H3 Lysine 4 Is Acetylated at Active Gene Promoters and Is Regulated by H3 Lysine 4 Methylation

Benoit Guillemette1,2, Paul Drogaris2, Hsiu-Hsu Sophia Lin1, Harry Armstrong3, Kyoko Hiragami-Hamada3, Axel Imhof4, Éric Bonneil2, Pierre Thibault2,5, Alain Verreault6,7, Richard J. Festenstein1,7*

1 Department of Medicine, Imperial College London, Hammersmith Hospital Campus, London, United Kingdom, 2 Institute for Research in Immunology and Cancer, Université de Montréal, Montreal, Canada, 3 Laboratory for Chromatin Dynamics, Riken Kobe Institute, Centre for Developmental Biology, Kobe, Hyogo, Japan, 4 Adolf-Butenandt Institute, Ludwig-Maximilians-University Munich (LMU), Center of Integrated Protein Science (CIPS), Munich, Germany, 5 Department de Chimie, Université de Montréal, Montreal, Canada, 6 Département de Pathologie et Biologie Cellulaire, Université de Montréal, Montreal, Canada, 7 Medical Research Council Clinical Sciences Centre, London, United Kingdom

Abstract

Methylation of histone H3 lysine 4 (H3K4me) is an evolutionarily conserved modification whose role in the regulation of gene expression has been extensively studied. In contrast, the function of H3K4 acetylation (H3K4ac) has received little attention because of a lack of tools to separate its function from that of H3K4me. Here we show that, in addition to being methylated, H3K4 is also acetylated in budding yeast. Genetic studies reveal that the histone acetyltransferases (HATs) Gcn5 and Rtt109 contribute to H3K4 acetylation in vivo. Whilst removal of H3K4ac from euchromatin mainly requires the histone deacetylase (HDAC) Hst1, Sir2 is needed for H3K4 deacetylation in heterochromatin. Using genome-wide chromatin immunoprecipitation (ChIP), we show that H3K4ac is enriched at promoters of actively transcribed genes and located just upstream of H3K4 tri-methylation (H3K4me3), a pattern that has been conserved in human cells. We find that the Set1-containing complex (COMPASS), which promotes H3K4me2 and -me3, also serves to limit the abundance of H3K4ac at gene promoters. In addition, we identify a group of genes that have high levels of H3K4ac in their promoters and are inadequately expressed in H3-K4R, but not in set1Δ mutant strains, suggesting that H3K4ac plays a positive role in transcription. Our results reveal a novel regulatory feature of promoter-proximal chromatin, involving mutually exclusive histone modifications of the same histone residue (H3K4ac and H3K4me).

Introduction

Histones are covalently modified on many lysine residues. The outcome of these modifications is either to modify chromatin structure directly or provide docking sites for the binding of non-histone proteins [1]. The same chemical modification (e.g. methylation) on different residues can lead to distinct outcomes. Moreover, some histone modifications function in a combinatorial fashion to generate different functional outcomes [2-4]. This led to the notion that histone modifications may represent an epigenetic code that influences gene expression and serves as a “memory” of cell identity during development of cell lineages [5-6]. The recent development of high resolution mass spectrometry has enabled the identification of a great number of new histone modifications [7-9]. Elucidation of the functions of these new modifications is greatly facilitated in model organisms where, in contrast to vertebrate cells, histone gene mutations that abolish specific modifications can be readily introduced.

Histone H3 lysine 4 is a highly studied residue whose modification is important for many biological processes in a wide range of species [10-11]. The genomic localisation of H3K4 methylation (H3K4me) has been conserved through evolution. It is highly regulated and generally associated with transcriptionally active genes [12-18]. H3K4 tri-methylation (H3K4me3) is a hallmark of transcriptional start sites and is generally followed by H3K4me2 and H3K4me1 along gene coding regions [19-22]. The multiple functions of H3K4 are mediated by a number of chromatin-associated proteins that selectively bind to some of the four methylation states of H3K4: unmethylated, mono-, di- or trimethylated [23-37]. In yeast, H3K4 is methylated by Set1, a SET domain containing protein and a homolog of Trithorax. Set1 is part of a complex termed COMPASS (complex of proteins associated with Set1) [38-42]. The regulation of the different forms of H3K4me is complex and requires not only the components of COMPASS, but also a trans-histone pathway which involves mono-ubiquitylation of H2B lysine 123 [43-44].

A close link exists between H3K4me2 and -me3 and acetylation of the H3 tail. H3K4me3 is often found together with acetylation of other residues (i.e. K9, K14, K18, K23 and K27) on the same H3 molecules [45-49]. In a subset of yeast genes, H3K4me3 is
Author Summary

In the nucleus of mammals and yeast, DNA is packaged by forming complexes with histone proteins in a structure called the nucleosome, the basic building block of chromatin. The tails of the histones protrude from the nucleosome and can be marked on many amino acid residues by chemical modifications such as methylation and acetylation. A highly studied modification, which is robustly associated with active gene promoters, is histone H3 lysine 4 methylation (H3K4me). We describe here a novel modification, histone H3 lysine 4 acetylation (H3K4ac), which can occur on the same lysine of the histone H3 tail (but not at the same time as methylation). We have identified the enzymes responsible for depositing and removing this mark and mapped its distribution throughout the yeast genome. We found that H3K4ac is present on active genes and is important for the full expression of a subset of them. Strikingly, H3K4 acetylation was found in the same promoters as H3K4me and contributes to regulate the abundance and localization of H3K4ac. This example of cross-talk between two different modifications of the same residue has fundamental implications for understanding how genes are activated and how their packaging in the nucleus controls this process.

directly binds to the PHD finger domain of Yng1, a subunit of the NuA3 histone acetyltransferase (HAT) complex that modifies H3K14, which couples the acetylation and methylation of H3 on different residues [33]. In contrast, through the recruitment of the SET3 complex, H3K4me2 in coding regions promotes deacetylation of H3 in the wake of RNA polymerase II (RNAPol II) [50]. These results suggest that there is a highly dynamic and coordinated interplay between histone H3K4 methylation and the enzymes that control H3 acetylation during transcription.

Despite extensive studies of histone H3K4 methylation, the functional implications of other modifications that occur on the same residue have not been investigated. Here, we identified H3K4 acetylation (H3K4ac) in S. cerevisiae using mass spectrometry and a highly specific antibody that we developed. We found that GCN5 and, to a lesser extent, RTT109 are needed for both H3K4ac and H3K9ac in vivo. H3K4 acetylation in euchromatin is mainly dependent upon HST1, and partly on SIR2. In contrast, removal of H3K4ac from heterochromatin requires SIR2 only. Genome-wide ChIP experiments revealed that H3K4ac is generally found upstream of H3K4me3 in active gene promoters, a pattern which has been conserved at many human CD4+ T-cell promoters [51]. We further demonstrate that H3K4me2 and -me3 mediated by the COMPASS complex limits global levels of H3K4ac at promoters and prevents it from spreading into the 5'-ends of coding regions. Using a genetic approach to separate the functions of H3K4ac and H3K4me, we identified a subset of S. cerevisiae genes whose expression depends upon H3K4ac, but not H3K4me. Altogether, our results strongly support a positive role for H3K4ac in gene transcription and identify a novel interplay between two modifications of the same histone residue.

Results

Histone H3 lysine 4 is acetylated in Saccharomyces cerevisiae

One of the most studied and conserved histone modifications is H3K4 methylation (H3K4me), which is tightly linked to transcription in many species [10]. Because of this, and the fact that methylation and acetylation of the same lysine residue are mutually exclusive, we investigated whether H3K4ac was present in S. cerevisiae, a powerful model organism for genetic analysis. For this purpose, we raised an antibody against H3K4ac and confirmed its specificity with peptide binding assays and immunoblotting competition assays (Figure 1A, 1B). Previous studies have detected the presence of H3K4ac in human cells and an antibody was made commercially available, however the specificity of this antibody was questioned as it showed cross-reactivity against the acetylated H4 N-terminal tail and H3K9ac peptides [51-52]. In contrast, our antibody does not show any strong cross-reactivity against H3K9ac or tetra-acetylated H4 peptides (Figure 1C).

We readily detected a single H3K4ac band in immunoblots of extracts prepared from wild-type (WT), S. cerevisiae cells (Figure 1D). Importantly, there was no H3K4ac signal in extracts from cells that express either H3-K4R or H3-K4Q as the only source of H3 (Figure 1D). We also confirmed the presence of H3K4ac by mass spectrometry (MS). Histones were isolated from exponentially growing yeast cells (Figure 1E). After trypsin digestion, histone peptides were analysed by nano-LC MS/MS. In histones purified from wild-type cells, we identified a doubly charged precursor ion with the expected mass-to-charge ratio (m/z) for the tryptic peptide 3-TKacQTAR-8. The m/z ratio for the monoisotopic form of this precursor ion was determined with high accuracy as 373.7113 (Figure 1F). The experimental mass of the non-protonated peptide corresponding to this precursor ion (745.4070 Da) was in excellent agreement (~1.6 ppm) with the theoretical mass expected for a K4-acetylated peptide (745.4082 Da) and clearly different from the predicted mass of a peptide containing trimethylated K4 (745.4592 Da). Fragmentation of this precursor peptide by collision-activated dissociation resulted in $b$- and $y$-type ion series (Figure 1G) with diagnostic fragment ions (e.g. the mass difference between the $y3$ and $y5$ fragments and the mass of the $b2$ fragment) that unambiguously proved the presence of an acetyl group on H3K4. However, technical limitations precluded us from determining the abundance of H3K4ac by MS. Using synthetic peptides, we found that the tryptic peptide containing H3K4ac was extremely poorly retained during reverse phase HPLC. Because of this we were not able to accurately assess the stoichiometry of H3K4 acetylation.

To investigate the presence of H3K4ac in chromatin, we performed chromatin immunoprecipitation (ChIP) in strains expressing either H3-WT or H3-K4R. Our initial screen of various genomic regions by qPCR revealed a strong enrichment of H3K4ac (relative to ChIP of n on-modified H3) at some gene promoters (MAE1, PHO85, RPL25) a modest enrichment at other genes (BNA5, MCM1, BAP2, BAP5) and a very low enrichment at a region ~1 Kb from telomere III-R (TELO3R) (Figure 1H). These results are consistent with our genome-wide ChIP-chip data showing an enrichment of H3K4ac in euchromatin at the promoters of highly transcribed genes (see below). We performed control experiments to confirm the target lysine specificity of our antibody in ChIP assays. The ChIP signal obtained with the H3K4ac antibody dropped significantly at all regions tested in the H3-K4R strain (Figure 1H). As an average for all the promoters tested, the signal obtained by ChIP with our H3K4ac antibody in the WT strain is about 7-fold higher than the non-specific signal (noise) derived from the H3-K4R strain. The average signal-to-noise ratio increases to 9-fold when considering only the four promoters where the signal is most abundant in WT cells. Certain promoters show a higher background, such as the RPL25 promoter (3-fold signal-to-noise), which may reflect some degree of non-specific binding of...
Figure 1. H3K4 Is Acetylated in *S. cerevisiae*. (A) Synthetic peptides derived from the histone H3 N-terminal tail (residues 1 to 10) with modifications at different residues were applied to a nitrocellulose membrane. The membrane was incubated with the H3K4ac antibody, a horseradish peroxidase-conjugated anti-rabbit IgG, and signals visualised by chemiluminescence (left). Ponceau S staining showing that all the peptides bound to nitrocellulose (right). (B) Immunoblots carried out with nuclear extracts from mouse thymocytes. The membrane was cut into strips and incubated with H3K4ac antibody that was pre-incubated with the indicated competitor peptides (1 μg/ml), resulting in a molar ratio of
our H3K4ac to other acetylated lysine residues present in proteins in the vicinity of the RPL25 promoter. Nevertheless, our H3K4ac-specific enrichment values are comparable with previously published ChIP data using acetyl-lysine specific antibodies, where the signal-to-noise ratio ranged from 3 to 10-fold [53]. These results validate the specificity of our antibody in ChIP assays and confirm the presence of H3K4ac in gene promoters.

H3K4 acetylation depends upon GCN5 and RTT109

To screen for potential HATs that act on H3K4, we monitored H3K4ac by immunoblotting extracts derived from strains carrying gene disruptions of previously identified HATs. Using this approach, we found that GCN5 and RTT109 were required for H3K4ac in vivo (Figure 2A, lanes 4 and 7). Consistent with a recent report [54], gcn5Δ and rtt109Δ single mutants also exhibited lower levels of H3K9ac than WT cells (Figure 2A). A gcn5Δ rtt109Δ double mutant showed levels of H3K4ac comparable to the background observed in histones from H3-K4R cells (Figure 2B, 2C), suggesting that, as is the case for H3K9ac, GCN5 and RTT109 are responsible for essentially all H3K4ac in yeast.

H3K4 deacetylation is dependent upon HST1 and SIR2

To identify potential HDACs responsible for H3K4 deacetylation we repeated our immunoblotting screen with extracts from different HDAC deletion mutants. This revealed that H3K4 deacetylation is at least in part, dependent upon HST1 and HD1A (Figure 2D, lanes 2 and 7). Deletion of HD1A also led to an increase in H3K9ac, whereas the hst1Δ mutant showed an increase in H3K4ac but not H3K9ac (Figure 2D, bottom panel). Because of this, we focussed our experiments on Hst1. S. cerevisiae Hst1 is a member of a family of five NAD+-dependent HDACs, known as sirtuins because of their homology to Sir2. These enzymes are readily inhibited by nicotinamide, one of the products of the deacetylation reaction. Consistent with this, exposure of WT cells to nicotinamide caused an increase in H3K4ac (Figure S1). Treatment of hst1Δ cells with nicotinamide led to a further increase in H3K4ac (Figure S1), which suggested that other sirtuins also contribute to H3K4 deacetylation. We analysed H3K4ac in a collection of sirtuin double mutants and found that deletion of SIR2 in cells lacking Hst1 increased H3K4ac above the levels observed in WT cells and hst1Δ single mutants (Figure 2E, 2F, lanes 1-3). This suggests that SIR2 can compensate for the loss of HST1 for global deacetylation of H3K4. In S. cerevisiae, Sir2 promotes heterochromatin formation through histone deacetylation in specific chromosomal regions, namely the silent mating type loci (HMLα and HMRα), sub-telomeric regions and a subset of ribosomal DNA (rDNA) repeats [55]. By immunoblotting whole-cell extracts, we found no global increase in H3K4ac in a sir2Δ single mutant (Figure 2D, lanes 1 and 11). We hypothesised that, in WT cells, Sir2 might deacetylate H3K4 mainly at heterochromatic loci, which represent a relatively small fraction of the genome. To test this hypothesis, we performed ChIP in WT, hst1Δ, sir2Δ and hst1Δ sir2Δ double mutant cells at different euchromatic and heterochromatic regions known to be deacetylated by Hst1 and Sir2 respectively; the promoters of BNA5 and BTN2, targeted by Hst1 [56], a sub-telomeric region (TEL03R) and the silent mating type locus HMLα, targeted by Sir2 [55]. We also analysed an origin of replication (ARS1233) and the BSC1 gene promoter, which are not known to be targeted by either Sir2 or Hst1. The sir2Δ single mutation elevated H3K4ac at the TEL03R and HMLα heterochromatic loci to levels comparable to those observed in the hst1Δ sir2Δ double mutant (Figure 2G). The deletion of HST1 had virtually no effect at these regions. Therefore, we conclude that H3K4 acetylation in heterochromatic regions is solely dependent upon SIR2. Conversely, deletion of HST1 increased H3K4ac at the promoters of BNA5 and BTN2, but not at other euchromatic regions (Figure 2G). The SIR2 deletion affected H3K4ac only slightly at those loci whereas the hst1Δ sir2Δ double mutant showed higher levels of H3K4ac than in any of the single mutants (Figure 2G). This is consistent with a previous study [57] and suggests that Sir2 can also contribute to deacetylate H3K4 at Hst1-targeted loci.

Genome-wide presence of H3K4ac in euchromatin and active promoters

To localise H3K4ac throughout the genome, DNA recovered from ChIPs was analysed with high-resolution tiling arrays (Affymetrix 1.0R). Nucleosome density was controlled by normalising H3K4ac to the results of a ChIP experiment performed from the same cell lysates using an antibody against the non-modified C-terminal tail of H3 (H3-C).

The data showed that H3K4ac peaks are distributed along all chromosomes and relatively low levels of H3K4ac are observed at most sub-telomeric regions (Figure S2). This is most apparent on chromosome III, where H3K4ac is markedly depleted in large sub-telomeric regions that encompass HMLα and HMRα (Figure S2), where Sir2 was previously shown to promote histone deacetylation. Closer manual inspection of the results using the UCSC genome browser [58] indicated that the strongest peaks of H3K4ac are located at highly transcribed genes, such as ribosomal protein genes. Two examples (RPL37A and RPS31) are highlighted in Figure 3A. We performed a systematic analysis aimed at determining whether H3K4ac was generally enriched at promoters. For this purpose, we aligned H3K4ac data derived from genes with upstream intergenic regions (IGRs) that contain divergent promoters. Based on this criterion, these IGRs are devoid of DNA sequences corresponding to 3′-untranslated regions (3′-UTRs) and transcriptional termination sequences. This analysis revealed that H3K4ac peaks slightly upstream of the 5′-ends of genes (Figure 3B). Furthermore, the degree of H3K4ac enrichment correlated with transcriptional activity (as measured in [59]). For the most highly transcribed genes (50+ mRNA/hr), H3K4ac peaks upstream and appears to spread into coding regions (Figure 3B). In less frequently transcribed genes, H3K4ac also peaks just upstream of 5′-ends, but sharply drops in coding regions (Figure 3B). We performed a similar analysis for genes that have converging 3′-ends. The corresponding IGRs contain
3′-UTRs and transcriptional termination sequences, but are devoid of promoters. These chromosomal regions revealed only a modest enrichment for H3K4ac in the most highly transcribed gene group (Figure 3C, coordinates 0.0 and beyond). This effect probably results from the aforementioned spreading of H3K4ac peaks over the whole body of highly transcribed genes (compare

---

**Figure 2. HATs and HDACs That Contribute to H3K4ac In Vivo.** (A–B) Whole-cell lysates prepared from strains with deletions of known HAT encoding genes were analysed by immunoblotting with the indicated antibodies. (C) Quantification of the immunoblots shown in (B). Signals for each band were expressed in Arbitrary Units (AU) and, after background subtraction (A Ub), normalised to the H3-C signal measured in each strain. (D–E) Whole-cell lysates obtained from strains with deletions of known HDAC encoding genes were analysed by immunoblotting with the indicated antibodies. (F) Quantification of the immunoblots shown in (E), calculated as described above. (G) Chromatin immunoprecipitation (ChIP) was performed by quantitative PCR to determine the abundance of H3K4ac at different loci in WT, hst1Δ, sir2Δ or hst1Δ sir2Δ strains. The six target regions were divided into three groups: regions targeted by Sir2, gene promoters targeted by Hst1 and other gene regions not targeted by Sir2 or Hst1. The TEL03R primers amplify a portion of ARS319, a unique sequence region approximately 1 Kb from the telomeric repeats on the right arm of chromosome III. Results are expressed as a ratio of ChIP signals obtained from the same extracts with antibodies against H3K4ac and a non-modified H3 C-terminal peptide.

doi:10.1371/journal.pgen.1001354.g002
From this data, it is clear that H3K4ac peaks at gene promoters and the degree of enrichment is proportional to the rate of transcription.

H3K4ac is upstream of H3K4me3 at gene promoters

We found that H3K4ac was enriched at transcriptionally active promoters (Figure 3B) and it has been reported that H3K4me3 is also present at this location in active genes [20]. To explore the relationship between H3K4ac and H3K4me3, we compared our genome-wide H3K4ac localisation data with the previously published high-resolution data for H3K4me1, -me2 and -me3 [60]. Strong peaks of H3K4ac were generally located upstream of H3K4me3 in gene promoters, followed by the typical pattern of H3K4me2 and H3K4me1 enrichment along coding regions (Figure 4A and more examples in Figure S3). There is a considerable overlap between H3K4ac with H3K4me3, although their localisation patterns are not identical. We repeated the H3K4me3 ChIP-chip under our conditions (as described for H3K4ac) and our results (data not shown) produced an enrichment pattern nearly identical to the Kirmizis et al. dataset [60].

To investigate whether this pattern was evolutionarily conserved, we examined the localisation of H3K4 modifications in human CD4+ lymphocytes from previously published ChIP-seq data [19,51]. Manual inspection of the data with the UCSC genome browser revealed H3K4ac and H3K4me3 overlapping at many transcriptional start sites, but H3K4ac was slightly upstream of H3K4me3 (representative examples are shown in Figure S4). Interestingly, H3K4ac peaks also overlap with RNApol II more closely than H3K4me3 at these genes (Figure S4), suggesting a function for H3K4ac in transcriptional regulation in human cells.

From these data, we conclude that H3K4ac is present at transcribed gene promoters, and is adjacent to a unidirectional gradient of H3K4me3, -me2 and -me1 that proceeds from promoters into coding regions. This pattern of H3K4 modifications has been conserved during evolution.

Genome-wide overlap of H3K4ac and H3K4me3 at promoters

To investigate and compare the proportion of promoters that are enriched in H3K4ac or H3K4me3, we transformed the log-ratio data into Z-scores (see Materials and Methods). This was performed to normalise datasets with different scales, thus allowing a direct comparison of the H3K4ac and H3K4me3 datasets. We then established a threshold value above which a promoter was judged enriched in H3K4ac or H3K4me3 relative to H3. When the cut-off was set at the 90th percentile (only promoters with

Figure 3B and 3C). From this data, it is clear that H3K4ac peaks at gene promoters and the degree of enrichment is proportional to the rate of transcription.
Regulation and Function of H3K4ac in Yeast

A

chr1:
670001 680001 690001 700001 710001 720001 730001
H3K4ac
H3K4me3
H3K4me2
H3K4me1

B

IGRs with divergent promoters

H3K4ac / H3
H3K4me3 / H3

50+ mRNA / hr
16-50 mRNA / hr
4-16 mRNA / hr
1-4 mRNA / hr
0-1 mRNA / hr

Position relative to gene start

(0.744, 3.80) (0.744, 3.80)

C

Cut-off: 90th percentile
Total: 5768 genes

H3K4ac: 2216
H3K4me3: 2682

D

Cut-off: 80th percentile
Total: 5768 genes

H3K4ac: 3379
H3K4me3: 4527

E

Cut-off: 70th percentile
Total: 5768 genes

H3K4ac: 4131
H3K4me3: 5019

F

Cut-off
90th 80th 70th
H3K4ac 38% 59% 72%
H3K4me3 46% 78% 87%
H3K4ac + H3K4me3 76% 93% 96%
H3K4me3 63% 69% 79%
enrichment values scoring in the top 10% of all values), H3K4ac was found to be enriched at 38% of all gene promoters, whereas H3K4me3 was enriched at 46% of promoters (Figure 4C and 4F). Of the promoters enriched in H3K4ac, 74% were also enriched in H3K4me3. This value increased to 93% when the cut-off was set to the top 90th percentile (Figure 4D and 4F) and 96% at the top 70th percentile (Figure 4E and 4F), indicating that most promoters enriched in H3K4ac also have a significant enrichment in H3K4me3. However, a smaller proportion of H3K4me3-enriched promoters also contain H3K4ac: 63%, 69% and 79% for the top 90th, 80th and 70th percentile cut-offs respectively (Figure 4F).

Figure 4. H3K4ac Is Located Upstream of H3K4me3. (A) The H3K4ac / H3 (black) ChIP-chip data were aligned with the H3K4me3 (burgundy), -me2 (purple) and -me1 (blue) data from [60] and displayed in the UCSC genome browser (http://genome.ucsc.edu). Each vertical line represents one probe in the dataset and the intensity of the color is proportional to the enrichment of the modification. For clarity, only the probes with a log2 ratio above 0 are shown for each dataset. (B) Systematic alignment of H3K4ac / H3 (red shades) and H3K4me3 / H3 (blue shades) ChIP-chip data at intergenic regions (IGRs) with divergent promoters (as in Figure 3B). The raw ChIP-chip data were converted into Z-scores in order to be compared on the same scale (see Material and Methods). (C-E) Venn diagrams illustrating the overlapping number of promoters that are enriched for H3K4ac or H3K4me3. The cut-off indicates the H3K4(mod) / H3-C Z-score threshold at which a given promoter was judged to be enriched in either H3K4ac or H3K4me3. For example, with a cut-off at the 90th percentile (C), the red circle contains the number of promoters that have H3K4ac / H3 signal ratios in the top 10% of the genome. (F) A table displaying the data from the Venn diagrams as percentages.

Set1-mediated H3K4me2 and me3 limit the accumulation of H3K4ac

Since H3K4ac and H3K4me3 overlap considerably at the promoters of many genes, we sought to determine if there is a competition for the lysine 4 substrate. Based on mass spectrometry, we found that a SET1 deletion caused a global increase in H3K4 acetylation (Figure 5A). This result was corroborated by immunoblotting data showing that H3K4ac, but not H3K9ac was elevated in set1Δ cells (Figure 5B, 5C). The immunoblotting result truly reflected an increase in H3K4ac in set1Δ cells, rather than cross-reaction to other sites of acetylation, because no signal was observed in set1Δ H3-K4R mutant cells (Figure 5D). In addition, an H3K4ac peptide, but not H3K9ac or non-modified K4 peptides, could compete the signal observed with the K4-ac antibody in WT and set1Δ cells (Figure 5E). Set1 is part of COMPASS, a complex that has multiple subunits which contribute to the different degrees of H3K4 methylation (me1, me2 or me3) [61-63]. To determine which subunits of COMPASS were important to limit the accumulation of H3K4ac, we performed immunoblots using strains with single gene deletions of all the COMPASS subunits. Cells lacking SET1, BRE2, SDCI, SWS3 or SWD1 have in common a complete absence of H3K4me2 and me3, but mutations of these genes differentially affect H3K4me1 (Figure 5F). For instance, bre2Δ and seclΔ mutants have WT levels of H3K4me1 (Figure 5F, lanes 1, 3 and 5), whereas set1Δ, suddΔ and sweΔ1 mutants lack all forms of H3K4me (lanes 2, 6 and 8). Nonetheless, relative to WT cells, all these mutants increased H3K4ac to similar degrees (Figure 5F, compare lane 1 with lanes 2, 3, 5, 6 and 8). An sppΔ mutant with nearly normal levels of H3K4me2, but reduced H3K4me3 showed a moderate increase of H3K4ac (Figure 5F, lanes 1 and 7). In striking contrast, an shg1Δ mutant that retained normal levels of H3K4me2 and H3K4me3 did not show a significant increase in H3K4ac compared with WT cells (Figure 5F, lanes 1 and 4). These results argue that the increase in H3K4ac observed in several mutants of the COMPASS complex occurs mainly because of the absence of H3K4me2 and me3, rather than a lack of H3K4me1.

Since a global reduction in H3K4me2 and me3 led to an increase in H3K4ac, we wished to investigate if the opposite was also true. Immunoblots of cell lysates prepared from goa5Δ and goa3Δ set1Δ109Δ strains, with strongly reduced H3K4ac, showed no significant increase in any form of H3K4me4 (Figure 2B, 2C). Conversely, we observed no apparent decrease in H3K4me1, me2 or me3 in cells with elevated levels of H3K4ac (hst1Δ or hst1Δ set2Δ. (Figure 2E, 2F). Therefore, although H3K4ac increases in mutant cells lacking H3K4me2 and me3, changes in H3K4ac do not influence global levels of H3K4 methylation. These results suggest that the relative abundance of H3K4ac does not merely reflect a simple competition for the H3K4 substrate.

Set1 regulates H3K4ac at promoters and the 5′ end of coding regions

To see if the deletion of SET1 led to a change in the genome-wide distribution of H3K4ac, we performed ChIP-chip experiments to compare the localisation of H3K4ac in set1Δ and WT cells. Manual inspection of the data in the UCSC Genome Browser revealed changes in the distribution and the relative abundance of H3K4ac in set1Δ cells, at several gene promoters. For example, at the FKH1 and SUR7 genes, H3K4-ac is increased at the promoter region and spreads towards the coding region in the set1Δ strain (Figure 5G, 5H). Systematic alignment of divergent promoter regions showed that the deletion of SET1 led to a global increase in the relative abundance of H3K4ac at gene promoters (Figure 5I, blue versus black dots). We also observed a slight shift of H3K4ac towards the 5′ ends of the coding region in set1Δ cells (Figure 5J). To better assess the changes in localisation of H3K4-ac in the set1Δ strain, the H3K4-ac ChIP-chip data from WT cells were subtracted from those of set1Δ cells. This analysis clearly showed that the strongest difference in H3K4-ac occurs at position 0 relative to gene start (Figure 5I, green dots), which corresponds to the 5′ ends of coding regions. The fact that the shoulder of the H3K4-ac peak in the set1Δ strain is skewed over downstream sequences argues that this increase is not merely due to increased peak signal in set1Δ cells, since this would lead to a symmetric increase in each shoulder. Instead, this shows that, on average, H3K4-ac spreads further into the 5′ ends of coding regions in set1Δ cells. We also observed a global decrease in H3K4-ac at the 3′ ends of coding regions in set1Δ mutants (Figure 5I). The significance of this decrease is unknown. Nevertheless, we can conclude that the global increase in H3K4-ac observed in set1Δ mutant cells by MS and immunoblotting occurs more prominently at the promoters and 5′ ends of coding regions relative to other regions.

The global average increase of H3K4-ac in set1Δ cells likely occurs at a subset of genes and not all genes. Manual inspection of the datasets clearly shows that the H3K4-ac pattern remains unaffected at certain genes, for example GAL20 and YML053c (Figure 5H). To provide further evidence that the increase in H3K4-ac observed in the set1Δ strain did not occur equally at all genes, we performed ChIP-qPCR experiments in WT and set1Δ strains at three genes: CPY2, ARG7 and FKH1. These genes were selected because they respectively showed little, moderate and
large changes in H3K4ac in set1Δ strains according to our initial observations in the UCSC Genome Browser. Our ChIP-qPCR analyses confirmed the ChIP-chip results for H3K4ac in the set1Δ strain (Figure S5A-S5C). Therefore, although the absence of H3K4me2 and me3 leads to a global increase in H3K4ac (Figure 5A, 5B), ChIP assays demonstrate that the increase in H3K4ac is more prominent at some genes than others.

H3K4ac and gene expression

Since H3K4ac is enriched at highly transcribed gene promoters, we explored the possibility that H3K4ac might play a role in gene transcription. Genetic analysis of the function of H3K4ac is complicated by the fact that mutation of H3K4 abolishes both acetylation and methylation. To circumvent this problem, we compared the global mRNA profile of a set1Δ strain, which lacks...
Figure 6. Global mRNA Expression Profiles of set1Δ and H3-K4R Mutant Strains. (A) Graph of mRNA expression fold-change relative to WT cells in the H3-K4R (blue) and set1Δ (yellow) strains for all the analysed genes. The genes were ranked according to log2 fold-change in the H3-K4R strain and a sliding average (window = 50, step = 1) was applied to the data on the y-axis. The bottom panels are heat map illustrations of the log2 fold-change, where yellow indicates an increase in mRNA abundance in the mutant strains relative to WT cells and blue indicates a decrease. (B–C) Venn diagrams showing the overlap between groups of genes up-regulated (B) or down-regulated (C) at least two-fold when either the H3-K4R or the set1Δ strain is compared with WT cells. (D) Graph of the H3K4ac / H3 ratio at the promoter regions of each gene (average for from −200 to +1bp relative to the start codon) derived from the ChIP-chip data as a function of their log2 fold-change in mRNA abundance in the H3-K4R mutant strain.
H3K4me but retains H3K4ac, with that of an H3-K4R mutant where both H3K4 acetylation and methylation are lost. In order to get a global view of the expression profiles in both mutants, we ranked the genes by log2 fold change in mutant strains relative to WT cells (Figure 6A). At one end of the spectrum, we found many genes that are up-regulated in both the H3-K4R and the set1Δ mutant strains (Figure 6A, left part). Many of these genes are up-regulated only in H3-K4R mutant cells (Figure 6A, 6B). One possibility is that expression of some of these genes may be affected simply because of the lysine-to-arginine mutations, rather than a lack of modification. However, there is considerable overlap between the genes up-regulated in H3-K4R and set1Δ mutant strains (Figure 6B). We conclude that the repressed state of these genes in WT cells is likely to be dependent on H3K4me, rather than H3K4ac. This is consistent with previous reports showing a repressive function for SET1 [64-66].

At the other end of the spectrum, we found a group of genes which were down-regulated in the H3-K4R strain, but were unaffected in the set1Δ strain (Figure 6A, right part), suggesting a positive role for H3K4ac in the expression of these genes. We identified 37 genes that were down-regulated 2-fold or more in the H3-K4R strain and 44 in the set1Δ strain. Strikingly, there was no overlap between these two groups of genes (Figure 6C), suggesting a function of H3K4 in gene expression that is independent of methylation. We next compared the H3K4ac abundance at promoter regions in WT (from our ChIP-chip dataset) with the expression change of the gene in the H3-K4R strain. The genes that are down-regulated in the H3-K4R strain have high levels of H3K4ac at their promoters in WT cells (Figure 6D), which is consistent with a direct role of H3K4ac in gene expression. Gene ontology term enrichment analysis of the genes down-regulated 2-fold or more in the H3-K4R strain, but whose expression was not affected in a set1Δ strain, identified genes encoding amino acid transporters (p = 1.42×10⁻⁴; BAP2/YBR068C, TAT1/YBR069C, BAP3/YDR046C, GNPI/YDR508C, TAT2/YOL020W). Inspection of our ChIP-chip data for these genes confirmed that they all had high levels of H3K4ac at their promoters in WT cells (Figure 6E-6H). We confirmed by RT-qPCR that these genes are negatively regulated in a H3-K4R strain, but unaffected in a set1Δ strain (Figure 6I-6M). In contrast to the other RNAs that we monitored, the expression of the TAT2 RNA was unexpectedly restored in a set1Δ H3-K4R double mutant strain, suggesting an indirect effect of the SET1 deletion on this gene (Figure 6M). Taken together, our data suggest that H3K4ac has a positive and direct role in the transcription of a subset of yeast genes.

Discussion

Histone H3K4 methylation is an extensively studied modification in a wide range of organisms, and particularly in the budding yeast Saccharomyces cerevisiae. Using MS and highly specific antibodies we demonstrated that H3K4 is also acetylated in budding yeast. An earlier study of histone modifications had shown that both H3K4ac and H3K4me exist in human, mouse and ciliates [32] and, very recently, in Schizosaccharomyces pombe [67]. The versatility of genetic tools available in S. cerevisiae, and the ability to mutate histone H3K4, has enabled us to uncover a positive function for H3K4ac in the expression of a subset of genes that carry H3K4ac in their promoters. The presence of H3K4ac at active gene promoters has been conserved in human CD4+ cells [51], which suggests a similar role for H3K4ac in gene expression in mammals. We also discovered that the COMPASS complex and H3K4me2 and mc3 appear to globally limit the abundance of H3K4ac at promoters and its spreading into the 5'-coding regions of many genes.

Acetylation of the H3 N-terminal tail by specific HATs

Our targeted screen of known HAT-encoding genes has allowed us to identify GCN5 and RTT109 as responsible for H3K4ac in vivo. Gcn5 is the catalytic subunit of the ADA, SAGA, SLIK/ SALS transcriptional co-activator complexes [68] and has been highly conserved during evolution [69]. Our results are consistent with the fact that Gcn5 has been shown to acetylate multiple lysine residues within the H3 N-terminal tail [70-71] and is localised to active gene promoters dispersed throughout the genome [56]. Interestingly, RTT109 is also important for the acetylation of H3K4. Furthermore, the gen5 Δ rtt109Δ double mutant has markedly less H3K4ac than the single mutants. Collectively, our results indicate that GCN5 and RTT109 promote H3K4ac via different pathways and/or in different contexts. If they were fully redundant, there should be no decrease in H3K4ac in the single mutants, which is not the case. However, we cannot exclude the possibility that they may also have partially redundant functions in mediating H3K4ac. RTT109 encodes a HAT that mediates H3K5ac [72-75], which is involved in nucleosome assembly during DNA replication and the DNA damage response [76-78]. In addition, Rtt109 has recently been implicated in acetyllating H3K9 and H3K27 in vivo and in vitro [54,79-80]. As is the case for H3K4ac, H3K9ac and H3K27ac are virtually undetectable by immunoblotting and mass spectrometry in gen5Δ rtt109Δ cells [79]. Therefore, the same HATs are responsible for the bulk of both H3K4 and H3K9 acetylation.

H3 N-terminal tail acetylation and gene transcription

The genome-wide localisation pattern of H3K4ac showed strong peaks at the promoters of active genes. This enrichment has also been observed in the genome-wide patterns of other acetylated lysines in the H3 N-terminal tail, such as H3K9ac, H3K14ac and H3K18ac [20-21], suggesting that these modifications often occur together on chromatin. Our microarray analysis identified a relatively small group of genes (37 genes down-regulated by 2-fold or more, Figure 6C) that require H3K4ac, but not H3K4me, for normal expression. However, based on our ChIP-chip data, many other promoters contain high levels of H3K4ac. It is tempting to speculate that acetylation of H3K4 functions in a partially redundant manner with other sites of acetylation at many promoters to increase accessibility to the transcriptional initiation machinery and thereby facilitate gene expression. Interestingly, our gene ontology term analyses revealed a group of amino acid transporter genes (BAP2, BAP3, TAT1, TAT2, GNPI) that are co-regulated by levels of extracellular amino acids [81-84]. An interesting possibility is that the transcription factors involved in their expression require H3K4ac either alone
Regional and functional specificity of HST1 and SIR2

We identified a role for HST1 in global deacetylation of H3K4 in euchromatin. HST1 encodes a class III HDAC found in at least two complexes. Hst1 is part of a complex with Sum1 and Rfm1 [85-86]. Interestingly, the deletion of SUM1 did not result in a significant global increase in H3K4ac based on immunoblotting (data not shown). Hst1 has also been found as a sub-stoichiometric subunit of the SET3 complex (SET3C) [87], which represses middle-sporulation gene during mitosis [88]. However, neither deletion of HOS2 (encoding the main HDAC in the complex) (Figure 2C), nor that of any other components of SET3C led to a global increase in H3K4ac (data not shown). Our results suggest that Hst1 might act in a non-targeted manner to promote global deacetylation of H3K4. Alternatively, Hst1 might be part of another, as yet unidentified complex involved in H3K4 deacetylation.

Interestingly, we also observed some redundancy between HST1 and SIR2 for deacetylation of H3K4 in euchromatic regions. In specific conditions, Hst1 and Sir2 have been shown to act outside of the chromosomal regions where they “normally” exert their functions. For example, overexpression of HST1, but not SIR2 or HST3, can specifically compensate for the loss of silencing at HMRα that occurs in a sir2Δ mutant [89]. Our results are also consistent with studies showing a strong connection between these two genes. Both genes share 71% identity in their sequence over 84% of the length of HST1 [90]. SIR2 was previously shown to affect the expression of euchromatic genes when HTZ1 and SET1 were deleted [91] and to interfere with the firing of a number of replication origins [92]. Moreover, studies of chimaeric proteins demonstrated that the N-terminal domains of Hst1 and Sir2 control the chromosomal location and function of both proteins. In contrast, other domains of Hst1 and Sir2 could be swapped, supporting the notion that these enzymes have similar substrate specificity [93]. We propose here that H3K4 is an important target for deacetylation by both Hst1 and Sir2. HST1 and SIR2 are both part of the same phylogenetic branch (subgroup Ia) of NAD+-dependent HDACs (class III) that also includes human SIRT1 and Drosophila Sir2 [94], which are, therefore, likely candidates for deacetylation of H3K4 in these organisms.

Patterns of mutually exclusive histone modifications

The relationship between H3K9ac and H3K4me differs from that of other modifications that occur on the same lysine residue. The best studied case is that of H3K9, which is either acetylated or methylated. H3K9ac and H3K9me are present at different genomic regions [51] and have opposing roles in transcriptional regulation. H3K9ac is found in promoter regions and stimulates transcription through the recruitment of TFII D [95], whereas H3K9me2 and me3 contribute to gene repression and pericentric heterochromatin structure by binding HP1 proteins through their chromo-domains [96-97]. In this case, deacetylation of H3K9 is essential for the establishment of H3K9 methylation [98-100]. Another example is H3K36ac and H3K36me3, which are respectively present in promoters and coding regions of transcriptionally active genes [101]. In contrast to these modifications, H3K3-ac overlaps considerably with H3K4me3 in promoter regions. Furthermore, H3K4me2/me3 globally limit the levels of H3K9-ac, which suggests the existence of mechanisms to establish the correct patterns of localisation of these mutually exclusive modifications. For example, H3K4me2/me3 could prevent H3K9-ac from occurring in gene coding regions by simple competition for the H3K4 substrate. Another possibility is that H3K4 methylation might be a prerequisite step in a negative feedback loop that enables Hst1 to fine tune the levels of H3K4 acetylation in promoters and 5′-coding regions. Mechanisms that confine H3K4ac to some promoters may contribute to prevent spurious transcriptional initiation events [102] or attenuation of transcriptional initiation [66]. The potential to restrict the spreading of histone H3K4ac suggests a novel function for H3K4 methylation and reveals a previously unrecognised layer of chromatin regulation linked to the regulation of transcription in Drosophila.

Materials and Methods

H3K4ac antibody production and validation

Immunisation procedures and animal handling were carried out by Eurogentec. Sera from ten animals were screened prior to immunisation. Rabbits were immunised with the following peptide: ART[acetyl-K]QTARKSC. The efficiency of antibody production was monitored using ELISA. The antibody was affinity-purified using the same peptide coupled to a Sulfolink column (Thermo) and its specificity tested by dot blots with synthetic peptides and experiments where 1 μg/ml of the peptides were incubated with the antibody prior to immunoblotting.

Yeast strains and media

For most experiments, yeast strains were grown at 30°C in YPD (1% yeast extract, 2% peptone, 2% dextrose). A complete list of yeast strains is available in Table S1.

Whole-cell lysates and immunoblots

Yeast whole-cell lysates were prepared using an alkaline lysis protocol [103]. Essentially, 5×10^7 cells were resuspended in 500 μl of distilled water and 500 μl of 0.2 M NaOH was added. After 5 min at room temperature, cells were spun down, and resuspended in 100 μl SDS-PAGE sample buffer, boiled for 3 min and spun down again. About 7 μl (equivalent to 3.5×10^6 cells) of supernatant was typically loaded per lane and, after electrophoresis through a 15%-polyacrylamide gel, proteins were transferred to PVDF membranes using a semi-dry apparatus with 29 mM Tris, 193 mM glycine, 0.02% SDS, 5% methanol for 2 h at 1 mA /cm^2 (set maximum:15 V). We used the following dilutions for antibody incubations: anti-H3K4ac: 1/500, anti-H3K9Ac (Cell Signaling): 1/1000 diluted, anti-H3K9ac (Millipore 07-539): 1/10 000, anti-H3K56ac (Millipore 07-677): 1/5000.

Histone sample preparation and mass spectrometry

Histones were acid extracted from setΔ hstΔ cells essentially as described previously [104], except that we added 100 mM sodium butyrate and 100 mM nicotinamide in the nuclear isolation buffer and wash buffers. H3 was purified and digested as previously described [105]. Intact core histones (approximately 10 μg of total protein acid extract) were separated using an Agilent 1200 HPLC system equipped with a fraction collector. Separations were performed using an ACE C8 column (5 μm, 300 Å, 150×4.6 mm i.d., with a solvent system consisting of 0.1% trifluoroacetic acid (TFA) in water (v/v) (A) and 0.1% TFA in acetonitrile (v/v) (B). Gradient elution was performed from 5–70% B in 60 minutes at 0.7 ml/min. Fractions were collected in conical tubes in 60 second time slices. Individual histone peaks that eluted over multiple fractions were pooled together. Histone fractions were evaporated in a Speed-Vac. Dried fractions were resuspended in 0.1 M ammonium bicarbonate (without pH adjustment) and digested overnight at 37°C using 1 μg of trypsin. Samples were acidified
with 5% TFA in water (v/v) prior to LC-MS/MS analysis. Tryptic
digests of histone extracts were injected onto a Thermo Electron
LTQ-Orbitrap XL mass spectrometer equipped with an Eksigent
nanoLC separation module. Samples were loaded onto a C18
trapping column at 10 μL/min using 0.2% formic acid (v/v) in
water. Peptides were eluted onto a C18 analytical column (10
cm x 150 μm I.d.), at 0.6 μL/min. Gradient elution was performed
using a solvent system of 0.2% formic acid in water (A), and 0.2%
formic acid in acetonitrile (B). Peptides were separated by gradient
elution from 0 to 70% B in 60 minutes. For mass calibration, we
used an internal lock mass [protonated (Si(CH3)2O)4]+: m/z
445.12057. In each data collection cycle, one full MS scan (m/z
300–2000) was acquired in the Orbitrap (60000 resolution setting,
automatic gain control (AGC) target of 106), followed by 3 data-
dependent MS/MS scans in the LTQ (AGC target 10000;
threshold 5000) for the 3 most abundant ions and collision induced
dissociation (CID) for fragmentation.

Chromatin immunoprecipitation (ChIP)

Extracts containing fragments for chromatin immunoprecipitation
(ChIP) were prepared as follows. 200 ml of exponentially
growing cells (O.D.600 of 0.6 to 0.8) in YPD were treated with 1%
formaldehyde at room temperature for 10 minutes. Formaldehyde
was quenched with 0.125 M of glycine and cells were washed three
times in cold water. Pellets (approximately 1.2 x 109 cells) were
resuspended in 1.2 ml of lysis buffer (50 mM HEPES-KOH pH
7.5, 140 mM NaCl, 1% Triton X-100, 0.1% Na-deoxycholate,
1 mM EDTA, 1 mM PMSF, 30 mM sodium butyrate, complete
EDTA-free protease inhibitor cocktail, Roche), and split into three
tubes. After adding one volume of glass beads to each sample, cells
were disrupted using a bead beater (Disruptor Genie, Scientific
Industries) at maximal speed for 2h at 4°C. After removing the
glass beads, the lysates were transferred to fresh tubes and
sonicated for 15 minutes (30 seconds ON, 30 seconds OFF) at high
intensity in a Bioruptor (Diagenode) connected to a water cooler at
4°C. Lysates were clarified by centrifugation and pooled into a 2 ml
tube. After addition of 800 μl of fresh lysis buffer and mixing,
lysates were clarified again by centrifugation. 4 μl of lysate (1% of
the whole cell extract, WCE) was kept aside as an input control.
For immunoprecipitation, aliquots of 400 μl were prepared for each
ChIP and mixed with the appropriate antibody: either 5 μl of anti-
H3K4-ac, 5 μl of anti-H3-C (Abcam ab1791), 3 μl of anti-
H3K4me3 (Abcam ab8580) for at least 1h at 4°C. Before use,
Dynabeads M-280 coupled to sheep anti-rabbit IgG (Invitrogen)
(30 μl per ChIP) were washed in lysis buffer containing 5 mg/ml
bovine serum albumin. The beads were added to each lysate and
incubated at 4°C for at least 1 h. Beads were washed twice with
lysis buffer, twice with lysis buffer containing 500 mM NaCl, twice
in wash buffer (10 mM Tris-HCl pH 8.0, 250 mM LiCl, 0.5%
Nonidet-P40, 0.5% Na-deoxycholate, 1 mM EDTA) and once in
TE (10 mM Tris-HCl pH 8.0, 1 mM EDTA). To elute the DNA,
100 μl of a 10% Chelex 100 (Biorad) suspension in water was
delivered to the beads (as well as to the 1% WCE) and incubated at
100°C for 10 minutes. Tubes were then cooled and treated with
0.2 μg/μl ribonuclease A at 30°C for 30 min, followed by 0.2 μg/
μl proteinase K at 50°C for 30 min. Samples were incubated again
at 100°C for 10 min to inactivate the proteinase K and then
cooled down and spun down to get rid of debris. 80 μl of each
supernatant was kept at –20°C for qPCR or ChIP-chip analysis.

ChIP-chip

All ChIP-on-chip datasets have been deposited in NCBI’s
Gene Expression Omnibus [106] and are accessible through GEO
Series accession number GSE27307 [http://www.ncbi.nlm.
nih.gov/geo/query/acc.cgi?acc=GSE27307]. Samples were
processed according to the Affymetrix protocol for amplification,
fragmentation and labelling of DNA for hybridisation to a GeneChip S. cerevisiae Tiling 1.0R Array (www.affymetrix.com). All ChIP-chips were performed in triplicate from independent
colonies. Pre-processing of the data was carried out with the
Affymetrix Tiling Analysis Software with default settings at 200bp
bandwidth and expressed as a log2 ratio of the ChIP signal
obtained for modified H3 (K4ac or K4me3) over that obtained
from non-modified C-terminal H3 ChIP. The datasets used in
Figure 4C and Figure 5F, 5G were normalised using the standard
score (Z-score) equation: 

\[ Z = \frac{x - \mu}{\sigma} \]

where \( x \) is the log2

\[ \text{H3K4(mod) / H3-C} \]

test value for each probe; \( \mu \) is the mean raw value of all probes and \( \sigma \) is the standard deviation of the raw
values for all probes. The resulting datasets have a normal distribution with a mean value of 0 and a standard
deviation value of 1, and thus can be compared directly on the same scales.

Data was displayed on the UCSC genome browser with the
October 2003 S.cerevisiae genome assembly [http://genome.ucsc.
.edu/]. Systematic alignment of ChIP-chip data to gene promoters and
terminators were performed using the database management tools
provided in the web application Galaxy [http://main.g2.bx.
psu.edu/] and graphics were created with R [http://www.
r-project.org/].

Promoter enrichment analyses and Venn diagrams

Gene promoters were defined as a genomic region ~500 bp to
+100 bp relative to gene start. Promoters containing Z-score
values above the threshold value (90th percentile: \( Z > 1.28 \); 80th
percentile: \( Z > 0.84 \); 70th percentile \( Z > 0.52 \)) were compared
using the web application BioVenn [http://www.cmbi.ru.nl/cdd/biovenn/].

RNA extraction and expression analysis using microarrays

Total RNA was extracted from three independent colonies
using the hot phenol method as described previously [107]. A total
of 7 μg of RNA were processed for microarray analysis using the
One-Cycle Target Labeling package from Affymetrix and
hybridised to a GeneChip Yeast Genome 2.0 Array following
the manufacturer’s instructions [http://www.affymetrix.com/].
Probe intensity data was pre-processed using the mas5 program
of the R-Bioconductor software [http://www.bioconductor.org/
index.html] [108]. The data from either \( H3/K4R \) or \( set1\Delta \) mutants
were normalised to their WT counterpart and the genes were
sorted according to log2 fold change. The log2 fold change
values for all genes are presented in Table S2 and represented graphically
in Figure S6. For RT-qPCR analyses, 1 μg of total RNA was
treated with DNase I, which was then inactivated following the
manufacturer’s instructions (DNA-free kit, Ambion). RT was
performed using the First-Strand cDNA Synthesis Kit with
MLV RT (Invitrogen) and oligo-dT primers, following the
manufacturer’s instructions.

Quantitative PCR

1 μl of the ChIP sample was mixed with 50 nM of region-
specific primers and SYBR Green JumpStart Taq ReadyMix
(Sigma) in a total volume of 20 μl and analysed on an Opticon
real-time PCR machine (MJ Research/Bio-Rad). Relative
binding values were extrapolated by subtracting the Ct of non-
modified H3-C to that of modified H3 (K4ac or K4me3), and
converting the ACt with the following formula: 

\[ x = 2^{-ACt} \]

where \( x \) represents the relative binding value of modified H3 on non-
modified H3-C.
Supporting Information

Figure S1 Nicotinamide Treatment Increases H3K4ac in WT and set1Δ cells. Whole-cell lysates from cells treated with 0, 5 or 25 mM nicotinamide for the indicated times were analysed by immunoblotting.

Found at: doi:10.1371/journal.pgen.1001354.s001 (0.40 MB TIF)

Figure S2 Genome-Wide Location of H3K4ac along the 16 S. cerevisiae Chromosomes. The log2 of the H3K4ac / H3 derived from ChIP-chip data was aligned to the chromosomes in the UCSC genome browser (http://genome.ucsc.edu). The graphs are drawn to scale for chromosome length, except for the chromosome XII, on which the repeated ribosomal DNA locus is represented drawn to scale for chromosome length, except for the chromosome

The y-axis is scaled according to maximal and minimal values for each individual chromosome. In general, regions that are enriched (peaks) or depleted (troughs) for H3K4ac are interspersed and evenly distributed across all chromosomes. One notable exception is the ends of chromosome III, where large regions that extend from telomeres to beyond the silent mating type loci (HMLα and HMRα; highlighted) are depleted in H3K4ac.

Found at: doi:10.1371/journal.pgen.1001354.s002 (2.00 MB TIF)

Figure S3 Localisation of H3K4-ac, H3K4me3, -me2 and -me1 at several genomic regions in yeast. The log2 of the H3K4-ac / H3 derived from ChIP-chip data was aligned to the chromosomes in the UCSC genome browser and aligned with the H3K4me1, me2 and me3 data published previously [59]. Each vertical line represents one probe in the dataset and the intensity of the color is proportional to the enrichment of the modification. For clarity, only the probes with a log2 ratio above 0 are shown for each dataset.

Found at: doi:10.1371/journal.pgen.1001354.s003 (1.05 MB TIF)

Figure S4 Localisation of H3K4 Modifications (H3K4ac, H3K4me3, H3K4me2, H3K4me1) and RNA polymerase II (Pol II) in Human CD4 T Cells. ChIP-seq data from human CD4+ lymphocytes previously published by the Zhao laboratory [19,51] were displayed on the UCSC genome browser (http://genome.ucsc.edu/). Genes are illustrated at the bottom of each panel with an arrow indicating the transcriptional start site and direction.

Found at: doi:10.1371/journal.pgen.1001354.s004 (2.50 MB TIF)

Figure S5 ChIP-qPCR confirmation of H3K4ac regulation by SET1 at specific gene promoters. We performed ChIP against either H3K4ac (top row), H3K4me2 (middle row) or H3K4me3 (bottom row) in WT and set1Δ cells. We analysed the enrichment over three genomic regions by qPCR: CPA2 (A), ARG7 (B) and FKH1 (C) which showed low, moderate and high increase in H3K4ac in set1Δ cells compared to WT in the coding region in our ChIP-chip analyses. Modification-specific ChIPs were normalised with ChIPs against non-modified H3-C using the same whole cell extract and experiments were done in biological triplicates.

Found at: doi:10.1371/journal.pgen.1001354.s005 (0.51 MB TIF)

Figure S6 Gene Expression Changes in H3-K4R and set1Δ Mutants Relative to WT Cells. All genes were color-coded and ranked according to log2 fold change in the H3-K4R strain or the set1Δ strain versus WT cells. On the right, the top and bottom part of the graph were cropped to reveal the general overlap between the genes that are up-regulated (yellow), but not down-regulated (blue). The right part of the figure contains the list of all genes with a fold-change equal or greater than two in the H3-K4R strain. A complete list of all the genes and their fold-change values is presented in Table S2.

Found at: doi:10.1371/journal.pgen.1001354.s006 (2.17 MB TIF)

Table S1 Yeast Strains Used in this Study.

Found at: doi:10.1371/journal.pgen.1001354.s007 (0.07 MB DOC)

Table S2 Genes and their fold-change values.

Found at: doi:10.1371/journal.pgen.1001354.s008 (1.59 MB XLS)

Acknowledgments

We are grateful to Drs. A. Morillon, S. Dent, M. Grunstein, J. Greenblatt, J. Boeke, and V. Gelli for the gift of strains. We also thank L. Aragon, M. Ledu, P.-E. Jacques, S. Drozin, S. Lemieux, L. Game, J. Dennis, T. Bradley, P. Wijchers, R. Allshire, and members of the Festenstein and Verreault labs for helpful discussions.

Author Contributions

Conceived and designed the experiments: BG AV RJF. Performed the experiments: BG PD HHSL HA AI E ’B PT. Analyzed the data: BG PD HHSL HA AI E ’B PT. Wrote the paper: BG AV RJF.

References

1. Kouzarides T (2007) Chromatin modifications and their function. Cell 129: 693–705.
2. Sun ZW, Allis CD (2002) Ubiquitination of histone H2B regulates H3 methylation and gene silencing in yeast. Nature 418: 104–108.
3. Dover J, Schneider J, Tawiah-Boateng MA, Wood A, Dean K, et al. (2002) Methylation of histone H3 by COMPASS requires ubiquitination of histone H2B by Rad6. J Biol Chem 277: 23638–23671.
4. Fischle W, Tseng BS, Dormann HL, Ueberheide BM, Garcia BA, et al. (2005) Regulation of H3K4-methylation can be used to distinguish between transcriptionally active and inactive human genes. Nature 436: 1116–1122.
5. Jenuwein T, Allis CD (2001) Translating the histone code. Science 293: 1074–1080.
6. Turner BM (2002) Cellular memory and the histone code. Cell 111: 261–291.
7. Telle MB, Jensen ON (2007) Functional proteomics in histone research and epigenetics. Expert Rev Proteomics 4: 491–505.
8. Brumback B, Phanstiel D, Coss JJ (2008) Unraveling the histone’s potential: a proteomics perspective. Epigenetics 3: 254–257.
9. Zhang L, Eugenin EE, Parthun MR, Fresta MA (2005) Identification of novel histone post-translational modifications by peptide mass fingerprinting. Chromosoma 112: 77–86.
10. Shilatifard A (2008) Molecular implementation and physiological roles for histone H3 lysine 4 (H3K4) methylation. Curr Opin Cell Biol 20: 341–348.
11. Ruthebusl AJ, Allis CD, Wysocka J (2007) Methylation of lysine 4 on histone H3: intricacy of writing and reading a single epigenetic mark. Mol Cell 25: 15–30.
12. Bernstein BE, Humphrey EL, Erlich RL, Schneider R, Bounam P, et al. (2002) Methylation of histone H3 Lys 4 in coding regions of active genes. Proc Natl Acad Sci U S A 99: 8695–8700.
13. Strahl BD, Olba R, Cook RG, Allis CD (1999) Methylation of histone H3 at lysine 4 is highly conserved and correlates with transcriptionally active nuclei in Tetrahymena. Proc Natl Acad Sci U S A 96: 14967–14972.
14. Schneider R, Bannister AJ, Myers FA, Thorne AW, Crane-Robinson C, et al. (2003) Histone H3 lysine 4 methylation patterns in higher eukaryotic genes. Nat Cell Biol.
15. Schulze D, MacAlpine DM, Scalzo D, Wirbelauer C, Kooperberg C, et al. (2004) The histone modification pattern of active genes revealed through genome-wide chromatin analysis of a higher eukaryote. Genes Dev 18: 1263–1271.
16. Santos-Rosa H, Schneider R, Bannister AJ, Sherriff J, Bernstein BE, et al. (2002) Active genes are tri-methylated at K4 of histone H3. Nature 419: 407–411.
17. Guenther MG, Levine SS, Boyer LA, Jaenisch R, Young RA (2007) A chromatin landmark and transcription initiation at most promoters in human cells. Cell 130: 77–88.
18. Ng HH, Robert F, Young RA, Struhl K (2003) Targeted recruitment of Set1 histone methylase by elongating Pol II provides a localized mark and memory of recent transcriptional activity. Mol Cell 11: 709–719. 
19. Barski A, Cuddapah S, Cui K, Roh TY, Schones DE, et al. (2007) High-resolution profiling of histone modifications in the human genome. Cell 129: 823–837.
24. Wysocka J, Swogor T, Xu H, Milne TA, Kwon SY, et al. (2006) A PHD finger of NURF couples histone H3 lysine 4 trimethylation with chromatin remodelling. Nature 442: 96–99.

25. Li H, Ilin S, Wang W, Duncan EM, Wysocka J, et al. (2006) Molecular basis for site-specific read-out of histone H3K4me3 by the BPTF PHD finger of NURF. Nature 442: 91–95.

26. Shi X, Hong T, Walter KL, Ewalt M, Michishita E, et al. (2006) ING2 PHD domain links histone H3 lysine 4 methylation to active gene repression. Nature 442: 85–90.

27. Martin DG, Baetz K, Shi X, Walter KL, MacDonald VE, et al. (2006) The Ynp1p plant homeodomain is a methyl-histone binding module that recognizes lysine 4-methylated histone H3. Mol Cell 26: 7671–7679.

28. Shiro K, Sachdevia J, Walter KL, Khorrami S, Schneider JF, et al. (2002) Protein-provide analysis in Saccharomyces cerevisiae identifies several PHD fingers as novel direct and selective binding modules of histone H3 methylated at either lysine 4 or lysine 36. J Biol Chem 276: 2450–2455.

29. Lan F, Collins RE, De Cegli R, Alpatov R, Horton JR, et al. (2007) High-throughput genomics analysis in Saccharomyces cerevisiae identifies several PHD fingers as novel direct and selective binding modules of histone H3 methylated at either lysine 4 or lysine 36. J Biol Chem 282: 7641–7653.

30. Roisen F, Pokholok DK, Harbison CT, Levine S, Cole M, et al. (2005) Organizational differences in post-translational modifications in histones H3 and H4. J Biol Chem 280: 7641–7653.

31. Suka N, Suka Y, Carmen AA, Wu J, Grunstein M (2001) Highly specific antibodies determine histone acetylation site usage in yeast heterochromatin and euchromatin. Mol Cell 7: 473–479.

32. Fillingham J, Rech J, Silva AG, Suter B, Emilii A, et al. (2008) Chaperone control of the activity and specificity of the histone H3 acetyltransferase Rtt109. Mol Cell 30: 434–453.

33. Rusche LN, Kirkmaier AL, Rinn J (2005) The establishment, inheritance, and function of silenced chromatin in Saccharomyces cerevisiae. Annu Rev Biochem 74: 481–516.

34. Robert F, Pokholok DK, Hannett NM, Rinaldi NJ, Chandy M, et al. (2004) Global position and recruitment of H3ATs and H3DATs in the yeast genome. Mol Cell 16: 199–209.

35. Lokhmotov MA, Rusche LN (2007) Substitution as a mechanism for genetic robustness: the duplicated deacetylases Hist1p and Sir2p in Saccharomyces cerevisiae. PLoS Genet 3: e126. doi:10.1371/journal.pgen.0030126.

36. Karolchik D, Baertsch R, Diekhans M, Furey TS, Hinrichs A, et al. (2003) The UCSC Genome Browser Database. Nucleic Acids Res 31: 55–59.

37. Holberg FC, Jennings EG, Wyrick JJ, Lee TI, Hengartner CJ, et al. (1998) Dissecting the regulatory circuitry of a eukaryotic genome. Cell 95: 717–728.

38. Kirmizis A, Santos-Rosa H, Penkett CJ, Singer MA, Veeman M, et al. (2007) Arginine methylation at histone H3R2 controls deposition of H3K4 methylation. J Biol Chem 282: 4929–4932.

39. Deich PM, Dichtl B, Schaff D, Rogaschek, Wilm M, et al. (2006) Protein-provide analysis in Saccharomyces cerevisiae identifies several PHD fingers as novel direct and selective binding modules of histone H3 methylated at either lysine 4 or lysine 36. J Biol Chem 282: 7641–7653.

40. Nightingale KP, Gendreizig S, White DA, Bradbury C, Hoffelder F, et al. (2007) Cross-talk between histone modifications in response to histone deacetylase inhibitors: ML14 links histone H3 acetylation and histone H3K4 methylation. J Biol Chem 282: 4408–4416.

41. Young NL, Dimaggio PD, Hsu Y, Madian MD, Balbanc RC, Flandus CA, et al. (2009) High-throughput characterization of combinatorial histone codes. Mol Cell Proteomics. 8. 3053–3070.
91. Venkatasubrahmanyam S, Hwang WW, Meneghini MD, Tong AH, Derbyshire MK, Weinstock KG, Strathern JN (1996) HST1, a new member of the SIR2 family of genes. Yeast 12: 631–640.
92. Crampton A, Chang F, Pappas DL, Jr., Frisch RL, Weinstein M (2008) An ARS element inhibits DNA replication through a SIR2-dependent mechanism. Mol Cell 30: 156–166.
93. Mead J, McCord R, Youngster L, Sharma M, Gartenberg MR, et al. (2007) Switching the gene-specific and regional silencing specificities of the Hst1 and Sir2 histone deacetylases. Mol Cell Biol 27: 2466–2475.
94. Frye RA (2000) Phylogenetic classification of prokaryotic and eukaryotic Sir2-like proteins. Biochem Biophys Res Commun 273: 793–798.
95. Apalzi T, Chen G, Thanos D (2002) Deciphering the transcriptional histone acetylation code for a human gene. Cell 111: 381–392.
96. Lacnner M, O’Carroll D, Rea S, Mechler K, Jennewein T (2001) Methyltransferase of histone H3 lysine 9 creates a binding site for H3P proteins. Nature 410: 116–120.
97. Bannister AJ, Zegerman P, Partridge JF, Misra EA, Thomas JO, et al. (2001) Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. Nature 410: 120–124.
98. Shankaranarayana GD, Motamedi MR, Meazed D, Grewe SI (2003) Sir2 regulates histone H3 lysine 9 methylation and heterochromatin assembly in fission yeast.Curr Biol 13: 1240–1246.
99. Nakayama J, Rice JC, Strahl BD, Allis CD, Grewe SI (2003) Role of histone H3 lysine 9 methylation in epigenetic control of heterochromatin assembly. Science 292: 110–113.
100. Rea S, Eisenhaber F, O’Carroll D, Strahl BD, Sun ZW, et al. (2000) Regulation of chromatin structure by site-specific histone H3 methyltransferases. Nature 406: 593–599.
101. Morris SA, Rao B, Garcia BA, Hake SB, Diaz RJ, et al. (2007) Identification of histone H3 lysine 36 acetylation as a highly conserved histone modification. J Biol Chem 282: 7632–7640.
102. Pinkaya M, Morlín A (2009) Histone H3 lysine 4 di-methylation: A novel mark for transcriptional fidelity? Epigenetics 4.
103. Kushnirou VV (2000) Rapid and reliable protein extraction from yeast. Yeast 16: 857–860.
104. Edmondson DJ, Smith MM, Roth SY (1996) Repression domain of the yeast transcriptional silencer. Yeast 11: 291–304.
105. Mudhar H, Mehta AK, Way M, Price FM, Hake SB, et al. (1999) SUM1 and HST1 repress middle sporulation-specific gene expression during meiosis in Saccharomyces cerevisiae. Proc Natl Acad Sci U S A 96: 2893–2895.
106. Bannister AJ, Zegerman P, Partridge JF, Misra EA, Thomas JO, et al. (2001) Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. Nature 410: 120–124.
107. Shankaranarayana GD, Motamedi MR, Meazed D, Grewe SI (2003) Sir2 regulates histone H3 lysine 9 methylation and heterochromatin assembly in fission yeast. Curr Biol 13: 1240–1246.
108. Nakayama J, Rice JC, Strahl BD, Allis CD, Grewe SI (2003) Role of histone H3 lysine 9 methylation in epigenetic control of heterochromatin assembly. Science 292: 110–113.