UNIVERSITY OF CALIFORNIA SAN DIEGO

Changes in Chromatin Structure During Mitosis and in Response to Acute BRD4 Degradation

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Biology

by

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2020

The Thesis of Linyu Zhou is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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LIST OF ABBREVIATIONS

NDR	Nucleosome Depleted Region
HDACs	Histone Deacetylases
TSS	Transcription Start Site
BRD4	Bromodomain-containing protein 4
SE	Super Enhancer
dTAG system	Degradation tag system
HOMER	Hypergeometric Optimization of Motif EnRichment
WCE	Whole Cell Extract
MEG	Mitotically Enriched Gene
GREAT	Genomic Regions Enrichment of Annotations Tool
TF	Transcription Factor

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ABSTRACT OF THE THESIS

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Professor Cornelis Murre, Co-Chair

Chromatin organization and accessibility is an important layer for regulating gene expression. We wanted to learn how chromatin changes by exploring either data of cells going through mitosis or cells responding to an acute removal of a key regulator, BRD4.

Nucleosome Depleted Regions (NDRs) are active genomic locations for transcription machinery or other regulatory factors to bind. In mitosis, cells lose higher-order chromatin structure and have drastic reduction in transcription level. We are interested in revealing chromatin structure changes at enhancers during mitosis in terms of NDR patterns of histone

marks, especially for enhancers relating to TSSs that were shown to have distinctive NDR patterns previously. We found disappearance of NDRs at all enhancers because of entry of a nucleosome, which has methylated but deacetylated histones. By analyzing data for H3K9ac following the inhibition of HDACs, we confirmed the key involvement of HDACs in maintaining mitotic NDR pattern at enhancers, by observing acetylated histones at enhancer centers with decreasing signal in surrounding regions.

To explore immediate chromatin structure changes after BRD4 removal, we used an inducible degradation system utilizing dTAG molecules to link target proteins to cells' ubiquitin proteasome machinery. We analyzed ATAC-seq following the dTAG treatment, to identify changes in open chromatin. We found that after 48-hour of degrading BRD4, new chromatin opening/closing sites appear: interestingly, some exposed sites are involved in cell death regulation and increasing cancer incidence. Thus, BRD4 might contribute to maintenance of chromatin structure by keeping regular opening sites exposed for interactions while silencing others.

CHAPTER 1: INTRODUCTION

DNA molecules are wrapped around an octamer of histone proteins to form nucleosomes, which are then packaged to dense complexes called chromatin (Annunziato, 2008) (**Figure 1**). This compacted but organized structure not only reinforces DNA during cell division, but also is involved in regulation of gene expression. As an example, it can promote fast retrieval of the target piece of genetic information; together with regulatory factors, proper dosage of transcripts can be produced to achieve precise control of cellular activities and in-time responses to changes of the environment.

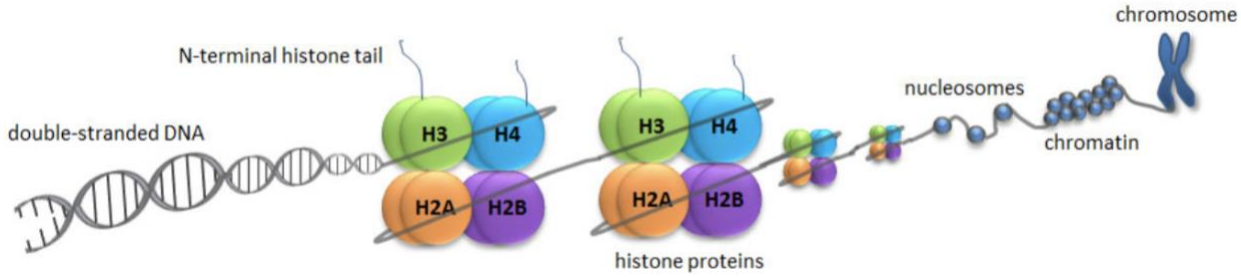

Figure 1: Schematic representation of the DNA molecule packaged to nucleosomes, chromatin, and chromosome. (Reprinted from What is Epigenetics) DNA is represented in grey, and the colored circles represent the octamer of histone proteins.

The central dogma sets the framework of gene expression -- genetic information encoded in DNA is transcribed to RNA, and then translated into proteins. In reality this process is more complicated, and cells integrate multiple regulatory steps to achieve proper levels of protein production. One of the regulatory layers is chromatin accessibility, which is a reversible process that works by changing nucleosome structure and localization. For example, histone acetylation removes the positive charge on the histone proteins, which impairs the strength of the interaction between its N-terminus and DNA. Thus, histone acetylation is involved in the switch of

chromatin from its condensed form to a more relaxed form and enabling increased gene transcription (Gallinari et al., 2007).

Multicellular organisms use a variety of epigenetic mechanisms during cell growth, division and differentiation, in order to achieve precise control of gene expression in different cell types. During mitosis, cells go through breakdown of the nuclear envelope, reduction of transcription levels and condensation of the chromatin (Palozola et al., 2017; Antonin & Neumann, 2016). While the chromatin structure is disrupted during the process, it is necessary to recover the original organization of the chromatin afterwards to maintain cellular activities. Immediately after mitosis, interphase chromatin structure is reestablished and transcription resumes (Belmont and Bruce, 1994). However, how do cells maintain the precisely defined chromatin structure, together with how epigenetic marks help maintain cell identity during the process remain to be explored.

A recent study by the Goren Lab and his colleagues at the Simon Lab (Javasky et al., 2018), proposed that the epigenetic landscape is likely to be preserved during mitosis with few exceptions: global reduction in histone acetylation, increase in histone phosphorylation and deacetylation of the nucleosome that enters the nucleosome depleted region (NDR) of most genes. Specifically, in this study for TSS-associated NDRs, four gene groups were identified based on the NDR pattern of histone modifications H3K4me3, H3K27ac, and H3K9ac (**Figure 2A, 2B**) (Javasky et al., 2018). In particular, Group 1 retains NDR during mitosis; Group 2 loses NDR because of the entry of a nucleosome with only methylated histones; Group 3 loses NDR as well because of entering nucleosome, which contains both methylated and acetylated histones; Group 4 has no NDR at all (**Figure 2B, 2C**). They proposed a model to explain why most genes have deacetylated nucleosome entering NDRs during mitosis: the new nucleosome is formed by

shifting surrounding nucleosomes to NDRs, and HDACs are deacetylating its histones actively during mitosis. This proposed model was supported by treating mitotic cells with the global HDAC inhibitor TSA, where the NDR-occupying nucleosomes are observed to have acetylated histones instead (**Figure 2D, 2E, 2F**).

Inspired by the previous study, in this work our aim is to further investigate the behavior of the corresponding enhancers of the four gene groups.

Enhancers are cis-acting regulatory factors that can be bound by activators to promote transcription (Blackwood & Kadonaga, 1998). Comparing NDR patterns at enhancer centers from interphase to mitosis, we expected to see either enhancers can also be separated into distinguishable clusters based on NDR pattern as promoters did; or no difference among groups, which can potentially be explained by the disruption of long-distance regulation when cells lose 3D organization of chromatin during mitosis. By revealing the nucleosome occupancy of enhancers, we may give a more complete picture of the epigenetic landscape of cells during mitosis. For example, if both the promoters and enhancers for certain genes remain highly accessible during mitosis, we might be able to show the existence of long-distance interactions in cell. We can then further explore their functionalities in reactivation of normal transcription after mitosis. Alternatively, if enhancers are silenced while promoters are active, we might then conclude mitotic cells lack distal regulations from enhancers. We continued to use HeLa-S3 cells as the modeling system and used computational approaches to describe the epigenetic landscape at enhancers in mitotic and interphase cells: 3 histone modifications (H3K4me1, H3K9ac, H3K27ac) are used to capture major chromatin features.

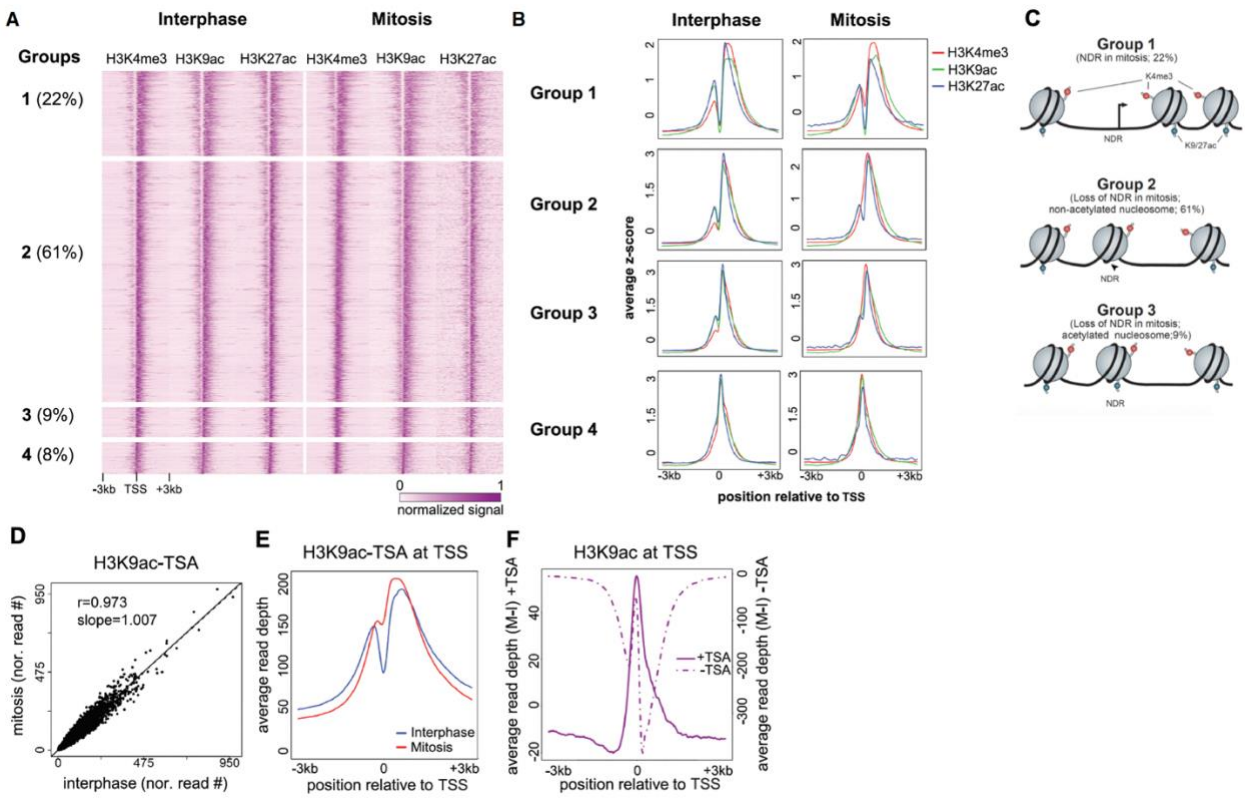

Figure 2: Characterization of NDRs at TSS with or without TSA treatment. (Reprinted and edited from Javasky et al., 2018: A, B, D, E, F correspond to Fig 5A, 5B, 6B, 6C, 6D). (A) Heatmap indicating normalized signal of H3K4me3, H3K9ac, H3K27ac at ± 3kb region near TSS of 5731 genes, divided into 4 groups. (B) Metagene plots indicating average z-score for each modification at ± 3kb regions around TSS for each group. (C) Schematic visualizing the mitotic NDR pattern for group 1-3 observed in (A) and (B). (D) Scatterplot showing normalized read count in mitosis vs. interphase at H3K9ac peaks in TSA-treated cells. (E) Metagene plot for H3K9ac at TSS after TSA treatment. (F) Delta metagene plot for H3K9ac at TSS with (left) or without TSA treatment (right).

Another example of changes in chromatin is the response to a reduction level of a key regulator. Bromodomain-containing protein 4 (BRD4) is involved in organizing super-enhancers (SEs), regulating expression of oncogenes, and maintaining stability of the genome (Donati, Lorenzini, & Ciarrocchi, 2018). Specifically, it interacts with hyperacetylated histone regions on the chromatin and remains on transcriptionally active regulatory elements. Because of its key

functionality, it has been a target for cancer therapy -- inhibition BRD4 represses oncogenes in a cell-specific manner that causes cancer cell death. We aimed to know how removal of this protein could affect chromatin structure immediately in the cell, and how genome accessibility changes correspondingly.

To achieve this goal, we used a HEK293 cell line with BRD4 tagged by FKPB12 that enables induced degradation following treatment with a small molecule (dTAG) (Nabet et al., 2018). This system can perform immediate target-specific protein degradation through the usage of dTAG molecule, which can link the target protein selectively with ubiquitin proteasome machinery in the cell. We used ATAC-seq to capture chromatin accessibility changes.

Overall, we use computational approaches to capture how the genomic chromatin structure is modified in response to various changes in the cell. And our results provide some evidence to explain the decrease of enhancers accessibility during mitosis and the role of BRD4 in maintaining chromatin structure, which set a prelude to reveal the actual underlying mechanisms of those processes.

CHAPTER 2: METHODS

Mitosis Data Accession

For the first part of my work, the ChIP-seq datasets from Javasky et al., 2018 were obtained from NCBI Gene Expression Omnibus (GEO; http://www.mcbi.nlm.nih.gov/geo) under accession number GSE108173. As a summary, HeLa-S3 cells were used as the model system, and samples were either synchronized or unsynchronized with one additional sample treated with TSA. ChIP-seq was performed using monoclonal antibodies of H3K9ac, H3K27ac, H3K4me1. Together we had 4 different histone modifications under different conditions, where each contains two interphase and mitosis samples, and whole cell extract (WCE) in interphase and mitosis were used as the corresponding control groups.

Bioinformatic Analysis of Mitosis Data

Keeping consistency with the analysis done in Javasky et al., 2018, the ChIP-seq reads were aligned to human genome hg19 using Bowtie (Langmead and Salzberg 2012). The duplicated alignments were removed using Picard Tools MarkDuplicates (http://broadinstitute.github.io/picard/). The bam files were filtered and sorted by SAMTools (Li et al., 2009). Finally, if any regions fall into the ENCODE hg19 blacklist, they are removed from analysis.

HOMER was used to make tag directories for all histone modifications and to detect peaks in mitosis and interphase samples separately, where corresponding WCE datasets were used as control (Heinz et al. 2010). Default histone style parameters were used. To maintain consistency with the previous work, the promoter and enhancer list were obtained from Elisheva

Javasky: the former was generated by merging mitotic and interphase H3K4me3, H3K9ac, and H3K27ac ChIP-seq peaks with TSS from UCSC (Karolchik et al. 2004); the latter was generated by merging mitotic and interphase H3K4me1, H3K9ac, and H3K27ac ChIP-Seq peaks with UCSC EP300 HeLa-S3 peaks. An overlap of 500bp was required for merging to occur. For both TSS and enhancer list, we picked the center 100bp reads for each histone modification as its NDR occupancy. We then sorted the 2 lists based on the sum of all histone modifications' interphase NDR occupancy. The division to 4 groups (as in Figure 2A) was done using the percentage given.

To identify enhancers that potentially regulate each group of genes, we used BEDTools slop and intersect functions to find enhancers that are in the vicinity of ±100kb of TSS for each group (Quinlan and Hall 2010). Since some enhancers appear in multiple groups that can obscure the NDR pattern for each group, we excluded those from analysis and kept only enhancers that were unique to one group. As a result, 475, 1012, 142, 123 enhancers were found for the four groups of promoters, respectively.

The signals of all the histone modifications (H3K4me1, H3K9ac, H3K27ac) falling into ± 3kb of enhancer centers, with bin size of 10bp, was generated from HOMER annotatePeaks. By default, the read depth was normalized to 10 million reads. The distance matrix was visualized on heatmaps and metagene plots. The heatmaps were sorted by the sum of NDR occupancy (center 100bp reads) in interphase for all histone modifications, and the depths of each row were normalized to a range of 0 to 1. Each heatmap was generated using heatmap.2 in R {gplots} package. Metagene plots were produced by taking the coverage profile generated by HOMER annotatePeaks and plotted using R base graphics. Delta metagene plot was generated by taking

the difference between the normalized depth (Mitosis-interphase) for each histone mark, and plotted using R.

We also examined the changes of histone modifications level for mitotically enriched genes (MEGs) (Palozola et al., 2017). MEGs were defined as genes with more transcripts observed in mitosis compared to interphase. 466 of the MEGs were expressed in HeLa-S3 cells. We examined the enrichment of MEGs and their enhancers for H3K9ac, H3K27ac, and H3K4me1 by HOMER, using randomly generated genes as control. We plotted scatterplots of mitosis vs. interphase signals as Figure 2C, with linear regression line for each group for better visualization. We also merged MEGs and non-MEGs and attempted using K-means clustering (k=2) to separate MEGs from the other group.

Bioinformatic Analysis of BRD4 Data

The BRD4 degradation experiments were done by Mark Moyer in the Goren lab, where we acquired 5 samples: A. 4-hour dTAG treatment; B. 4-hour DMSO treatment; C. 4-hour untreated; D. 48-hour dTAG treatment; E. 49-hour DMSO treatment. And we had one sample for each different treatment. The raw ATAC-seq data was aligned to the human genome hg38 and processed by the Center for Epigenomics at UCSD.

I started from the metadata that was cleaned and filtered to bam files. HOMER was used to make tag directories and findPeaks. IGV was used to check if real peaks were detected since ATAC-Seq gave broader signals (Thorvaldsdottir et al., 2013). After that, for samples with the same treatment time, BEDTools intersect was used to separate overlapping and unique peaks in each group, and it was also used to find regions overlapping with TSS. In HOMER mergePeaks was used to merge peaks of same treatment time, and getDifferentialPeaks was used to find 4-fold differential peaks that were more expressed in dTAG samples or vice versa.

Specifically, for the 48h treatment, all differential peaks were further filtered using hclust function in R by performing hierarchical clustering. By inspecting the dendrogram, we found 3 clear clusters: one clearly more enriched in DMSO, one more enriched in dTAG, and one has vague patterns. Taking the first 2 clusters, heatmap was generated using data matrix from HOMER annotatePeaks and heatmap.2 function in R. Metagene plots were generated accordingly. For the cleaned-up differential peaks, GREAT was used to perform subsequent Gene Ontology (GO) analysis (McLean et al., 2010).

CHAPTER 3: RESULTS

CHAPTER 3.1: LOSS OF NDR AT ENHANCER CENTER DURING MITOSIS

To address the first question of our interest – to what extent are enhancers involved in mitotic gene expression, we used NDR occupancy for different histone modifications at the center of enhancers as the indicator. Since it was harder to define enhancer regions and to find active enhancers, we first verified if our enhancer list indeed captured ones that had more support of being active. A combination of histone modifications H3K4me1, H3K27ac and H3K9ac was used as marks to support that the enhancer identified are potentially active or primed (Rada-Iglesias, 2018). We observed the presence of NDR at enhancer centers of H3K4me1, H3K9ac, and H3K27ac during interphase as a general pattern (**Figure 3A**): the surrounding regions have relatively higher density of signal, with the lack of signal at center. This was also confirmed by the metagene plots of the top 10% and 20% enhancers with the strongest NDR pattern (**Figure 3B**) and visualization of one typical enhancer (**Figure 3C**). The lack of nucleosome occupancy at enhancer centers in interphase indicates potential transcription factor binding sites, in line with the notion that these enhancers are potentially active.

We next looked at how the enhancers for different gene groups differ. We narrowed the enhancer list down to corresponding unique enhancers for the 4 gene groups in Figure 2A within a distance of ±100kb of TSS. Here in order to reveal main chromatin features at active enhancers, we still focused on the behavior of H3K4me1, H3K9ac, H3K27ac at enhancer center (**Figure 4**). For all 3 histone modifications, we observed that the NDR pattern change from interphase to mitosis is similar when comparing between different groups of enhancers. As a general pattern, despite the general reduction of signal in mitotic cells, all enhancers we observed

mimicked Group 2 in Figure 2A, which lose their NDR in mitosis because of the entry of a nucleosome that is methylated but deacetylated. Overall, we concluded that the NDRs of the enhancers we identified show a consistent pattern in all groups, regardless of the NDRs of the associated TSSs.

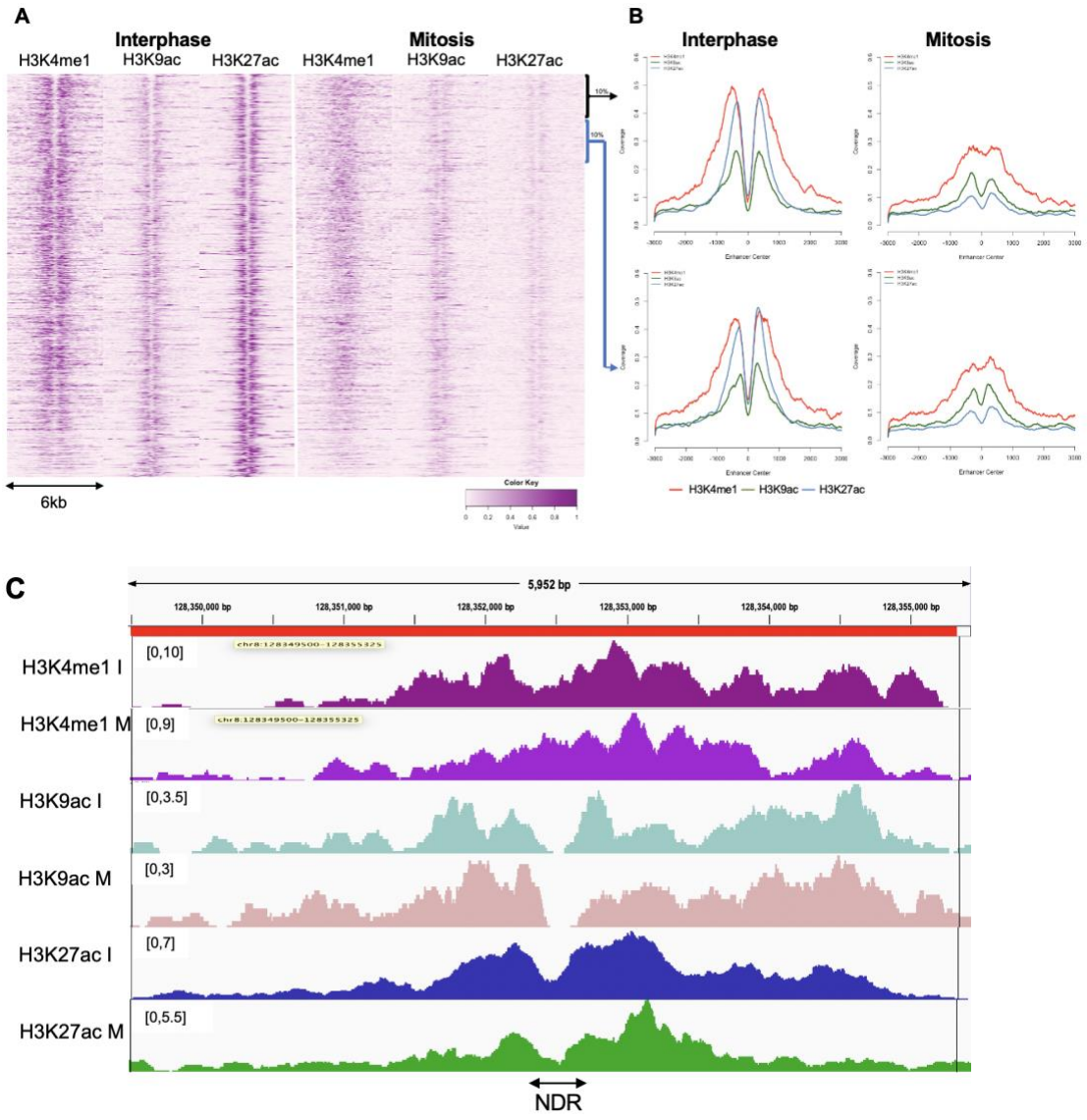

Figure 3: Characteristics of the active enhancers we identified. (A) Heatmap showing normalized signal of H3K4me1, H3K9ac, H3K27ac that were 6kb around enhancer centers in interphase and mitosis samples. A total of 4276 enhancers were ordered based on NDR occupancy from strongest to weakest. (B) Metagene plots were drawn for top 10% and 20% enhancers showing average coverage for each modification at 6kb regions around the center. (C) IGV visualization of one enhancer identified and its NDR pattern in interphase and mitosis.

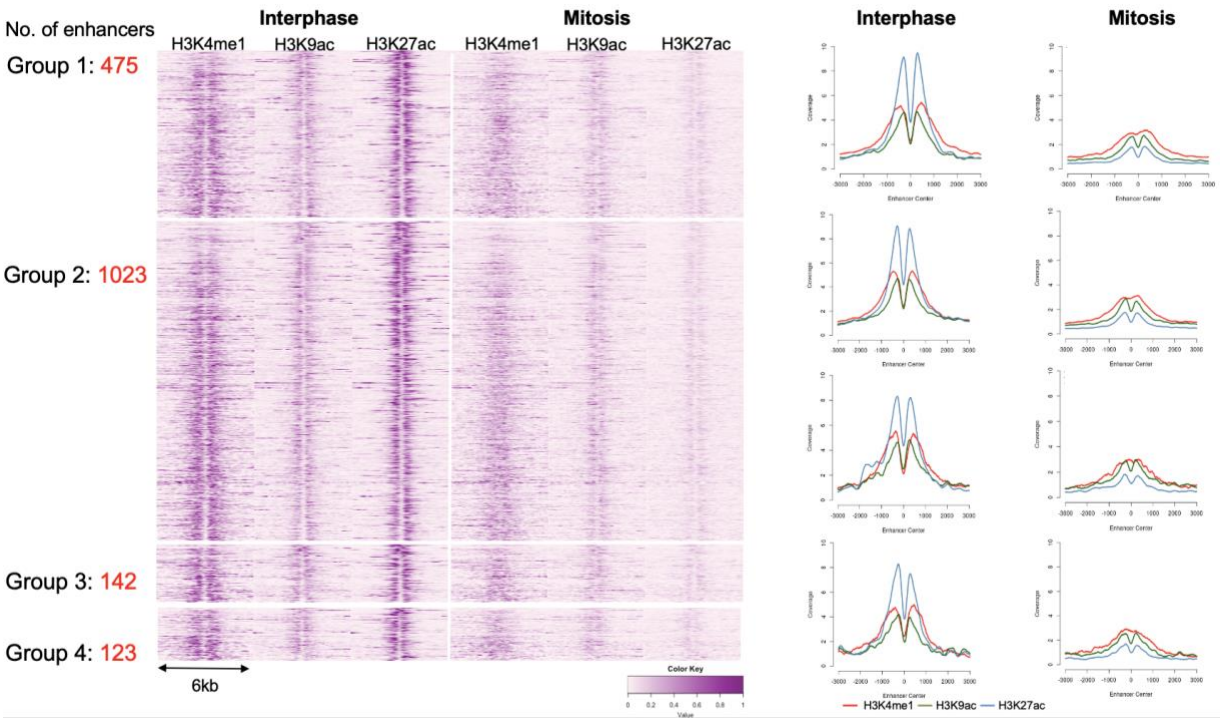

Figure 4: NDR at enhancer centers was lost in mitosis for all enhancer groups. Heatmap and Metagene plots showing normalized signals of H3K4me1, H3K9ac, H3K27ac 6kb around enhancer centers in interphase and mitosis samples. Number of enhancers identified is on the left in red. All enhancers are unique to 1 of the 4 gene groups, 1761 in total.

We observed the disappearance of NDR, showing enhancers are less accessible in mitosis. We also saw the pattern to be similar across all groups, while it is not the case for TSS: it might be explained by the fact that enhancers are cis-regulatory elements, which fail to reach their regulatory sites during mitosis because of chromatin condensation. However, TSSs are more flexible so that in mitosis different sites exhibit differences in accessibility. Our result of loss of NDR at enhancer during mitosis was consistent with previous studies, demonstrating transcription during mitosis while enhancer activity level was low (Palozola et al., 2017). As a side analysis, we examined the histone modifications of 1) mitotically enriched genes (MEGs) in Palozola et al., 2017 that were supposed to have more transcripts in mitosis (HUH7 cell-line) and

2) their corresponding enhancers. However, potentially due to cell line discrepancy (HeLa-S3 vs HUH7), we didn't see MEGs and their enhancers had remarkably different mitosis to interphase histone marks change (H3K4me1, H3K9ac, H3K27ac) compared to random genes as control.

CHAPTER 3.2: HDAC ACTIVITY HELPS TO MAINTAIN MITOTIC NDR PATTERN

After observing the occurrence of deacetylated nucleosomes at NDRs in mitosis, it remains unexplained how the newly entered nucleosome is formed and how it is deacetylated. Therefore, we wanted to test whether our observation upholds the model proposed in Javasky et al., 2018, that the new nucleosome is formed by shifting nearby ones, and the histones are actively deacetylated by HDACs. We reanalyzed the ChIP-seq data of H3K9ac treated with the global HDAC inhibitor TSA at enhancer centers, using the untreated H3K9ac as control. As a result, after inhibiting HDACs in mitotic cells, the TSA-treated group is now acetylated at enhancer center, as opposed to the deacetylated pattern seen in the untreated group (**Figure 5A**). Aiming to reveal how the read depth of surrounding regions near the enhancer center shifts from interphase to mitosis, we used the delta metagene plots to show the changes in reads for both the treated and the untreated group (See Methods). We observed a sharp relative increase of signal for the TSA-treated H3K9ac sample with reduction of signal at the surrounding region (about 750bp near the center); however, the pattern is not observed in the untreated group due to global mitotic deacetylation. (**Figure 5B**) To conclude, our result upholds the model that HDACs activity contributes to deacetylation of the newly entered nucleosome at the enhancer center.

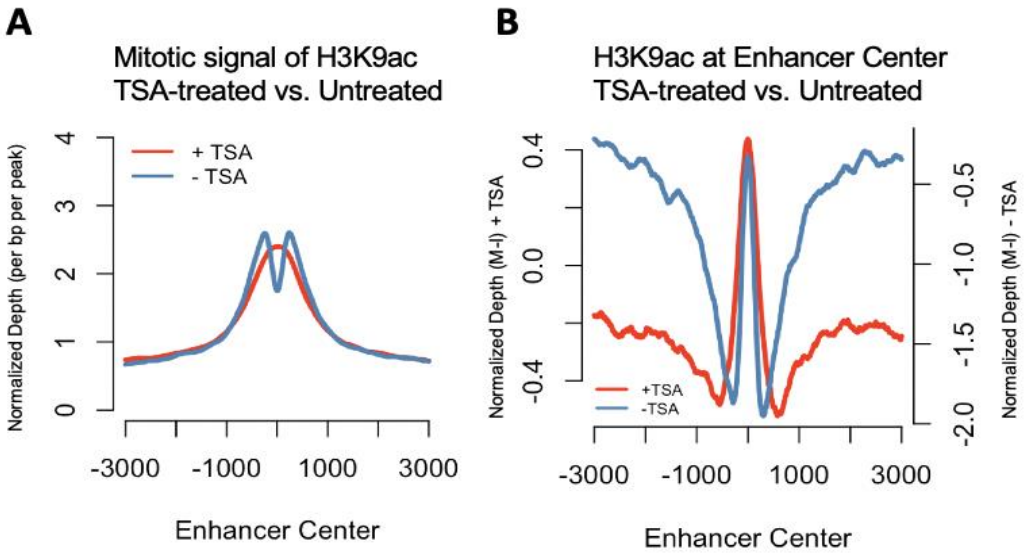

Figure 5: Nearby nucleosome shift and active HDAC deacetylation might explain NDR disappearance. (A) Metagene plot of mitotic signals for H3K9ac with TSA treatment or without TSA treatment. (B) Delta metagene plot showing relative increase of signal at enhancer center for H3K9ac with TSA-treatment (red line, left y axis) coupled with reduction of signal near the center. The pattern is not observed for the untreated group (blue line, right y axis). The relative change of signal is measured by subtracting normalized interphase reads from mitotic reads.

CHAPTER 3.3: CHANGES TO CHROMATIN OPENING FOLLOWING ACUTE REMOVAL OF BRD4

Besides chromatin accessibility change in response to mitosis, we would also like to know how chromatin structure changes when degrading a key protein in interphase, during which cells have normal transcription activities. We found that following the removal of BRD4 protein, the chromatin structure can change in key loci. We performed a preliminary experiment treating cells with dTAG for 4 hours to degrade cellular BRD4, and used ATAC-seq to reveal chromatin changes. Three samples were involved in this experiment: dTAG treatment of 4 hours, DMSO treatment of 4 hours, and untreated. As a result, we confirmed the different treatments give distinctive sets of peaks, which indicate open chromatin regions (**Figure 6A**). However, when we planned to obtain some statistical power by finding differential peaks that are distinctive from common peaks with default P-value < 0.0001, we got very few differential peaks (184 when comparing dTAG to DMSO, 155 for untreated vs dTAG, 62 for untreated vs DMSO). In conclusion, this experiment showed some changes in chromatin accessibility after degrading BRD4 protein using dTAG, however the changes are not significant.

Aiming to capture a more profound effect by degrading BRD4 protein, we extended the treatment time to 48 hours. We had 2 new samples: dTAG treatment of 48 hours and DMSO treatment of 48 hours, and we picked DMSO as control as it is the solvent carrying dTAG. Following the same procedure to find differentially enriched peaks between the groups (p-value < 0.0001), we obtained 2,293 peaks that are more enriched in DMSO treatment, meaning removing BRD4 we lost the accessibility at those openings on the chromatin. Besides, we found 1,530 peaks that are more enriched in dTAG, meaning the treatment reveals some new openings (**Figure 6B, 6C** as examples).

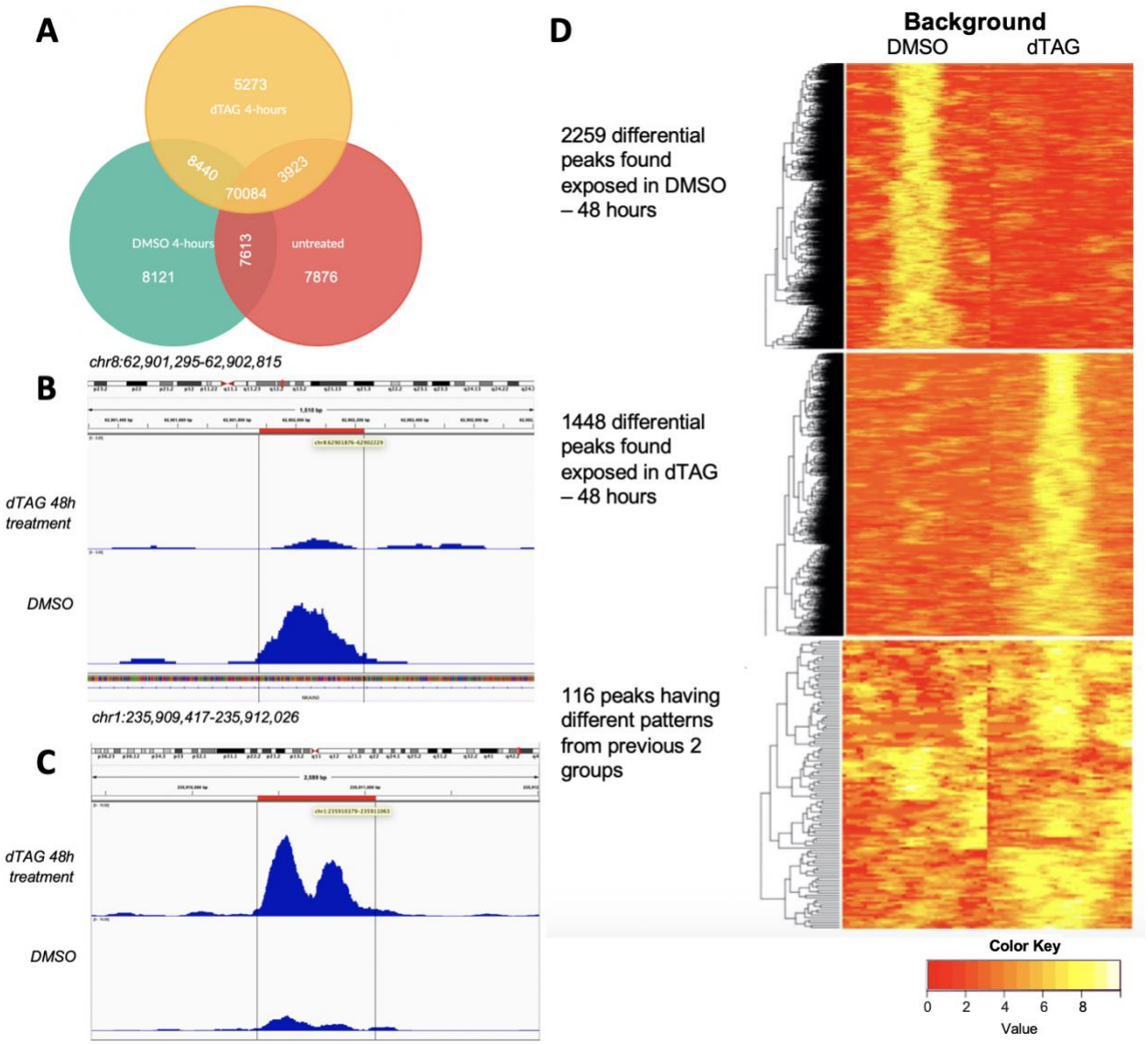

Figure 6: Degradation of BRD4 elicits changes in chromatin openings. (A) Venn diagram represents the overlaps within the 3 peak sets: 4 hours dTAG treatment, DMSO treatment and untreated as control. Numbers indicate the number of peaks. (B) Example of a peak that is less enriched after dTAG 48 hours treatment. (C) Example of a peak that is less enriched after 48h dTAG treatment. (D) Heatmap showing the signals of filtered differential peaks at the center 0.5kb of peak regions, using DMSO tags (left) and dTAG tags (right) as background. Hierarchical clustering was used to separate them into 3 groups.

Hierarchical clustering was done to further clean up the peaks into differential peaks highly accessible in DMSO treatment, in dTAG, and ones having unclear pattern (**Figure 6D**).

To summarize, our clustering and grouping results provided two sets of genes that are differentially exposed. In addition, we examined whether there exist similarities between peak sets of the same treatment with different treatment time (4 hours vs 48 hours). However, potentially due to the small number of differential peaks found in 4-hour treatment in general, we did not observe high enrichment of 4-hour treatment peaks at the center of differential peaks found highly enriched in the corresponding 48-hour treatment in both dTAG and DMSO.

To explore how the two sets of differentially expressed peaks differ in functionality, we checked the TSS distribution of the two groups of differential peaks. However, we obtained very similar ratio of TSS as a fraction of the total number of peaks (171 for DMSO, 142 for dTAG), showing that after dTAG treatment, locations on chromatin that are newly exposed or lost did not differ significantly in terms of TSS availability.

Finally, we performed GO analysis using GREAT (McLean et al., 2010), which provided interesting results: a number of peaks occur after removing BRD4 protein are involved in GDP binding and RNA polymerase II core promoter sequence-specific DNA binding, and stress-induced cell death. This analysis also identified multiple linked abnormal mouse phenotypes, such as increased testis tumor incidence, abnormal fetal cardiomyocyte proliferation. These somewhat matched disorders related to BRD4 gene in humans, like nut midline carcinoma (GeneCards) or other cancers (Donati et al., 2018). Our results provide initial insight on the role that BRD4 plays in regulation of gene expression, and by acute removal of this protein we can further study its impact on chromatin structure.

CHAPTER 4: DISCUSSION

In this study, we aimed to explore chromatin accessibility when disrupting normal chromatin structures present in interphase: mitosis or degradation of a protein that has an important role in the transcriptional machinery. We used bioinformatic approaches to reveal the enhancers' behavior before and after mitosis, and the exposure sites variations after degrading BRD4 with dTAG.

We first found that NDR pattern of enhancers do not exhibit distinct clusters as the genes they are potentially regulating. As a general pattern, NDRs disappear during mitosis for all enhancers, which can be explained by the entrance of a nucleosome that has methylated but deacetylated histones. This observation of general lack of accessibility of enhancers (demonstrated by an occupied NDR) is consistent with previous studies (Palozola et al., 2017), where they found long-distance interactions across the genome are notably reduced in mitosis: mitotic eRNA level is significantly lower than its asynchronous counterpart, and it recovers in early mitotic exit. This observation also fits with Dekker group's finding that the highly compartmentalized 3-D organization of chromatin in interphase disappears when transit to mitosis. (Naumova et al., 2013) The breakdown of the 3-D organization could mean that enhancers are not required to regulate their target promoters during mitosis.

We then explored where the nucleosome came from and how it was deacetylated, and the result exhibited that after inhibiting HDAC activity, acetylated histones are observed at the enhancer region coupled with a decrease in reads at surrounding regions. Our result extended what was seen in TSS (Javasky et al., 2018) to enhancers, and upheld the model they proposed to explain the disappearance of NDR.

Based on all observations we had for enhancer activity during mitosis, our result provides some evidence for the idea that the expression of some genes in mitosis could be controlled by a subset (of different) mechanisms from the ones utilized during interphase. In literature, several different models are proposed to explain the reduction of transcription level in mitosis, and one is that in general proximal promoters are more accessible, but not distal regulatory elements. (Hsiung et al., 2015) Even though the enhancer-promoter loops are disassembled, a low level of transcription is maintained because transcription factors remain at promoter or bound to chromatin (Palozola, Lerner, & Zaret, 2019). Overall, our findings supported the idea that long distance regulations by enhancers is silenced during mitosis, while at promoters some chromatin accessibility is retained, and low level of gene expression exists.

For the second part of the analysis, we concluded that the rapid degradation of BRD4 protein might shift part of chromatin structure -- by looking at the GO analysis results, removal of BRD4 causes a lot of sites that are normally not accessible to be exposed, such as genes functioning in stress-induced cell death, which matches the role of BRD4 in gene stability (Donati et al., 2018). With the absence of BRD4, those sites can be wrongly targeted to cause irregularities in gene expression. This analysis provided some insights to what locations on the chromatin are exposed or disappear, however how precisely BRD4 degradation causes 3D chromatin structure change and whether the original structure can be recovered or not, require further analysis.

There are some limitations to our current analysis, for example, it is technically difficult to identify with high confidence the particular enhancers that regulate the gene groups of our interest, and it is possible that some enhancers we found in proximity are not the true enhancers or regulate a different gene. Another difficulty we faced is that unfortunately due to Covid-19,

we didn't perform proposed experimental verifications, which is the inhibition of one specific histone deacetylase HDAC3. Since HDAC3 remains on chromatin during all stages of mitosis, we proposed it might regulate mitotic histone. By inhibiting HDAC3, we might reveal clearer patterns of mitotic acetylation level, instead of inhibiting all HDAC in a global manner, and we will perform the experiment in the future as a verification. In addition, potential future work could involve studying whether mitotic expressed genes, which are actively transcribed during mitosis (Palozola et al., 2017) are regulated by TFs that reside in the promoters whereas the silent one is regulated only by distal TFs.

For the analysis of BRD4 degradation, because of time constraints we did not have biological replicates and that limited our ability to use several methods to conclude with statistical power that before and after the dTAG treatment the peaks differ significantly. But as a preliminary result, we showed that BRD4 degradation led to various potential changes and adding biological replicates can be a future direction to give some robustness of our results. Also, as we have shown, the treatment time might affect the exposed regions, so in the future it might be worthwhile to explore the optimal treatment time that we can capture observable chromatin structure changes while not disrupting the cellular activity excessively. As another future direction, knowing this protein interacts heavily with hyperacetylated histone regions, we can also perform ChIP-seq experiments with a few histone acetylation marks to reveal the state of chromatin before and after dTAG treatment.

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