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RESEARCH ARTICLE

The Role of Epigenetics in Gene Regulation: A Study of Histone Modifications

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ABSTRACT: Epigenetics, the study of genetic changes to chromatin that can impact gene expression, has emerged as a significant component of understanding cellular behavior and disease development. This work delves into the relationship between histone modifications and gene expression to explore the impact of epigenetic changes on gene behavior. Modification of Histone proteins in chromatin modifies the binding of histones to DNA. This influences gene accessibility to proteins such as RNA polymerase, thereby regulating gene expression levels. Histone modifications influence cellular behavior and play a critical role in biological processes, such as memory formation and cancer development. This paper explores the typical patterns of various histone modifications across genes, investigating how they interact and are related to gene expression. By examining these relationships, this study aims to understand the significant role of epigenetics in modulating gene behavior and its broader implications in biological systems. Epigenetics Research is crucial for developing drugs and understanding the inheritance of gene expression patterns in daughter cells and developmental biology. The latter deals with cellular differentiation and tissue-specific gene expression. This research examines the interplay between histone modifications and gene expression.

KEYWORDS: Animal Sciences, Genetics, Chromatin, DNA, Histone Modifications.

Introduction

Inheritance has long been related to the transmission of genetic information encoded within the DNA strands passed down from one generation of living organisms to the next. However, recent advancements in genetics have uncovered another layer of complexity in hereditary processes: epigenetics.[1] DNA sequence and the detailed assembly of DNA and proteins within the nucleus are crucial mediators of cellular behavior and inheritance. Recent studies have revealed that epigenetic modifications to chromatin also play a critical role in this regard.[2-4] These modifications, including changes to histone proteins, influence gene expression and affect basic yet important biological processes ranging from memory formation to developing diseases like cancer.

At the core of chromatin structure lies histone proteins, which are large biomolecules that DNA is wound around DNA forming nucleosomes. The modification of histones affects the accessibility of DNA, thus regulating gene expression levels. Histones can either promote or hinder the binding of regulatory proteins, such as the transcription factor TFIID, thereby regulating and modulating gene activity within the cell.

This research project focuses on the detailed interplay between histone modifications and gene expression, aiming to explain the underlying mechanisms of epigenetic regulation. By analyzing the positions and levels of histone modifications across genes and relating them to levels of transcription factors, RNA Polymerase, and nucleosome levels, this paper aims to explore the impact of histone modifications on transcription in active and inactive genes. In this work, typical patterns of various histone modifications across genes can be seen to unravel the complex and detailed web of epigenetics.[5] Central to this question is exploring how histone modifications correlate with gene expression profiles. By deciphering whether specific histone marks are associated with active or repressed genes, this paper aims to gain insights into the relevance of epigenetic modifications in shaping gene behavior and cellular processes. This study aims to reevaluate established research of transcriptional regulation by integrating ChIP-seq, MNase-seq, and NET-seq datasets within a unified metagene framework in yeast, *Saccharomyces cerevisiae*. The primary focus is to systematically examine how specific histone modifications like H3K4 methylation, H3 and H4 acetylation, and H2B ubiquitination relate to nucleosome positioning, transcription factor occupancy, and new RNA synthesis at and around the transcription start site (TSS). This work reexamines their interdependence by aligning and correlating transcription across thousands of genes. Through this approach, the study refines current understanding of how chromatin structure and chemical histone modifications such as methylations, acetylation, and ubiquitination dynamically interact to regulate transcription initiation and elongation.

The remainder of this paper is organized as follows. In this Section, the background and layout of the scope of our study are briefly described. Section 3 describes the methodology used to analyze the data sets and examine the role of histone modifications on gene expression. Section 4 describes the results obtained from our study, followed by a discussion of key findings in Section 5. Finally, the paper concludes with a summary of the study and areas and aspects that need further exploration.

Background:

Epigenetics refers to heritable changes in gene function that do not involve alterations to the underlying DNA sequence.

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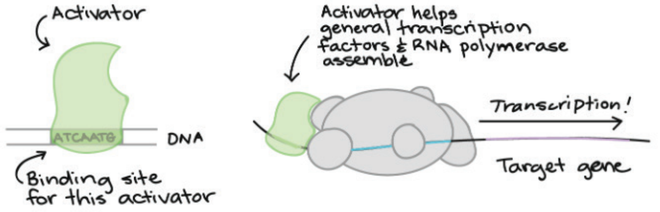

Figure 1: An activator, such as TFIID, a transcription factor that promotes transcriptional initiation, binds to a target DNA sequence. The subsequent binding of RNA polymerase and transcription factors to the activator initiates transcription. Transcription activators, as seen in Figure 1, are also impacted by histone modifications.

The N-terminus tails of histones undergo various chemical modifications, including methylation, acetylation, phosphorylation, and ubiquitination. These modifications alter the chromatin structure, influencing how tightly or loosely the DNA is packaged around histones. In turn, these histone modifications can regulate access to DNA by affecting the levels and/or types of transcription factors, repressors, and RNA polymerase present, subsequently changing levels of gene expression.

Various studies[1-3] have identified specific patterns of histone modifications that correlate with distinct gene activities in the context of transcriptional regulation for specific histone modifications. For example, histone acetylation is generally associated with open chromatin and active gene transcription. In contrast, histone methylation at certain lysine residues can mark either active or repressive chromatin states depending on the context.

The Transcription Start Site (TSS) in genes is where RNA polymerase binds to DNA to initiate transcription. Different histone modifications in the vicinity of the TSS affect chromatin structure, therefore regulating the accessibility of DNA to RNA polymerase. All histones show a common trend in affecting chromatin structure and regulating transcription. Histone modifications such as the promoter-associated H3K4me3 and the gene-body-enriched H3K36me3 show distinct positional patterns around the TSS that either promote or repress transcription.

Goal and Scope of the Present Study:

This study explores the role of histone modifications on gene expression using the yeast genome *Saccharomyces cerevisiae* as an example.[6] Though the study is limited to examining yeast, patterns of how histone modifications affect gene expression in this species represent how histones affect gene expression in other organisms as well. To limit the scope of the study and focus on understanding the fundamental role of histone modifications, the exploration is confined to certain histones and histone modification types. Histones considered in this study include H2, H3, H4, and H2B. Further, the exploration is limited to methylation, acetylation, and ubiquitination-based histone modifications.

■ Materials

This study draws on various datasets relating to the yeast genome *Saccharomyces cerevisiae*, which typically contains 6275 genes made available courtesy of the Cambridge Center of International Research. In this study, ChIP-seq data sets (Chromatin Immunoprecipitation Sequencing)* are used to identify regions of the genome enriched with specific histone modifications.[6] MNase-seq data (Micrococcal Nuclease Sequencing)* is used to assess nucleosome positioning and chromatin accessibility. By analyzing MNase digestion patterns, the influence of histone modifications on chromatin structure and DNA accessibility is explored, thereby impacting gene expression. MNase preferentially cuts exposed linker DNA but is blocked by tightly positioned nucleosomes. By analyzing MNase digestion patterns, we can determine where nucleosomes are positioned, how strongly they are packed, and how accessible the underlying DNA is. This is important because histone modifications often shift nucleosome positioning or stability, thereby altering chromatin accessibility and influencing whether transcription factors and RNA polymerase can access the DNA to initiate transcription.[7] This is repeated across the genome-wide dataset of histone modifications computationally. NET-seq data (Native Elongating Transcript Sequencing)* shows the level of RNA polymerase occupancy across genes, providing insights into active transcriptional activity.[8] This data is then analyzed in conjunction with histone modification profiles obtained from plotting histone levels across the gene body, and the correlation of specific modifications with transcription initiation, elongation, and termination processes is examined. This is carried out across the set of histone modifications considered.*

*Courtesy of the Cambridge Center of International Research (CCIR)

■ Methods

The analysis of the datasets and the analytical methods was completed as follows. First, a meta-gene data sequence of 5,331 functional genes in the S. cerevisiae yeast genome was created. Meta-genes embody a group of genes that refer to a collective gene expression analysis of histone-modification, MNase-seq, and NET-seq datasets, which was performed across a set of genes. They are used to summarize overall trends in transcriptional activity. The meta-genes data sequences were created by grouping genes exhibiting similar patterns of histone-modification enrichment profiles across genes and nucleosome levels.

Next, levels for each histone modification were plotted across the meta-gene. For each histone modification, 'average histone modification' refers to the mean ChIP-seq amount for that histone modification across all genes in the meta-gene. Gene bodies were divided into normalized bins relative to the TSS, and the signal in each bin was averaged across all genes of the same histone modification type to produce a quantitative meta-gene profile. For example, for the histone H3 lysine 4 methylation modification (H3K4me), three methylation states, monomethylation (H3K4me1), dimethylation (H3K4me2), and trimethylation (H3K4me3) were measured. The 'levels' of each modification refer to the ChIP-seq signal intensity associated with that modification at each genomic position. To generate an aggregate H3K4me profile across the meta-gene, the ChIP-seq signal intensities for H3K4me1, H3K4me2, and H3K4me3 were averaged along the gene body. Histone modification levels were computed and plotted relative to the transcription start site (TSS) because the TSS marks the region where transcription is initiated. By analyzing the positional patterns of multiple histone modifications near the TSS, we can assess how activating and repressive marks correlate with transcriptional activity, including promoter accessibility and the recruitment of transcription factors and RNA Polymerase, which aid in the initiation of transcription. This approach allows us to specifically explore how different histone modifications influence gene expression at the promoter and early gene body regions.

MNase-seq (Micrococcal Nuclease Sequencing), ChIP-seq (Chromatin Immunoprecipitation Sequencing), and NET-seq (Native Elongating Transcript Sequencing) data were then analyzed across the meta-gene to assess nucleosome positioning, histone modification levels, and transcriptional activity, respectively. ChIP-seq datasets for the S. cerevisiae genome were used to obtain amounts of transcription factors across genes. MNase-seq data were used to assess nucleosome positioning and chromatin accessibility. By analyzing MNase digestion patterns, the influence of histone modifications on chromatin structure and DNA accessibility was explored, thereby affecting gene expression. MNase-seq uses micrococcal nuclease to preferentially digest exposed DNA between nucleosomes, while DNA wrapped around nucleosomes is protected. By closely observing MNase digestion patterns, nucleosome positions and occupancy can be mapped across the genome. Since histone modifications can alter nucleosome stability and positioning, these patterns reveal how specific modifications influence chromatin accessibility. Chromatin accessibility, in turn, affects the ability of transcription factors and the transcriptional machinery to engage DNA, thereby impacting gene expression. Thus, MNase digestion patterns provide a quantitative measure of how histone modifications modulate chromatin structure and regulate transcriptional activity. NET-seq data were used to measure nascent RNA associated with RNA polymerase II, providing maps of transcription across genes. This helps determine where transcription is actively initiating, how RNA polymerase progresses along the gene body, and where elongation may pause or terminate, thereby linking specific histone modification patterns to transcriptional activity. In addition to the meta-gene analyses, a genome-wide correlation plot was generated comparing H3K4me3 ChIP-seq signal to NET-seq nascent transcription levels. For this analysis, gene-body averaged H3K4me3 ChIP-seq values were computed for all genes and plotted against their corresponding NET-seq read densities. The resulting scatter plot provided a quantitative metric of the relationship between histone modification abundance and transcriptional output. This correlation analysis was used to validate whether positional enrichment patterns observed in the meta-gene profiles translated into genome-wide transcriptional behavior. The inclusion of this correlation plot in our analysis allowed us to directly test whether histone modification levels were predictive of transcriptional activity. By quantifying the association between H3K4me3 enrichment and RNA polymerase engagement, this method complemented the meta-gene-based approach and strengthened the overall interpretation that histone modifications actively contribute to transcriptional regulation. NET-seq data was also used to be analyzed in conjunction with histone modification profiles obtained in Step 1 to obtain a correlation of specific histone modifications with transcription initiation, elongation, and termination processes.

Correlation patterns between histone modification plots and MNase-seq, ChIP-seq, and NET-seq data plots were analyzed to find the relationship between transcription and histone modifications. In addition, the levels of various histone modifications across the genome were analyzed, which revealed the characteristic positional patterns of activating and repressive marks such as acetylation (e.g., H2AK5ac, H4AK8) and methylation (e.g., H3K4me1/2/3, H3K36me3) across gene promoters and gene bodies. These patterns reflect how specific chemical groups attached to histone tails are distributed along the genome and differ among the core histones (H2A, H2B, H3, and H4) that form the nucleosome. By examining these modification profiles, we identified how each histone mark varies relative to others and how their enrichment aligns with transcriptionally active or inactive chromatin regions.

Finally, correlations between patterns of histone modification levels and levels of transcription factors, RNA polymerase, and nucleosomes were established. These trends were compared against similar known trends reported in the literature that link gene expression to histone modifications.[4,20,25]

■ Results

In this section, the results of the analysis of histone modification data are presented, and their correlation to transcription is analyzed.

1. MNase-seq Data:

Figure 2 shows the nucleosome level across genes with high and low transcription in the constructed meta-gene of the *S. cerevisiae* genome. Genes with high transcription were used for metabolic processes, specifically in synthesizing enzymes that catabolize glucose, whereas inactive genes were used by the yeast far less often and are used only in rare, specific conditions. The plot shows active genes have a large deviation in nucleosome levels before the TSS, indicating the DNA is available for transcription. In contrast, more chromatin is present before the TSS in inactive genes, decreasing the likelihood that RNA polymerase can bind and initiate transcription.

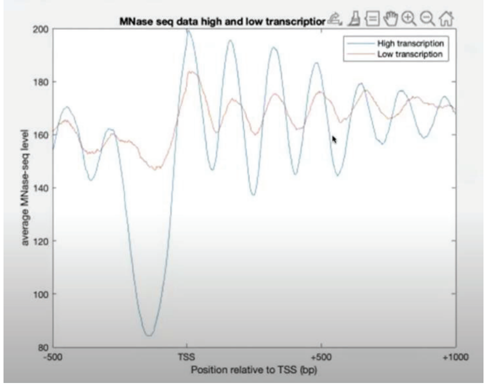

Figure 2: A plot of MNase-sequence data shows the nucleosome level across high-transcription and low-transcription genes. Active genes exhibit a sharp decline in nucleosome levels near the TSS, allowing RNA polymerase to bind and initiate transcription, whereas inactive genes have more nucleosomes before the TSS, which prevents transcription.

2. Net-Seq Data:

The Net-seq data plotted below shows the position at which RNA polymerase actively elongates and the density of RNA polymerase enzymes across the gene body (Figure 3). The position of RNA polymerase is primarily found at the TSS, where it initiates transcription, as indicated by the peak. RNA transcription then terminates at the end of the gene location, as evidenced by the significantly reduced density of RNA Polymerase near the gene index 1000 relative to the TSS, thus showing RNA Polymerase is not active in this position of the gene.

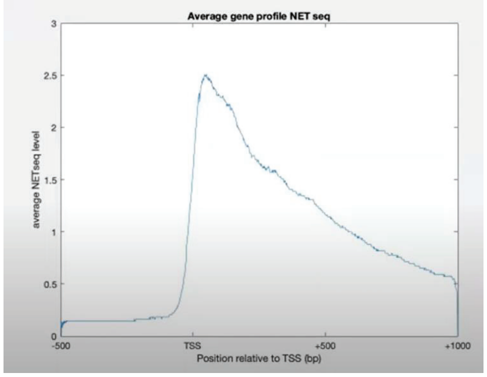

Figure 3: The Net-seq data show the locations of RNA polymerase enzymes across the gene body. Figure 3 shows that RNA polymerase levels peak at the TSS, where transcription begins, and decrease near the termination site, indicating the start and end of transcription.

3. ChIP-seq Data:

Figure 4 presents the ChIP-seq data analyzed for the S. cerevisiae genome, illustrating the distribution of transcription factors across gene bodies relative to the transcription start site (TSS). The figure reveals a pronounced enrichment of transcription factors near the TSS, indicating a high amount of transcription factors at this position. Histone modifications enriched at the TSS help recruit transcription factors, which, as seen in this figure, are found at the TSS, facilitating the initiation of transcription.

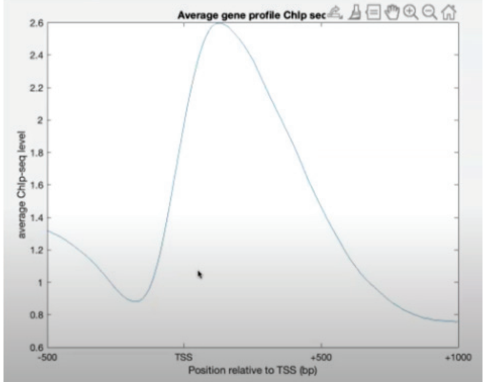

Figure 4: ChIP-seq data show transcription factors' locations across the gene body relative to the TSS. Transcription factors accumulate near the TSS, indicating their role in initiating transcription, as seen in Figure 4.

4. Average Level of H3K4 Methylation Across Genes:

This plot shows the level of H3K4 methylation across genes, including averaged levels across the H3K4me1, H3K4me2, and H3K4me3 modifications. H3K4 Methylation is associated with the transcription of genes and is therefore seen peaking at the TSS of actively transcribed genes. The dip observed just before the TSS corresponds to a H3K4 methylation-depleted region, where chromatin is open to allow transcriptional initiators and factors to access DNA, making it easier for RNA polymerase to bind to the DNA strand. In other words, the dip in H3K4 methylation indicates that fewer H3K4 methylation modifications are available, making chromatin less dense and signifying open chromatin. This histone modification also recruits transcription factors that aid in transcriptional initiation. H3K4 methylation peaks at the TSS, promoting transcription by facilitating open chromatin and recruiting transcription factors such as TFIID and CHD1, which bind to the methylated histone tail and help assemble the transcriptional initiation at the TSS.[19]

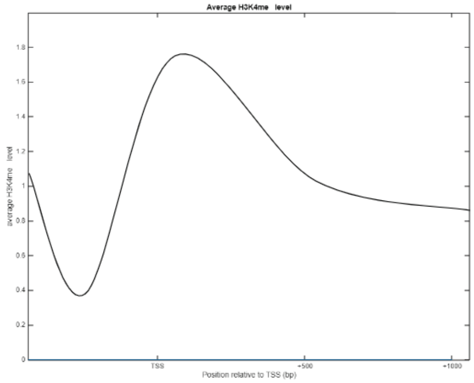

Figure 5: Average level of H3K4 methylation across the gene body relative to the TSS.

5. Average Level of H2AK5 Acetylation across genes:

Figure 6 displays the average levels of H2AK5 acetylation across genes, aligned relative to the transcription start site (TSS). The data show a clear peak of H2AK5 acetylation at the TSS, indicating that this modification is most abundant at the TSS of actively transcribed genes. This pattern suggests that H2AK5ac is associated with transcription initiation, consistent with its known role in loosening chromatin structure to facilitate access by the transcriptional factors and RNA polymerase,

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as can be seen by the dip before the TSS. In the context of this study, the higher level of H2AK5ac near the TSS supports the broader finding that active histone modifications mark open chromatin regions, promoting gene expression.

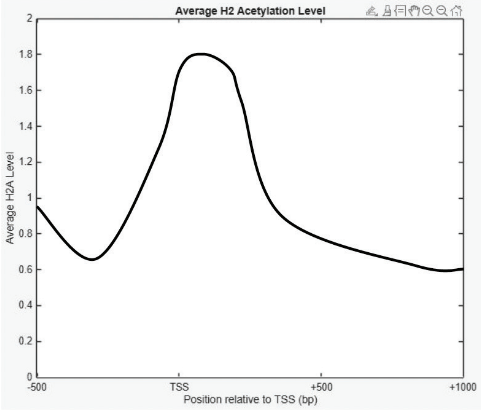

Figure 6: The level of H2AK5 acetylation across the gene body relative to TSS. H2AK5ac levels peak sharply at the TSS and decrease across the gene body.

6. Average Level of H3 Methylation Across Genes:

Figure 7 shows the level of other H3 methylations across genes. In this case, the levels do not peak at the TSS and are rather distributed across the gene body. H3 methylations promote transcription by stabilizing RNA polymerase II during elongation and by loosening nucleosomes, creating an open chromatin structure. This modification reduces nucleosome density and enhances chromatin accessibility, allowing RNA polymerase to move more efficiently along the DNA strand. Transcription is also facilitated by recruiting other transcription factors such as the FACT complex and Spt6, which interact with H3 methylation to reorganize nucleosomes and allow RNA polymerase II to move smoothly along the DNA strand.[20] that help make RNA polymerase move smoothly along the DNA strand. The plot shows the H3 methylation levels are distributed along the gene body, thereby promoting the recruitment of transcription factors that aid in the movement of RNA polymerase and facilitate transcription.

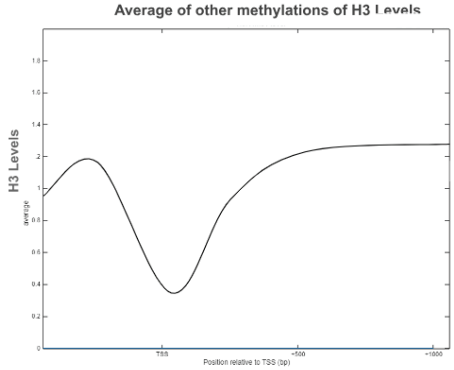

Figure 7: The average levels of H3 methylations across the gene body relative to the TSS. Histone 3 methylations are distributed along the gene body, which promotes transcription elongation by facilitating RNA polymerase movement.

7. Average Level of H3 Acetylation Across Genes:

Histone 3 acetylation peaks at the TSS of actively transcribed genes. A slight dip just upstream of the TSS corresponds to a histone-depleted region, where fewer histones are present, indicating a more open chromatin structure that allows transcription factors and RNA polymerase II to access the DNA. The high level of H3 acetylation at the TSS is consistent with its known role in reducing histone-DNA interactions, thereby loosening chromatin structure and facilitating transcription initiation by allowing RNA polymerase and transcription factors such as TFIID to easily initiate transcription and bind to the TSS region of the gene.

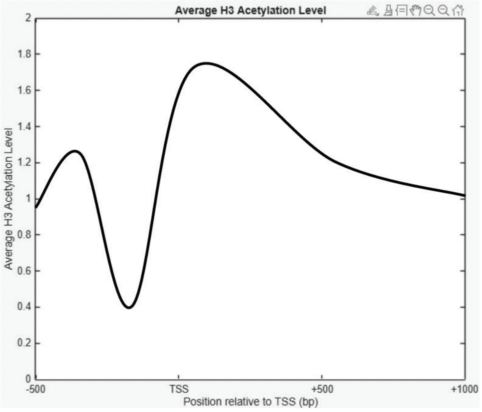

Figure 8: The Average histone 3 acetylations across the gene body relative to the TSS. Histone 3 acetylation peaks at the TSS, promoting transcription initiation by keeping an open chromatin structure, as seen in Figure 8.

8. Average Level of H4 Acetylation Across Genes:

Figure 9 depicts the level of H4 acetylation, which peaks at the TSS of actively transcribed genes. The dip before the TSS suggests an open chromatin structure, allowing RNA polymerase to bind easily to the DNA strand. H4 acetylation peaks at the TSS of actively transcribed genes, marking nucleosomes at the promoter. In addition, this modification recruits bromodomain-containing chromatin remodeling complexes, such as SWI/SNF, directly to the acetylated histone tails at the TSS and nearby nucleosomes.[21] These remodelers reposition nucleosomes downstream of the TSS, facilitating not only transcription initiation but also efficient elongation by RNA polymerase II along the gene body. Histone 4 acetylation peaks at the TSS, promoting transcription initiation by increasing DNA accessibility.

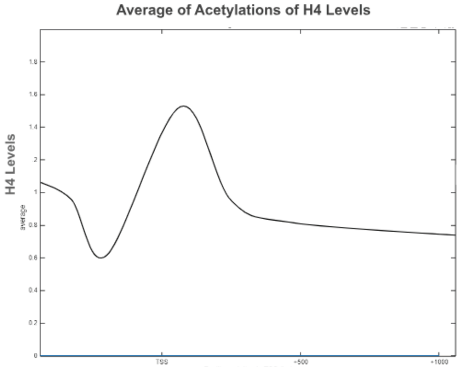

Figure 9: The Average level of H4 acetylation across the gene body relative to the TSS. Figure 9 shows H4 acetylation peaks at the TSS, promoting transcription initiation by increasing DNA accessibility.

9. Average Level of H2B123UB Across Genes:

The plot in Figure 10 shows the level of H2B123UB. It is seen to peak at the TSS, which is known to help with transcriptional elongation of RNA, thereby allowing RNA polymerase to move smoothly across the gene body. In addition, H2B123UB participates in histone crosstalk; in other words, the ubiquitination of H2B influences the methylation of adjacent histone tails (notably H3K4 and H3K79), thereby creating a chromatin environment more permissive for transcription. For example, ubiquitinated H2B has been shown to stimulate the activity of H3K4 methylation, which in turn creates binding sites for transcriptional regulators.[22,23] This histone modification also recruits transcriptional repressors in inactive genes.

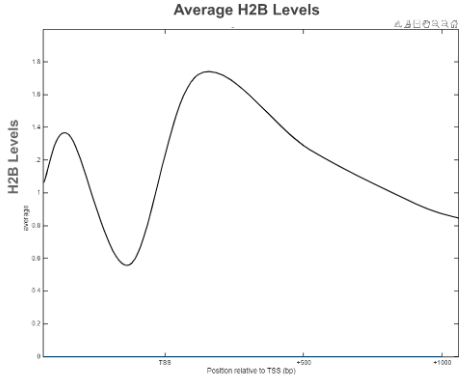

Figure 10: The level of histone H2B123Ub across the gene body relative to the TSS. H2B123Ub supports transcription elongation in active genes but also plays a role in gene repression when present in inactive genes, as seen in Figure 10.

10. Average Level of H3K9 and H3K27 Methylation Across Genes:

This plot shows the average amount of H3K9 and H3K27 methylation across genes relative to the TSS and transcription termination site (TTS). In actively transcribed genes, H3K9me and H3K27me levels are low at the TSS and gradually increase toward the TTS, reflecting their minor role in promoting the transcription of active genes. Instead, these modifications are associated with gene repression because they recruit chromatin-compacting proteins across the gene body that restrict access of RNA polymerase II and transcription factors to DNA.[2] In contrast, in inactive genes, H3K9me and H3K27me amounts are high upstream of the TSS, where their presence contributes to chromatin compaction and prevents transcription initiation, further repressing gene expression.[3]

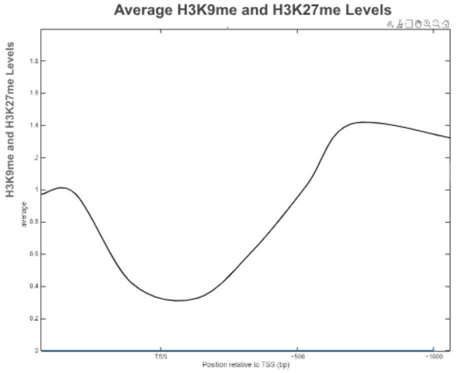

Figure 11: Average level of H3K9 and H3K27 methylations across the gene body. H3K9 and H3K27 methylations repress transcription by compacting chromatin and obstructing RNA polymerase binding.

11. ChIP-seq Data Across Active and Inactive Genes:

Figure 12 shows the enrichment of transcription factors across genes relative to the TSS in both high and low transcription genes. In active genes, general transcription factors such as TFIID, TBP (TATA-binding protein), and Spt6, along with elongation-associated factors like Spt16 (part of FACT) and Paf1, peak shortly downstream of the TSS. These factors facilitate transcription initiation and elongation by helping RNA polymerase II bind to promoters, stabilize its passage along nucleosomes, and coordinate chromatin remodeling. In contrast, in inactive genes, repressor proteins such as Ssn6-Tup1 or chromatin-bound histone readers may occupy similar regions, blocking RNA polymerase binding or impeding elongation. The varying binding of transcription factors in active versus inactive genes demonstrates how transcription factor recruitment regulates gene expression. In yeast, this ensures that only genes needed under specific conditions are expressed, while in higher eukaryotes, similar mechanisms allow precise control of gene transcription, contributing to cellular differentiation and response to environmental signals.

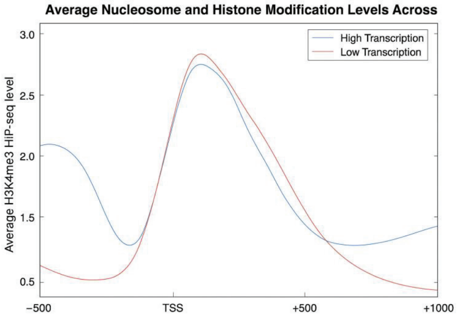

Figure 12: The ChIP-seq data show the locations of transcription factors across the gene body for genes with high and low transcription relative to the TSS. Transcription factors peak downstream of the TSS in highly transcribed genes, supporting RNA polymerase II elongation, while in lowly transcribed genes, factors are enriched near the promoter or upstream regions, consistent with repression of transcription initiation.

12. Histone Density Variation Across Genes:

In this study, the variation of levels of histone modifications was examined across the genome. Specifically, subsets of genes exhibiting high and low levels of histone were identified for each of the histone modifications. This was accomplished using two-way clustering of histone levels for each modification type using the K-median clustering algorithm.[31] A 1500 base-pair segment around the TSS was split into 30 equally-spaced intervals, and the mean histone level (density) was computed across these intervals. In the clustering scheme, the histone density over these 30 intervals was chosen to be the feature set, and the density vectors across the genes are the observations.

Figure 13 shows a high and low-histone level clustering for the H3K4me3 Histone modification, which is known to promote gene transcription. The plot shows more pronounced peaking of the histone level around the TSS for Cluster 1 (high-level) compared to Cluster 2 (low-level). This would in turn suggest a higher likelihood of the H3K4me3 Histone modification promoting gene transcription in the Cluster 1 genes compared to Cluster 2.

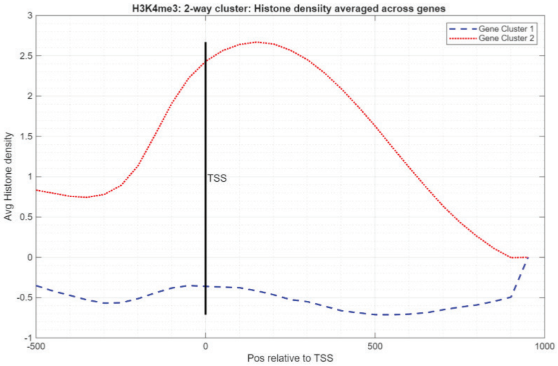

Figure 13: Average H3K4me3 density across gene bodies relative to the transcription start site (TSS), separated into two clusters. Cluster 1 (red) shows high enrichment downstream of the TSS, indicative of actively transcribed genes, while Cluster 2 (blue dashed) shows low enrichment, corresponding to lowly transcribed genes.

Figure 14 shows a high and low-histone level clustering for the H3K36me3 Histone modification, which is known to repress gene transcription in most cases. The plot shows more pronounced peaking of the histone level towards the end of the gene segment for Cluster 1 (high-level) compared to Cluster 2 (low-level). This finding suggests a higher likelihood of the H3K36me3 Histone modification in repressing gene transcription in the Cluster 1 genes compared to Cluster 2.

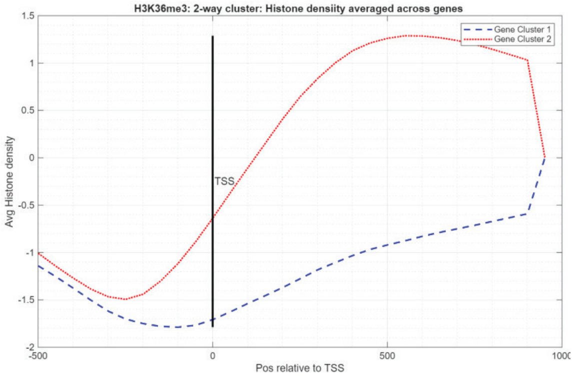

Figure 14: Average H3K36me3 density across gene bodies relative to the TSS, separated into two clusters. Cluster 2 (red) shows strong H3K36me3 accumulation toward the transcription termination site (TTS), consistent with genes undergoing elongation-coupled repression, while Cluster 1 (blue dashed) shows minimal enrichment across the gene body, characteristic of actively transcribed genes.

The bar plot in Figure 15 presents the variation of histone densities across genes, summarized for the entire histone set considered in this study. This plot shows the ratio of the number of genes exhibiting high histone levels relative to the number of genes exhibiting significantly lower levels of histone levels. The plot shows that different histone modifications are manifested to significantly varying degrees across genes.

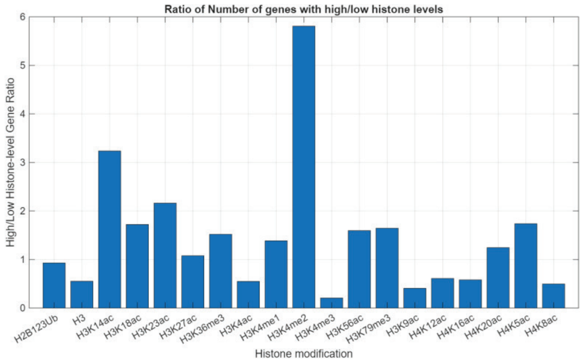

Figure 15: Ratio of the number of genes exhibiting a high level of histone modification relative to the gene subset with low histone levels. This data was obtained using a K-medioids-based two-way clustering scheme with signal over a fixed segment of base-pairs around the TSS as the features and genes as the observations.

13. Distribution of Histone Levels Relative to TSS:

In this section, we examine the distribution of histone levels over a gene segment in the vicinity of the TSS. Specifically, a gene segment ranging from 499 base-pairs on the leading side of the TSS and 1000 base-pairs to the trailing side of the TSS was considered. Histogramming of the histone level across this segment was then performed with the 5331-gene set treated as independent observations. This probabilistic analysis factors in the variation of histone levels across genes.

To assess the distribution of H3K4me3 across gene segments, we plotted the positions of minimum and maximum signal intensities relative to the TSS. The Max-signal histogram in Figure 14 shows a strong concentration of signal peaks near the TSS, consistent with H3K4me3's role in marking active promoters. The Min-signal histogram in Figure 15 shows a peak at the TTS and start of the gene, signifying its role as purely a transcription-promoting histone. The clustering of maximum signals around the TSS highlights its function in facilitating transcription initiation. These probabilistic profiles confirm that H3K4me3 is tightly localized to promoter regions and can reliably indicate transcriptional activity. These results reinforce the functional importance of H3K4me3's density across genes in transcriptional regulation and demonstrate that signal peak positions can be used to infer promoter activity and chromatin state across diverse gene sets.

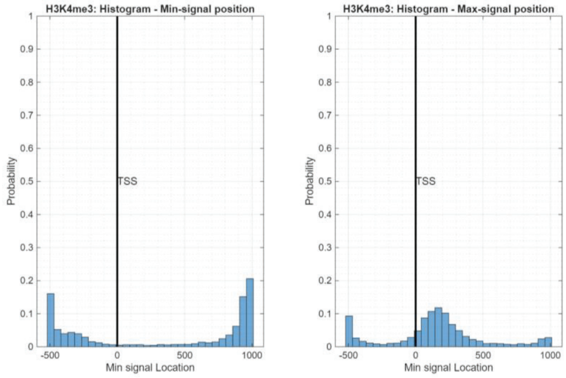

Figure 16: Histograms show the positions of minimum and maximum H3K4me3 signal intensities across genes relative to the TSS. Both distributions cluster sharply at the TSS, reflecting H3K4me3's localization to active promoters.

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To examine the signal distribution of H3K36me3, we plotted the probable positions of minimum and maximum signal intensities relative to the TSS. As shown in Figure 16, both histograms reveal that H3K36me3 signals are predominantly located towards the end of the gene, with maximum signal positions broadly distributed towards the TTS of the gene body, while the minimum-signal positions show the histones' reduced density at the start of the gene and the TSS showing its role as a transcriptional repressor. This pattern is consistent with H3K36me3's role as a repressive histone mark deposited during transcriptional elongation to suppress cryptic initiation and modulate gene output. These probabilistic plots highlight the gene-body localization of H3K36me3 and support its function in repressing transcriptional activity.

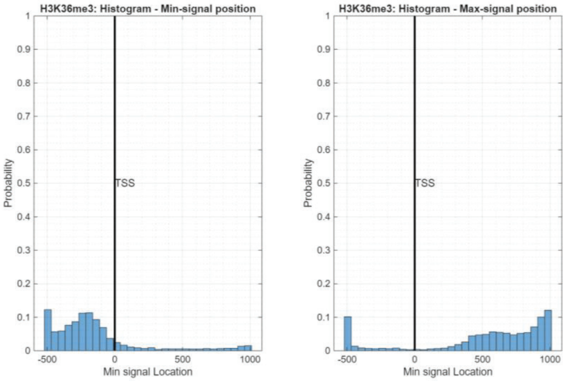

Figure 17: Histograms show the positions of minimum and maximum H3K36me3 signal intensities relative to the TSS. Maximum-signal positions are broadly distributed toward the TTS, reflecting H3K36me3 accumulation along the gene body, while minimum-signal positions cluster near the TSS. This distribution is consistent with H3K36me3's role as an elongation-linked repressive mark.

14. Correlation Between H3K4me3 Enrichment and Net-Seq Data:

To investigate the relationship between histone modifications and transcription, we compared gene-body H3K4me3 levels (measured by ChIP-seq) with nascent transcription (measured by NET-seq). Figure 17 shows a scatter plot of H3K4me3 ChIP-seq signal versus NET-seq signal across all analyzed genes. A positive correlation (r = 0.43) was observed, indicating that genes with higher H3K4me3 enrichment in their gene bodies tend to exhibit higher levels of transcription. This positive correlation is consistent with the known role of H3K4me3 as an activating histone mark, often enriched at promoters and early gene bodies of actively transcribed genes. The inclusion of this plot in our study provides quantitative evidence that H3K4me3 is functionally associated with transcriptional output in S. cerevisiae. By demonstrating this relationship, the figure supports our broader goal of linking specific histone modifications to transcriptional regulation. Specifically, it shows that H3K4me3 is not just present in genes, but its abundance quantitatively correlates with RNA polymerase engagement and transcriptional activity, validating its role as a marker of active gene expression in the context of our genome-wide analysis.

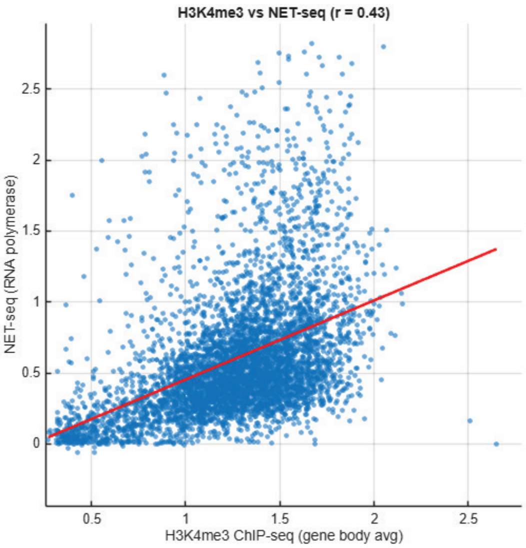

Figure 18: Scatter plot showing the relationship between gene-body H3K4me3 levels (ChIP-seq) and nascent transcription (NET-seq) across all analyzed genes in S. cerevisiae. A positive correlation (r = 0.43) indicates that higher H3K4me3 enrichment is associated with increased transcriptional activity because of increased amounts of RNA Polymerase at the TSS.

15. Histone Modification Summary Table:

Histone Modification:	Histone Role:	Observed Patterns:
1. H3K4 methylation	An activating histone modification that promotes transcription by recruiting transcription factors and RNA polymerase and opening chromatin at the TSS. H3K4me marks active promoters and stabilizes transcription initiation complexes.[18]	Strong peak immediately at the TSS in actively transcribed genes. A dip before this TSS reflects a histone-depleted region needed for RNA polymerase binding. High H3K4me3 correlates with increased NET-seq signal and transcription factor occupancy.
2. H2AK5 Acetylation	An activating acetylation mark that loosens chromatin, allowing transcription factors and RNA polymerase to access the promoter region. Associated with early transcription initiation.[20]	Sharp enrichment at the TSS with levels declining across the gene body. The dip before the TSS indicates open chromatin. Pattern reflects its role in promoter accessibility and transcription initiation.
3. H3 Methylation	Supports transcriptional elongation by stabilizing nucleosomes during RNA polymerase movement and recruiting elongation factors like FACT and Spt6.[10]	Distributed across the gene body rather than concentrated at the TSS. Levels increase towards the TTS of the gene body, reflecting a role in elongation rather than initiation.
4. H3 Acetylation	A strong transcription-activating modification associated with open chromatin, reduced nucleosome packing, and recruitment of TFIID and other initiation factors.[16]	High peak at the TSS in active genes with a small dip directly before the TSS (histone-depleted region). Enhances promoter accessibility and supports transcription initiation.
5. H4 Acetylation	Promotes transcription initiation by loosening chromatin at promoter regions and recruiting bromodomain-containing remodelers (e.g., SWI/SNF). Supports both initiation and early elongation.[29]	Strongly enriched at the TSS and decreases across the gene body. The dip before the TSS marks areas of open chromatin needed for RNA polymerase binding.
6. H2B	A ubiquitination mark that supports transcription elongation and activates methylation (H3K4 and H3K79) through histone crosstalk. Can also recruit repressive factors in inactive genes.[13,21,22]	Peaks at the TSS and early gene body. Supports RNA polymerase movement across genes but can also appear in inactive genes where it interacts with repressors.
7. H3K9, H3K27 and H3K36 Methylation	Repressive histone modifications that compact chromatin and prevent transcription initiation. - H3K9me and H3K27me: Promoter-proximal repression.[14] - H3K36me3: Deposited during elongation to suppress transcription and reduce gene output.[9]	In inactive genes there are high levels before the TSS, forming closed, compact chromatin(for H3K36me). In active genes there are low levels at the TSS but they increase toward the TTS.

Discussion

Histone Modification Enrichment in the Gene Body:

The results showed various patterns of histone modifications around genes and their effect on gene transcription.

Histone modifications, specifically H3K4me3, H3K4me1, and H3K4me2, exhibited enrichment near the TSS of the meta-gene, suggesting that they are likely to play a role in the transcription of active genes in S. cerevisiae. Results show a peak in amounts of H3K4me around the TSS. As shown by the Chip-seq data, the peak of transcription factors across the gene body also occurs at the TSS. This correlation is in line with H3K4me histone modifications recruiting transcription factors at the TSS for transcriptional initiation.[13] A similar peak around the TSS is also seen in the Net-seq data, showing RNA polymerase levels across the gene body. This relationship is further supported by the H3K4me3–NET-seq correlation (r = 0.43) seen in Figure 17, showing that higher levels of this activating mark correspond to increased nascent transcription. The high amount of H3K4me histones can recruit transcription factors.[11] Methylation contributes to an open chromatin structure, promoting transcriptional initiation.[9]

In contrast, other methylations, such as H3K36me3, were found to be more distributed over the gene body towards the TTS rather than concentrated at the TSS of the meta-gene. The meta-gene profiles show a clear separation of methylation states: H3K4 methylation concentrated at the TSS and H3K36 methylation increasing across the gene body. This pattern is not just correlative; it reflects the specialization of these methyl groups. H3K4me3 at the TSS functions as a landing for PHD-finger and Tudor-domain-containing proteins, which bind to the histones, allowing transcription factors to easily recruit transcription factors that stabilize the assembly of transcriptional initiation and early elongation factors, increasing transcriptional initiation at the TSS.[25] By contrast, H3K36 methylation deposited transcriptionally by elongation-coupled methyltransferases marks nucleosomes that have been traversed by RNA polymerase II and recruits chromatin regulators that act behind the polymerase.[10,26] In the data(Figure 18), the graded increase of H3K36me across gene bodies correlates quantitatively with NET-seq polymerase density, which is consistent with a role for H3K36me in coupling nucleosome state to polymerase processivity rather than simply reflecting past polymerase passage.[27-29]

H2AK5ac levels were found peaking at the TSS and steadily dropping towards the transcription termination site, suggesting a specific role in transcriptional initiation. The enrichment of H2AK5ac near the TSS likely facilitates transcription by promoting an open chromatin configuration, allowing easier access for RNA polymerase II and general transcription factors. This acetylation mark also serves as a recognition site for bromodomain-containing coactivators, which help recruit components of transcriptional initiation to the promoter.[24] The subsequent decline of H2AK5ac levels across the gene body suggests its primary involvement in the early stages of transcription, where the TSS accessibility and transcriptional initiation formation are most critical. Similarly, H4 acetylation is enriched at the TSS and decreased towards the transcription termination site. Among these modifications, methylation, ubiquitination, and acetylation are particularly significant. Each type of modification has distinct effects on histone function, leading to varying impacts on transcriptional activity. Methylation of histones generally occurs on lysine or arginine residues and can result in either transcriptional activation or repression, depending on the specific site. For example, H3K4me3 peaks at the TSS, consistent with previous reports that it recruits PHD- and Tudor-domain–containing proteins to facilitate pre-initiation complex assembly.[27]

Similarly, H2B123Ub and H3K36me show coordinated patterns along the gene body, supporting the concept of histone crosstalk in promoting transcriptional activity and also suppressing initiation.[24,26] Presenting this information here highlights how our findings corroborate and extend prior literature by mapping these modifications systematically across active and inactive gene regions in *S. cerevisiae*. The enzymes responsible for this modification are called methyltransferases. For instance, trimethylation of lysine 4 on histone H3 (H3K4me3) is enriched at the TSS of genes with high transcription. This modification is associated with gene activation and facilitates the recruitment of transcriptional machinery, enhancing transcription efficiency.[11] However, H3K27me3 is typically linked to gene repression.[12]

H3K4me3 is enriched at the TSS of genes with high transcription, promoting gene activation by serving as a binding platform for “reader” proteins containing PHD or Tudor domains, which recruit transcriptional activators, chromatin remodelers, and transcription factors such as TFIID. By stabilizing these factors at promoters, H3K4me3 increases accessibility of the DNA to RNA polymerase II, facilitating transcription initiation and early elongation.[5] Conversely, H3K27me3 results in repression by recruiting Polycomb repressive complexes (PRC1 and PRC2), which compact chromatin, limit nucleosome mobility, and prevent binding of transcription factors and RNA polymerase. In this way, H3K27me3 establishes a closed chromatin state that silences gene expression.[19] The level of H3K27 methylation is higher before the TSS, lower at the TSS, and higher at the transcription termination site, suggesting that it is a repressive histone.[11] High levels of H3K27me recruit Polycomb repressive complexes, which compact nucleosomes and reduce DNA accessibility, preventing RNA polymerase and transcription factors from binding.[29] Additionally, H3K27me interacts with other histone modifications, forming a coordinated network that reinforces gene-specific transcriptional states. Together, these observations indicate that H3K27 methylation actively enforces a stable, repressive chromatin environment in inactive genes.

Ubiquitination involves the attachment of ubiquitin molecules to specific lysine residues on histones, and this process is mediated by a group of enzymes known as ubiquitin ligases. Ubiquitination can have variable effects on transcriptional regulation. For example, H2B123Ub is associated with active transcription. However, this modification can recruit repressive factors that inhibit gene expression by promoting a closed chromatin conformation. H2B123Ub levels were higher near the transcription start sites of actively transcribed genes. This suggests a role in the initiation of transcription. H2B123Ub increases at the promoter of genes affecting gene expression by promoting transcription of certain genes while repressing others.[14] For example, transcription activators that are ubiquitinated are frequently degraded, thus repressing gene expression.[13]

Functionally, H3K36 methylation contributes to transcriptional repression by recruiting complexes (histone deacetylases and histone chaperones) that restore nucleosome structure and suppress spurious internal initiation. This explains why genes with strong amounts of H3K36me in our study show fewer signs of transcription when looking at NET-seq data: methylation helps re-establish a chromatin environment that prevents transcription within coding regions. Moreover, H2B123Ub and H3K4 methylation exhibit crosstalk. H2B123Ub enrichment at the TSS and early gene body coincides with downstream H3K4me and H3K79me levels, suggesting a cascade in which one influences the other. This interdependence implies that methylation patterns shape nucleosome dynamics not in isolation but as part of a coordinated modification network that helps transcriptional initiation and elongation. For example, acetylation of lysine residues on histones, including H4K8ac, neutralizes the positive charge of histones.[15] This reduces the closeness of histones to negatively charged DNA, loosening their grip on the DNA strand and making the chromatin structure more open and accessible.4 This open chromatin state allows transcription factors and RNA polymerase to access the DNA and initiate transcription. This is particularly relevant because peaks of H4 acetylation at the TSS correlate with regions of active transcription, indicating that this modification actively facilitates RNA polymerase and transcription factor binding in *S. cerevisiae*.[31] Therefore, H4K8ac and other H4 acetylation at promoters and enhancers increase gene expression. However, H3K9me and H3K27me are absent at the TSS of active genes because they are associated with gene repression.[6] These genes are not as crucial for yeast and are therefore repressed in most cases.[31]

Varying Histone Modifications and the Role of Histone Modification Patterns in Gene Expression:

Different histone modifications vary in location within genes. The methylation of histones can have repressive effects on transcription, but is also associated with transcription initiation, while the acetylation of histones is associated with transcriptionally active genes. Methylation can either repress or activate transcription depending on the specific residue and location within the gene; for example, H3K4me3 at promoters facilitates transcription initiation by recruiting transcription factors and coactivators, whereas H3K27me3 generally represses gene expression by promoting chromatin compaction.[13] Acetylation of histones, such as H3 and H4 lysine acetylation, is generally associated with transcriptionally active genes, particularly at the TSS, where it creates an open chromatin structure and increases DNA accessibility.[21]

However, the impact of acetylation can vary depending on the presence of other histone modifications and the local chromatin environment.[21]

In our study, observing peaks of H3 and H4 acetylation at the TSS supports their role in facilitating transcriptional initiation and aligns with the enrichment of RNA polymerase and transcription factors at these regions. Histone modifications can be enriched in specific gene regions, such as promoters, enhancers, or gene bodies.[15] At the same time, H3K4me histones were primarily found around the TSS.10 Certain histone modifications located in the gene body and towards the transcription termination site of genes also regulate gene expression. For example, H3K36me3 prevents premature transcriptional termination in the gene body, facilitates the movement of RNA polymerase along the gene body, and thus promotes gene expression.[16]

The ChIP-seq data show that peak levels of transcription factors across genes with low transcription and genes with high transcription are similar. Chip-seq data peaks after the TSS, showing there are many transcription factors and gene promoters at that position in the gene, and H3K4 histone modifications also peak after the TSS, showing that the level of H3K4 histone levels is high at this point. Analysis of the ChIP-seq data alongside the H3K4me data reveals a positive correlation at the TSS, where higher H3K4me levels coincide with increased binding of transcription factors.[19] This correlation is important because it provides evidence that H3K4me actively contributes to the recruitment of transcription factors that promote transcriptional initiation. This supports the interpretation that H3K4me acts as a functional recruiter for active transcription factors, linking histone modification to gene expression regulation in S. cerevisiae. MNase-seq data reveal differences in nucleosome occupancy between genes with high and low transcription. Genes with low transcription exhibit higher nucleosome levels immediately upstream of the TSS, creating a more compact chromatin structure that limits accessibility for RNA polymerase and transcription factors. In contrast, highly transcribed genes show a relative depletion of nucleosomes near the TSS, consistent with an open chromatin state that facilitates transcriptional initiation.[30] These patterns highlight the relationship between nucleosome positioning and gene activity, demonstrating how chromatin structure can directly influence transcriptional output.

An important difference seen in the MNase-seq data is that the level of nucleosomes for genes with low transcription was much higher than for genes before the TSS, which had high levels of transcription. In addition to this, the NET-seq analysis (Figure 18) revealed that genes with higher H3K4me3 levels also display higher nascent transcription, as shown by the positive correlation in our scatter plot. This indicates that H3K4me3 enrichment is not only associated with transcription factor binding but also quantitatively associated with RNA polymerase II occupancy along the gene body.

■ Future Directions

This study focused on the role of specific histone modifications and their nucleosome positioning on transcriptional activity. Future work is needed to determine the causal mechanisms underlying these relationships beyond what has already been reported in the literature. Targeted perturbations, such as mutating key histone residues or disrupting the enzymes responsible for their modifications, could clarify how individual histone modifications directly influence transcription initiation and elongation. Complementary approaches that include

ChIP-seq to assess factor binding, RNA-seq to measure transcript output, or high-resolution chromatin accessibility assays would provide a more detailed understanding of how histone modifications coordinate with transcriptional machinery. Additionally, exploring these dynamics under different environmental conditions or stress responses in *S. cerevisiae* could reveal how chromatin regulation adapts to changing cellular states. Overall, such studies would extend the insights gained from our meta-gene analyses and the larger body of work by other researchers in this area. In particular, this would help establish a mechanistic framework linking histone modifications to gene regulation.

Conclusion

In this study, the significant role of a select set of epigenetic histone modifications in S. cerevisiae was explored. The observed distributions of histone modification levels relative to the transcription start site and transcription termination site were found to be in agreement with these histone modifications being gene transcription promoters or repressors, as reported in prior studies. The research also depicts the varying levels of specific histone modifications across genes, highlighting their varying roles in different genes. The work also describes simple but powerful data-analytic methods to understand trans-gene trends of histone modifications. These methods can be improved and extended further in future studies to gain deeper insights into histone modification behaviors and their role in gene regulation.

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