FEBS Letters 589 (2015) 4061–4070

FEBS Letters

journal homepage: www.FEBSLetters.org

Global mapping of the regulatory interactions of histone residues

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ARTICLE INFO

Article history:
Received 3 September 2015
Revised 10 November 2015
Accepted 11 November 2015
Available online 19 November 2015

Edited by Ned Mantei

Keywords:
Histone residue
Gene expression profile
Nucleosome positioning
Histone modification

ABSTRACT

Histone residues can serve as platforms for specific regulatory function. Here we constructed a map of regulatory associations between histone residues and a wide spectrum of chromatin regulation factors based on gene expression changes by histone point mutations in *Saccharomyces cerevisiae*. Detailed analyses of this map revealed novel associations. Regarding the modulation of H3K4 and K36 methylation by Set1, Set2, or Jhd2, we proposed a role for H4K91 acetylation in early Pol II elongation, and for H4K16 deacetylation in late elongation and crosstalk with H3K4 demethylation for gene silencing. The association of H3K56 with nucleosome positioning suggested that this lysine residue and its acetylation might contribute to nucleosome mobility for transcription activation. Further insights into chromatin regulation are expected from this approach.

1. Introduction

As the structural units of chromatin, the core histones (H3, H4, H2A, and H2B) play a critical role in the epigenetic regulation of DNA. The biological significance and universality of their function are reflected by the remarkably high evolutionary conservation of the protein sequences from yeast to humans. The amino acid residues of histones can be partitioned into four major geographical domains: buried, disk (protein surface that does not contact DNA), lateral (protein surface that contacts DNA), and tail (protruding unstructured region).

To explore the functional role of each residue across the different histone domains, systematic mutant libraries were generated and screened for phenotypic changes in yeast [1,2]. The library of synthetic histone H3 and H4 mutants [1] was created by substituting each non-alanine residue with alanine to avoid deletion effects, while mutating alanine to serine. The mutant strains were tested for phenotypic changes in viability, transcriptional silencing, transcriptional elongation, response to DNA damage, response to microtubule disruption, and response to temperature shock. A database of the phenotypes of histone mutants collected from different studies, named HistoneHits, has been developed [3].

This library also included replacements of modifiable residues with amino acids mimicking modified and unmodified states. Post-translational modification (PTM) of histones includes acetylation, methylation, ubiquitination, phosphorylation, and sumoylation of lysine, arginine, serine, and threonine [4]. Although most of the modifications are observed in the histone tail, the globular domain also contains modifiable amino acids, including acetylated H3K56 and methylated H3K79 [5–9]. The histone code hypothesis [10] proposes that PTMs, alone or in combination, serve as selective binding platforms for regulatory proteins such as chromatin modifiers (CMs) and transcription factors (TFs).

However, modifiable residues cannot account for all histone functions. The modification of nucleosomes is preceded by the formation of nucleosomes through the interactions of histones with wrapping DNA. The nucleosome code hypothesis [11,12] predicts that DNA dictates its own physical packaging into the chromatin

Abbreviations: HR, histone residue; NUC, nucleosome; PTM, post-translational modification; CM, chromatin modifier; TF, transcription factor; NFR, nucleosome-free region; TSS, transcription start site; KS, Kolmogorov–Smirnov; HAT, histone acetyltransferase; HDAC, histone deacetylase; RNA polymerase II, Pol II; ORF, open reading frame

Author contributions: I.J. performed all data analysis and wrote the manuscript draft. J.S. and H.-S.L. performed the microarray and mononucleosome sequencing experiments. L.W.S. supervised the microarray and mononucleosome sequencing experiments. D.K. conceived the study design together with J.K.C. J.K.C. conceived the study, supervised the data analysis, and wrote the manuscript.

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http://dx.doi.org/10.1016/j.febslet.2015.11.016
0014-5793/

structure by modulating its binding with histone proteins. The importance of the DNA-histone interaction was illustrated by a viability test of the histone mutants. In the two mutation studies [1,2], most of the lethal substitution mutations were commonly mapped to the nucleosome lateral surface near the dyad axis or at the DNA entry/exit site, where histones make contact with DNA. The H2A and H2B essential residues were also located on the surface of nucleosomes [2].

Transcriptional regulation should be the primary mechanism by which each residue of histones contributes to phenotypic consequences. How nucleosome assembly and histone PTMs can influence gene transcription has been studied extensively, thanks to genomic technologies such as DNA microarray and next-generation sequencing. Various PTMs and nucleosome patterns near gene promoters have been intensively examined at the genome-wide level [13–20]. For example, the canonical promoter contains a nucleosome-free region (NFR) upstream of the transcription start site (TSS) and a +1 nucleosome downstream of the TSS. Several PTMs, including H3 or H4 acetylation and H3K4 methylation, are associated with active gene transcription.

In this work, we employed genomics approaches to study the function of individual residues of histone H3 and H4 by utilizing a public histone mutant library [1]. Gene expression microarrays were employed to measure the influence of each residue on transcription by comparing gene expression patterns in the histone mutants and wild type. We selected 123 mutants with the highest phenotypic effects for gene expression profiling. To determine whether certain mutants of high transcriptional importance alter nucleosome positioning, the mutated nucleosomes were purified and profiled by deep sequencing.

2. Materials and methods

2.1. Selection of histone mutants

Our mutant selection procedure was based on the HistoneHits database [3]. In essence, we used the alanine substitution mutants of H3 and H4, while discarding lethal mutants [1] and including the strains where the mutation was targeted to a known modifiable residue. The information of modifiability was obtained from the HistoneHits database except for H3K37, whose methylation was recently reported [44]. The degree of phenotypic change for each mutant was obtained from the same database, which collected phenotype scores observed in multiple experiments that belong to one of nine different categories. The nine categories and their readouts were: ribosomal silencing (growth on plates), telomeric silencing (colony color as gain of telomeric silencing), mating efficiency (enzyme assay and growth on plates), growth rate (growth on plates), DNA damage (growth on plates), Spt-phenotype (growth on plates), transcription elongation defect (growth on plates), K56 hyperacetylation suppression (growth on plates), and mating cassette silencing (growth on plates). The score for each experiment was represented as an integer ranging from −2 to 2 with a high absolute value indicating a higher degree of phenotypic change. The average for each category was calculated and then the average of the nine scores was obtained as the final measure for the degree of phenotype changes by each mutation. We first selected the mutants with the final phenotype score greater than 1 and then tried to filter out adjacent mutants that had similar response across the nine experimental conditions. To do so, we first identified the two adjacent mutants that had the same direction of response (the same sign of the phenotype score) in more than seven out of the nine conditions and then removed the one having the lower final phenotype score. H3Q5A was later added because H3Q5 was reported to crosstalk with H3K4me3 [2]. To investigate the effects of different acetylation states, we included H3K56R, H3K56Q, H4K16R, and H4K16Q. Arginine (R) substitution mimics unacetylated lysine and glutamine (Q) mimics acetylated lysine. Overall, a total of 123 mutants were profiled and compared against four replicates of the wild type by microarray experiments, totaling 127 microarray datasets.

2.2. Yeast strains and cell culture

We obtained the yeast histone mutant library from Open Biosystems (catalog number: YSC5106, none essential histone H3 & H4 mutant collection). The 2 ml glycerol stocks of selected mutants and the wild types were cultured in SD-ura medium for 22 h at 25 °C. After streaking on YPD plate at 25 °C, single colony was cultured in 2 ml SD-ura medium for 22 h at 25 °C, 500 μl out of which was subcultured in 10 ml SD-ura medium for 22 h at 25 °C.

2.3. Microarray hybridization

RNA was prepared using the RNeasy Mini Kit according to the manufacturer's instructions (Qiagen). After DNase treatment (TaKaRa Recombinant DNaseI), the first-strand complementary DNA was synthesized from 1 μg total RNA at 42 °C for 2 h, which was followed by second-strand cDNA synthesis at 16 °C for 2 h. The resulting double-stranded cDNA was purified. Fluorescence-labeled RNA was generated by carrying out an in vitro transcription reaction (double-stranded cDNA in nuclease-free water 16 μl, T7 rNTP mix 16 μl, T7 10× Reaction Buffer 4 μl, and T7 Enzyme Mix) at 37 °C for 16 h. The labeled RNA was subsequently purified and chemically fragmented at 70 °C for 15 min in fragmentation buffer (Ambion fragmentation reagent). The fragmented, labeled complementary RNA was hybridized to NimbleGen 12 × 135 K oligonucleotide microarrays at 42 °C for 16–20 h according to the provided instructions. The spotted microarrays of 60-mer oligonucleotide probes that represent 5777 yeast ORFs contained three to eight probes per gene with three replicates for each probe. The microarrays were washed in three consecutive steps by using the provided kit and the readouts were scanned using NimbleScan 2.5.26.

2.4. Microarray data processing

The raw microarray data was normalized by using VSN (Variance Stabilization and Normalization) algorithm [45]. This method utilized variance stabilizing transformation based on the parametric form $h(x) = \text{arcsinh}(a + bx)$, which is derived from a model of the variance-versus-mean dependence for microarray intensity data. For large intensities, h coincides with the log transformation, and Δh with the log ratio. Following the preprocessing by VSN, microarray batch effects were removed by means of the Combat algorithm [46]. We applied the non-parametric empirical Bayes frameworks to the VSN-normalized data. After removing batch effects, we obtained the relative gene expression changes as the log2 ratios between the mutants and wild type. The expression levels for the wild type were calculated as the mean of the replicates. A very high reproducibility ($R = 0.98$) was observed between the wild-type replicates. The genes that were up-regulated or down-regulated >1.5 fold were selected for each histone mutation. We performed gene-set enrichment analysis for these changed genes. To obtain a sufficient statistical power, we only considered gene cohorts that included more than 50 genes, resulting in 91 up-regulated gene cohorts and 71 down-regulated gene cohorts. Afterward, we conducted gene sent enrichment analysis for Gene Ontology (GO) terms by using DAVID (https://david.ncifcrf.gov/) and then performed hierarchical clustering for $-\log_{10}$