesin variants found in cancer within an isogenic system, revealing the relative roles of various cohesin perturbations on gene expression and maintenance of cellular identity.

Deciphering the nucleosome interactome

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In all eukaryotic cells, nucleosomes provide basic organization of genomic DNA and serve as signaling hubs for genome-templated processes. These functions are tuned by chromatin enzymes that introduce chemical modifications to nucleosomes and control their positioning and occupancy. Altered DNA methylation and histone modifications are recognized epigenetic defects in cancer, developmental and neurodegenerative diseases, yet our understanding of how the chromatin enzymes recognize their physiologic target, the nucleosome, is limited. High and medium resolution structures of chromatin enzymes bound to nucleosomes show multivalent interactions including histones and DNA. Nearly all reported interactions also include the anchoring of an arginine into a patch of acidic residues on the nucleosome surface. To assess the role of the acidic patch in nucleosome binding proteome-wide and to identify novel nucleosome binding hot-spots we performed a comprehensive, unbiased nucleosome interactome proteomic screen. This was accomplished through nucleosome affinity pulldowns from mouse embryonic stem cell nuclear lysates, using a reconstituted library of nucleosomes bearing mutations that collectively disrupt all exposed histone surfaces. To quantitatively compare binding differences across the library we utilized isobaric tandem mass tags (TMT) and multidimensional mass spectrometry. Remarkably, we found that over 50% of nucleosome binding proteins identified in our screen require the acidic patch for binding and that 18% and 8% of proteins also require one or two additional nucleosome disk surfaces. Perhaps more surprisingly, almost half of the nucleosome disk surface contributes only minimally to nucleosome binding nuclear proteome-wide. Overall, we paired about 300 proteins representing diverse nuclear functions including DNA methylation (DNMT1 and 3, Tet1), histone modification (HAT complexes, kinases), nucleosome remodeling (SWI/SNF), DNA repair (MRN complex), DNA replication (ORC complex), RNA processing, and cell cycle progression (APC/C) with specific nucleosome surface requirements. Altogether our results illuminate the pervasiveness of acidic patch binding, establish universal patterns of nucleosome recognition and provide a reliable resource that details the nucleosome interactome.

Elements at the 5' end of *Xist* harbor SPEN-independent transcriptional antiterminator activity

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Abstract

The *Xist* lncRNA requires Repeat A, a conserved RNA element located in its 5' end, to induce gene silencing during X-chromosome inactivation, a paradigm of epigenetic regulation. Intriguingly, Repeat A is also required for the production of *Xist*. While silencing by Repeat A requires the protein SPEN, how Repeat A promotes *Xist* production remains unclear. We report that in mouse embryonic stem cells, expression of a transgene comprising the first two kilobases of *Xist* (*Xist*-2kb) causes transcriptional readthrough of multiple downstream polyadenylation sequences. Readthrough required Repeat A and the ~750 nucleotides downstream but did not require SPEN. Despite associating with SPEN and chromatin, *Xist*-2kb did not robustly silence transcription, whereas a transgene comprising *Xist*'s first 5.5 kilobases robustly silenced transcription and read through its polyadenylation sequence. Longer, spliced *Xist* transgenes also induced robust silencing yet terminated efficiently. Thus, in contexts examined here, *Xist* requires sequence elements beyond its first two kilobases to robustly silence transcription, and the 5' end of *Xist* harbors SPEN-independent transcriptional antiterminator activity that can repress proximal cleavage and polyadenylation. In endogenous contexts, this antiterminator activity may help produce full-length *Xist* RNA while rendering the *Xist* locus resistant to silencing by the same repressive complexes that the lncRNA recruits to other genes.