Identification of Possible Demethylases for H4 Lysine 5 Methylation in *Saccharomyces Cerevisiae*

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Abstract

Histone methylation is a post-translational modification system that affects the side chains of lysine and arginine and is most prominent in histones H3 and H4. This epigenetic mechanism has been shown to regulate gene transcription by altering chromatin structure and has been linked to several human pathologies such as cancer, diabetes, autoimmune and neurodegenerative disorders. Although long considered a stable and permanent modification, histone methylation is a dynamic process that is indeed reversible, as indicated by the discovery of several histone demethylases that catalyze the demethylation of histones at key regulatory residues. While many histone demethylases and their associated targets have been identified, the identities of other demethylases for other residues have yet to be characterized. This study aims to identify possible demethylases for lysine residues 5, 8, and 12 in histone H4, which are associated with repression of gene expression in silent genomic regions. To identify the demethylase, we examined the methylation patterns of five strains of yeast lacking the following demethylases: Jhd1, Jhd2, Ecm5, Gis1, and Rph1. These strains were originally used by another study to identify possible demethylase in histone H3. We deleted each of these genes by homologous recombination in a wild type yeast strain. Nuclei were extracted from each of these strains and subject to Western blot analysis with antibodies against histones H3, H4 and H4 K5 methylation. We will present the western blot results showing enrichment of histones H3 and H4 in our nuclear extracts, and potential signal for H4 K5 methylation. This procedure will be followed by a Mass Spectroscopy analysis in order to observe a change in methylation in the ascertained demethylase mutant. Completion of this research should provide evidence of the regulation mechanism of histone demethylation at lysine residues 5, 8, and 12 on histone H4 which have yet to be characterized thus providing novel mechanistic insights into the regulation of histone methylation at these residues. Ultimately, improved molecular understanding of these dynamics may highlight new areas of epigenetic control that can become dysregulated in the progression of many diseases.

Background

Methylation of lysine (K) residues on histones is a major modification determinant for genome organization as well as formation of active and inactive regions of the genome. Lysines may be mono-, di-, or tri-methylated, where the degree of methylation is associated with differences in transcriptional states. In order to establish these methylation states, cells have enzymes that both add (lysine methyltransferases- KMTs) and remove (lysine demethylases- KDMs) methyl marks from specific lysines within the histones[1].

KDM	Function in Yeast
Jhd1	H3K36me or H3K36me2 demethylase
Jhd2	H3K4me3 demethylase
Rph1	H3K36me3 demethylase
Gis1	H3K36me or H3K36me2 demethylase
Ecm5	No evidence of demethylation activity

Table 1. Lysine demethylases in budding yeast and their functional characterization on histone H3.

Lysine demethyltransferases in budding yeast are highly conserved, and the human orthologs of these enzymes have been known to be deregulated in cancer, autoimmune and neurodegenerative disorders. It has been revealed that modifications on lysine residues 5, 8, and 12 of H4 are associated with genomic repression[3]. Studies have also shown that ribosomal protein Rpl23 in yeast is methylated at lysine residues by Rkm1 (ribosomal lysine (K) methyltransferase)[5]. Our aim is to functionally characterize novel sites of methylation on histone H4 at lysines 5,8, and 12 as well as ribosomal protein, Rpl23 in order to gain insight into the role of lysine methylation in a variety of human pathological processes.

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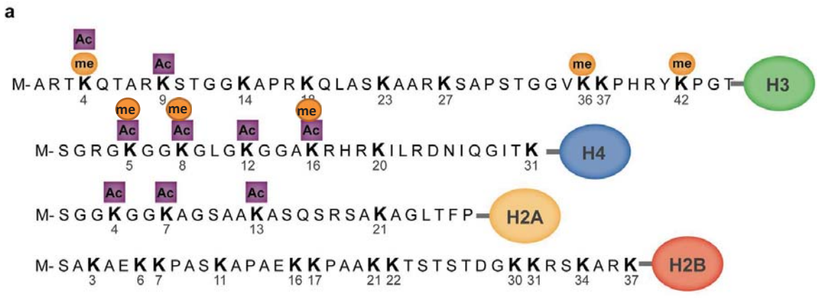

Figure 1. N-terminal tails of budding yeast histones. Well-characterized methylated and acetylated lysines are shown.

Methods

Generate knockout strain of our five mutants homologous recombination to insert Kanamycin.

Performed a small-scale accelerated nuclei protocol 2 that was used for the detection of histone modifications when antibody avidity is low.

Nuclei extracts of the strains were then resolved by SDS-PAGE, transferred to PVDF, and probed with primary & secondary antibodies directed against H3, H4, H4K5me1, and H2B, as apart of a Western Blot analysis.

Identification of a demethylase for H4K5me1

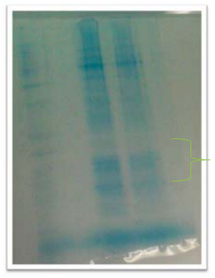

Figure 2. Coomassie Blue stained protein gel showing presence of histones on WT cells.

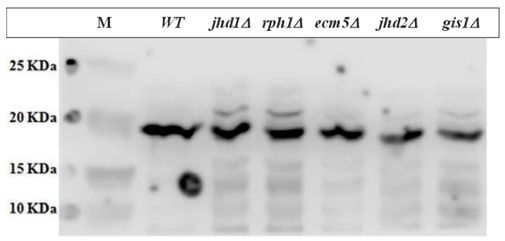

Figure 3. Nuclear extracts prepared from the indicated strains, run on SDS-PAGE and subjected to immunoblotting with anti-H3. There appears to be a strong signal for the H3 antibody.

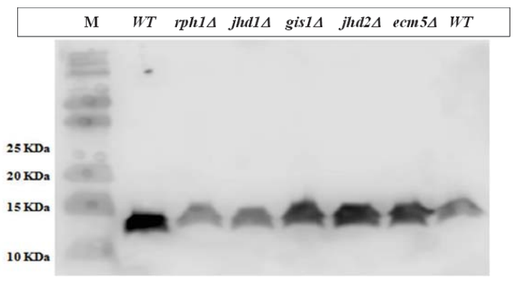

Figure 4. Nuclear extracts were prepared from the indicated strains, run on SDS-PAGE and subjected to immunoblotting with anti-H4.

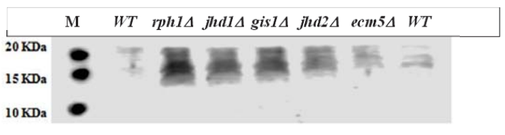

Figure 5. Nuclear extracts were prepared from the indicated strains, run on SDS-PAGE and subjected to immunoblotting with anti-H4K5me1. The results of our Western blot analysis with H4K5me1 antibody are inclusive as we are unable to discern the correct signal.

Identification of a demethylase for Rpl23ab

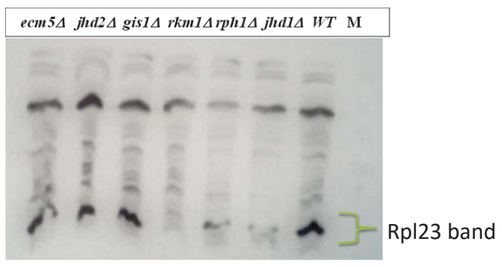

Figure 6. Nuclear extracts were prepared from the indicated strains, run on SDS-PAGE and subjected to immunoblotting with anti-methyl Rpl23.

Conclusions & Future Plans

- The H4K5me1 antibody does not appear specific enough using this approach, so we may need to purify histones from cells to improve the specificity of the western blot.
- The anti-methyl-Rpl23ab is specific enough to detect the methylated species (as shown by your rkm1Δ control), but the analysis needs to be repeated to ensure even loading on the SDS-PAGE in order to identify a potential demethylase.
- Once we are able to identify a potential demethylase for either methyl mark, the future directions will be:
  - To repeat H4K5me1 assays with purified histones from yeast
  - To repeat Rpl23ab assays to ensure equal protein loading to be able to interpret the results from the western blot
  - To verify the demethylation using *in vitro* demethylation assays
  - To validate the demethylation on both *in vitro* and *in vivo* samples using tandem mass spectrometry
  - To identify phenotypes associated with the demethylase that may be due to enhanced methylation at either of these sites

References

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