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A comprehensive library of histone mutants identifies nucleosomal residues required for H3K4 methylation

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Abstract

Methylation of histone 3 lysine 4 (H3K4) by yeast Set1-COMPASS requires prior monoubiquitination of histone H2B. To define whether other residues within the histones are also required for H3K4 methylation, we systematically generated a complete library of the alanine substitutions of all of the residues of the four core histones in *Saccharomyces cerevisiae*. From this study we discovered that 18 residues within the four histones are essential for viability on complete growth media. We also identified several *cis*-regulatory residues on the histone H3 N-terminal tail, including histone H3 lysine 14 (H3K14), which are required for normal levels of H3K4 trimethylation. Several previously uncharacterized *trans*-regulatory residues on histones H2A and H2B form a patch on nucleosomes and are required for methylation mediated by COMPASS. This library will be a valuable tool for defining the role of histone residues in processes requiring chromatin.

Chromatin is an essential platform for almost all DNA-templated processes, including replication, repair, recombination and transcription1-3. Nucleosomes, the fundamental unit of chromatin, consist of 147 base pairs (bp) of DNA wrapped around an octamer of histones consisting of two H2A-H2B dimers and a single H3-H4 tetramer, forming two nearly symmetrical halves in their tertiary structure4. The core histones are highly conserved proteins from yeast to humans, indicating the importance of each amino acid residue within the histones. Nucleosomes present a degree of structural constraint, and to circumvent these restrictions chromatin structure may be locally or globally altered either through the post-translational modification of histones1-3, interactions with other proteins such as ATP-dependent chromatin-remodeling complexes, or by replacement of core histones with histone variants5-7. Covalent modifications of histones include acetylation, methylation, ubiquitination, sumoylation and phosphorylation8,9. Most of the identified histone modifications are located in the histone tail, where modifying enzymes or modification-targeting proteins can gain relatively easy access. However, post-translational modifications within the core histones have also been reported2,10-14.

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AUTHOR CONTRIBUTIONS

S.N. and A.S. carried out the research and wrote the manuscript; B.W.S. and K.M.D. performed site-directed mutagenesis of histones and validation of the generated vectors; W.D.B. performed validation of the transformed yeast library; K.S.-H. and A.S. provided support and advice.

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A prevalent modification of histones that is associated with active transcription is H3K4 methylation[15-24]. More importantly, the lysine residues on histones can be mono-, di- or trimethylated by histone methyltransferases (HMTases), with each pattern of modification having a specific biological outcome[2,17,23]. Chromatin immunoprecipitation (ChIP)-based studies revealed that COMPASS and H3K4 trimethylation is found at the 5′ end of the coding region[15,16]. Several studies also demonstrated that the acetylation of H3K9 and H3K14 are also found at the 5′ ends of actively transcribed genes[25,26]. However, the relationship between H3 acetylation and H3 methylation is yet to be determined.

Methylation of H3K4 is catalyzed by the enzymatic activity of the macromolecular complex COMPASS, which contains the methyltransferase Set1 (ref. 2). Following the identification of Set1-COMPASS as the first H3K4 HMTase[15,18,19,21], it was demonstrated that the human homologs of Set1, the mixed lineage leukemia (MLL) proteins, MLL2-4, and Set1A and Set1B, were also found in COMPASS-like complexes capable of methylating H3K4 (ref. 27). Previously, we, and others, demonstrated that monoubiquitination of histone H2B on lysine 123 (H2BK123) is required for the proper methylation of H3K4 by COMPASS[28,29]. We now know that H2B monoubiquitination regulates H3K4 methylation via regulation of COMPASS catalytic activity. The molecular mechanism identified in yeast that regulates the implementation and removal of H3K4 methylation is conserved in humans[30-32]. Therefore, the lessons learned from yeast chromatin are highly valuable in defining the chromatin and transcriptional machinery in humans.

Not only is H2B monoubiquitination required for H3K4 methylation by COMPASS, but methylation of histone H3 arginine 2 (H3R2) also has an important role in regulating COMPASS's activity[33,34]. To determine how many other residues within the histones are required for proper H3 methylation by COMPASS, we systematically generated a library of alanine mutants of all residues of the four core histones in yeast *S. cerevisiae*. We call this library the scanning histone mutagenesis with alanine (SHIMA) library. This is an unbiased approach, which will facilitate determination of the importance and functional significance of all of the residues within histones. Given the conservation of residues between histones from yeast to humans, we initially predicted that many mutated residues within the histones would be required for viability, and therefore, be lethal. To our surprise, only 18 residues were found to be essential for viability on complete growth medium, three of which represented an extreme slow-growth phenotype. Recently, Matsubara and colleagues developed a strategy called global analysis of surfaces by point mutation (GLASP), where they mutated the surface residues of the four histones[35]. Their study resulted in the identification of eight essential residues within the four histones. The identification of additional essential residues here suggests the utility of SHIMA as a comprehensive and systematic mutant collection that will allow characterization of the functional importance of almost all residues within the core histones.

Using this entire comprehensive histone-mutant collection and our global proteomic screen (GPS) in *S. cerevisiae*, we then explored the network of histone cross-talk between histone H3K4 methylation and other residues within the histones[36]. With GPS, we have examined the extracts from the histone-mutant collection by western analysis using antibodies directed toward modified histones. We have identified several previously uncharacterized residues

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within histones acting either in *cis* or *trans* to regulate proper H3K4 methylation. Here we provide evidence for the existence of possible *cis*-cross-talk between histone H3K4 trimethylation and histone H3K14 acetylation. We have also identified residues that act *in trans* to regulate the pattern of histone H3K4 methylation, and they map to a patch on nucleosomes. This patch contains His112 and Arg119 of histone H2B, and Glu65, Leu66, Asn69 and Asp73 of histone H2A, all of which reside near H2BK123 when visualized on the three-dimensional structure of the nucleosome. Two of the residues are required for H2BK123 monoubiquitination, whereas the other residues may regulate COMPASS's activity independently of H2B monoubiquitination. This comprehensive library of histone mutants has been instrumental in defining the global regulation of histone cross-talk for H3K4 methylation. This collection will be a useful resource to the chromatin community for defining the role of histone residues in numerous processes involving chromatin.

RESULTS

Generation of the histone-mutant library

We have generated a complete library of alanine mutants at all residues of the four core histones, except at the naturally occurring alanine residues in yeast *S. cerevisiae* (Fig. 1a). We have named this library SHIMA. Plasmids containing alanine point mutations within the histone genes were generated by site-directed mutagenesis (Methods). Each plasmid was then sequenced for confirmation. The entire collection of the histone alanine mutant library in *S. cerevisiae* was generated by transforming the plasmids into yeast histone shuffle strains, either YBL574 containing *hht2/hhf2* encoding H3 and H4, or Y131 (Osley's lab) containing *hta1/htb1*, encoding H2A and H2B37. We carried out a strain selection for transformants, followed by a second single-colony selection on 5-fluoroorotic acid (5-FOA; Fig. 1b) to remove wild-type histone plasmids containing the *URA* gene. At this point, we identified the residues that are essential for cell survival under normal growth conditions (Fig. 1b). We carried out three additional selections by YPD-5-FOA to ensure the complete removal of wild-type histone plasmids before making the final stock of the library. Individual strains in the final yeast histone mutation library were sequenced for confirmation of the mutation and to determine the absence of the corresponding wild-type histone copy. The key for each plate is shown in Figure 2. This complete yeast mutation collection is now available to our colleagues.

Essential amino acid residues of yeast histones

Histone residues within nucleosomes are highly evolutionarily conserved. As a matter of fact, the amino acid residues in histones H2A and H2B are more than 70% conserved from yeast to humans, and in histones H3 and H4 more than 90% are conserved. When we decided to generate a complete alanine scanning collection of the histones, we considered its high evolutionary conservation and predicted that many of the mutants would be lethal. To our surprise, point mutations of less than 5% of the total residues of the four histones were lethal. Our comprehensive histone alanine scanning analysis identified only 18 residues within all of the core histones that were required for viability under normal growth conditions (Fig. 3a). All of the essential residues are found within the globular domains of histones (Fig. 3b). A few of these residues, marked by asterisk in Figure 3, are either slow

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growers or generate revertants as judged by growth after 5 d of slow or no growth. Although most of the post-translational modifications occur in histone tail domains, tail-less H3-H4, H2A and H2B histones are still capable of nucleosomal assembly *in vitro*. The histone H4 N-terminal tail is required for chromatin folding; however, it is dispensable for growth38. We have found that there are nine residues in histone H3 that are essential for viability: Tyr41, Leu48, Ile51, Gln55, Glu97, His113, Arg116, Thr118 and Asp123 (Fig. 3a). Five residues in histone H4 are also required for viability: Arg39, Arg40, Arg45, Tyr72 and Leu90. We have found that histone H2A and H2B together have only four residues required for yeast viability under normal growth conditions: three in H2A (Tyr58, Glu62 and Asp91) and one in H2B (Leu109) (Fig. 3a).

Most of the H3 and H4 essential residues are clustered on the lateral surface near the dyad axis or at the DNA entry-exit points (Fig. 3c,d), as mapped onto the crystal structure of the nucleosome determined previously60. These are the regions where histones make contact with DNA. Alanine substitutions at three of the five *Sin* (*SWI/SNF*-independent) mutation sites previously identified by genetic screens39, H3 R116A, H3 T118A and H4 R45A, are lethal. These residues are all located at the protein-DNA interaction interface formed by the L1L2 loops of the H3-H4 tetramer40. Additionally, H3 Arg116 makes a salt bridge with H3 Asp123 (ref. 41), which is also identified as an essential residue (Fig. 3a). Mutation of these residues caused a loss of the salt bridge and small changes in the conformation of the L2 loop. It has been demonstrated that H3 His113 also forms a hydrogen bond with H3 Asp123 (ref. 41). H3-H4 and H2A-H2B quasisymmetric heterodimers can be superimposed by rotating 180° around the axis (ref. 4). Indeed, *lrs* (loss of rDNA silencing) mutation sites are located at the other end of the H3-H4 heterodimer40,42. Even though these sites are structurally equivalent to the *Sin* mutation sites, none of these residues are required for viability (Fig. 3). H2A Arg42, H2B Thr85 and H2B Arg83 are the residues in the H2A-H2B heterodimer that are equivalent to H4 Arg45, H3 Thr118 and H3 Arg116, respectively40. However, none of these residues is required for viability (Fig. 3). This may be explained by the fact that the interactions between DNA and the histones are strongest at the nucleosome dyad4. Mutations in these regions may have more of an impact on histone-DNA interactions. The H2A and H2B essential residues are positioned on the surface of nucleosomes (Fig. 3c, red circle).

Cross-talk between histone residues

It has already been well established that the monoubiquitination of H2BK123 is required for proper H3K4 and H3K79 methylation by COMPASS and Dot1, respectively2. This mode of regulation is highly conserved from yeast to human. Yeast has had a fundamental role in paving the way to then defining the pathway of H3K4 methylation in mammalian cells. Almost all aspects of regulation of H3K4 methylation found in yeast2 were later shown to be similar in mammalian cells30-32,43. Rad6 is the E2 conjugating enzyme and was first reported in yeast to be required for proper H2BK123 monoubiquitination28,29,44,45. Bre1 is the E3 ligase for Rad6, physically associates with it and is required for its recruitment to chromatin45, and for cross-regulation from monoubiquitination to methylation45,46. In addition to Rad6 and Bre1, several other factors including the Paf1 complex, the Bur1-Bur2 kinase and the Ctk complex are required for proper H2BK123 monoubiquitination and

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H3K4 and H3K79 methylation16,17,47-52. Recently, it was demonstrated that the H2B monoubiquitination signal is translated via the Cps35 subunit of COMPASS53. Cps35 interacts with chromatin in an H2BK123 monoubiquitination-dependent manner, and its interaction with chromatin brings the COMPASS core and Cps35 together to activate the enzyme's trimethylase activity on chromatin53. This model describes how the interaction of COMPASS with Cps35 on chromatin will allow the activation of the enzyme and the methylation of the histones on chromatin, but not on the soluble histones53.

So far, we know that two identified residues (H2BK123 and H3R2) have important roles in proper methylation by COMPASS. Therefore, we wanted to determine whether any other residues within histones are required for proper H3K4 methylation. We performed a GPS analysis of the extracts from the entire histone-mutant collection. Our analysis has resulted in the identification of several previously uncharacterized key regulatory residues within the histones required for normal levels of H3K4 methylation (Figs. 4-6). The key for the position of the mutants is shown in Figure 2. First and foremost, we have identified a few consistently reproducible *cis*-regulatory residues within histone H3 regulating H3K4 trimethylation (Fig. 4). These include Arg2, Lys4, Gln5, Thr3 and Lys14 of H3, indicated on Figure 4a,b as residues I, II, III, IV and V, respectively. Of these, Lys4 mutated to alanine can no longer be methylated. Arg2 has already been shown to be required for substrate recognition by COMPASS33,34. Thr3 could be phosphorylated, and its phosphorylation could regulate COMPASS's activity; however, it is also possible that H3T3A mutation may result in epitope masking and therefore loss of immunoreactivity by the H3K4 polyclonal antibodies. Further studies should clarify these possibilities. Mutations in H3Q5 also result in the loss of the mono-, di- and trimethylation of H3K4. As many of these residues lay near the H3K4 site, their mutation could result in a defect in epitope recognition by the antibodies or defect in the substrate recognition by Set1-COMPASS.

Another *cis*-regulatory residue identified in this screen is H3K14 (Figs. 4a,b and 6). The H3K14A strain showed a specific loss of H3K4 trimethylation with no effect on H3K4 mono- or dimethylation (Fig. 6b). This residue is far from H3K4, and therefore its effect on H3K4 trimethylation cannot result from a defect in epitope recognition by the H3K4 trimethylation-specific antibodies. As H3K14 is a known site of acetylation, we tested the effect of the H3K14Q mutation to mimic a neutral, acetylated lysine, and an H3K14R mutant to mimic the basic, nonacetylated lysine (Fig. 6b, lanes 19-27). Both mutant strains lacked specific H3K4 trimethylation (Fig. 6b, lanes 19-27), suggesting that the regulation of H3K4 methylation is not simply electrostatic in nature. Both the H3K14 acetylation and the H3K4 global methylation patterns show a similar localization to the 5′ ends of actively transcribed genes25,26, and the presence of H3K4 trimethylation can be correlated with the hyperacetylation of histone H3 (ref. 54). Two enzyme complexes, GCN5-SAGA and Sas3-NuA3, are known to acetylate H3K14 (ref. 55). Interestingly, the deletion of Gcn5 results in decreased H3K4 methylation in the coding region of the *ARG1* ORF56, suggesting H3K14 acetylation levels could affect H3K4 methylation. Our finding that the H3K14A mutation abrogates H3K4 trimethylation indicates that H3K14 acetylation might regulate H3K4 trimethylation globally. Notably, the NuA3 subunit, Yng1, binds trimethylated H3K4 and facilitates H3K14 acetylation57,58, suggesting that these two modifications stabilize each

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other. Similarly, an acetyllysine binding protein such as the bromodomain-containing protein Rsc4 (ref. 59), could bind and stabilize acetylated H3K14, whereas Yng1 stabilizes trimethylated H3K4 by COMPASS. Indeed, we have identified COMPASS in a complex containing the RSC complex (data not shown), suggesting a possible physical link between these modifications. Our unbiased approach to understanding H3K4 methylation has thus generated hypotheses for future investigations into the mechanistic link between two highly studied histone modifications.

In addition to *cis*-regulatory residues identified within histone H3, we have also identified several *trans*-regulatory residues that specifically regulate H3K4 di- and trimethylation (Figs. 5 and 6). These include four residues within histone H2A—Glu65, Leu66, Asn69 and Asp73 (indicated on Fig. 5a,b as I, II, III and IV, respectively)—and three residues within histone H2B—Lys123, Arg119 and His112 (indicated on Fig. 5e,f as V, VI and VII, respectively). When we localized these residues on the crystal structure of the nucleosomes, they formed a patch near Lys123 of H2B, the site of monoubiquitination required for H3K4 methylation by COMPASS (Fig. 6c). We tested whether the mutation of residues within this patch could alter H2B monoubiquitination levels and found that only mutations in H2BH112 and H2AL66 result in a defect in H2B monoubiquitination (Fig. 6d). The other residues within the patch, H2AN69, H2AD73 and H2AE65, regulate COMPASS's activity independently of H2B monoubiquitination. We recently found that the Cps35 subunit of COMPASS interacts with chromatin in a monoubiquitination-dependent manner53 and that Cps35 is required for H3K4 methylation by COMPASS. Indeed, Cps35 loss phenocopies either Rad6 and/or H2BK123R mutations in regard to H3K4 di- and trimethylation. Conceivably, the residues within this patch that still have high levels of H2B monoubiquitination could be a part of a binding surface for proteins that recognize monoubiquitinated H2B in its nucleosomal context, or other factors, such as the Paf1 complex, that are also required for the association of COMPASS with transcribing polymerase.

DISCUSSION

In this study, we have generated a comprehensive library of all of the four core histones mutated at every single residue with an alanine. This library is now available to our colleagues who are interested in defining the functional and physiological relevance of histone residues in many processes requiring chromatin. As a proof of principle, using this library we have identified 18 residues within all four of the core histones that are essential for viability and proper growth under normal growth conditions. We have also identified several *cis*-regulatory residues on histone H3, including H3K14, which are required for the implementation of normal levels of H3K4 trimethylation. H3K14 is acetylated by both Gcn5 and Sas3, and this modification is associated within the early transcribed regions of active genes, similarly to the pattern of H3K4 trimethylation. Our study raises the possibility of a communication between H3K14 acetylation and H3K4 trimethylation.

Using this comprehensive library of histone mutants, we have also identified a *trans*-regulatory patch on chromatin containing several residues within histones H2A and H2B regulating H3K4 methylation. Given the high conservation of core histones from yeast to

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human, we predict that many of the corresponding residues in the human core histones will also be required for proper H3K4 methylation by the MLL complexes and the Set1A/B-containing complexes. Considering that translocations of MLL are associated with the pathogenesis of hematological malignancies, the identification of the role of these residues in the regulation of the enzymatic activity of the MLL complex will be of great interest.

We envision a plethora of studies that can take advantage of the availability of this comprehensive point-mutated histone collection. This collection can be used to define the role of different histone residues under many growth conditions, including sporulation. It is of great interest to define how signaling through chromatin allows chromatin compaction and spore formation. Analysis of epigenetic memory may also be facilitated by using this mutant collection, especially given that there are currently no identified, direct links between the known modified histone residues and this process. An unbiased approach using the entire histone-mutant collection will allow the identification of such possible residues. This collection can also be used for defining the role of individual histone residues and telomere-associated gene silencing. Application of this collection to methods such as the synthetic genome array (SGA) or just a simple synthetic analysis would allow one to determine the role of each histone residue with the deletion in a specific gene or the entire yeast deletion collection. In conclusion, the availability of this comprehensive histone-mutant collection is likely to facilitate numerous new insights into chromatin.

METHODS

Yeast strains and plasmids

We used plasmids pWZ414-F12, carrying *HHT2* and *HHF2*, and pZS145 (ref. 29), carrying *HTA1-Flag-HTB1 CEN HIS3* for this study. Previously described yeast shuffle strains, YBL 574 (ref. 37) and YBL 397 (Y131)29, were a gift from J. Workman.

Generation of histone-mutant library

Plasmids bearing alanine mutations in the *HTA1, HTB1, HHT2* and *HHF2* genes were systematically generated by site-directed mutagenesis. We performed site-directed mutagenesis in 96-well plates using the QuickChange II Site-Directed Mutagenesis Kit (Stratagene). Products were transformed into *E.cloni 10G ELITE* electrocompetent cells (Lucigen), using a 96-well electroporator, BTX model ECM 630 electro cell manipulator (Harvard Apparatus). Plasmids were prepared with a BioMekFX (Beckman-Coulter) using the *CosMCPrep* Kit (Agencourt). Mutated targets were confirmed by sequencing, using the following primers: HTA1seqF: 5′-cgaagcc agccagtggatcg-3′; HTA1seqR: 5′-gaagcagtttagttccttccgcct-3′; HTBseqF: 5′-ggca aatactaccttggttgg-3′; HTBseqR: 5′-tttcgagaacacaattttacaaccga-3′; HHT2seqF: 5′-gcgtgataacagcgtgttgtgc-3′; HHT2seqR: 5′-catgtcgttaaaagcattgcgaatag-3′; HHF2 seqF: 5′-gttgttcactcgcgcctggg-3′; and HHF2seqR: 3′-atatcgaattctggaggagca-5′.

Each plasmid was then transformed manually into yeast shuffle strains, Y131 for *HTA1* and *HTB1*, and YBL 574 for *HHT2* and *HHF2*, using a standard yeast-transformation protocol with minor modifications, and strains grown on a synthetic dropout medium lacking

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histidine, SC-His (for *hta1* and *htb1* mutants), or a medium lacking tryptophan, SC-Trp (for *hht2* and *hhf2* mutants). After 2 days of incubation, each transformant (20 μL) was plated onto 48-segment bioassay trays (Genetix) containing: either SC-HIS plus 5-FOA or SC-Trp plus 5-FOA to single-colony select cells that had lost the plasmid containing the wild-type histones. Each colony was inoculated into YPD medium plus 5-FOA in 96-well plates. All histone-mutant strains were again confirmed by sequencing, and the glycerol stocks of the histone-mutant library were generated.

Viability assays for the identification of essential residues within histones

Mutant and wild-type strains were grown to an optical density at 600 nm (OD_{600}) of 0.5. Next, four-fold serial dilutions of these strains were spotted on SC-Trp plates in the presence or absence of 5-FOA. The dilutions of the *hta1* and *htb1* mutant strains were spotted on SC-His plates in the presence or absence of 5-FOA. The plates were incubated at 30 °C for 72 h before being photographed.

Global proteomic screening for the identification of the residues required for proper methylation of H3K4

We carried out GPS as described previously36 using antibodies specific for H3K4 di- and trimethylation (Millipore).

Detection of H2B monoubiquitination

We prepared extracts from the histone H2A and H2B mutants that resulted in defects in H3K4 methylation, and subjected the extracts to western blot analysis. The plasmid used for the mutagenesis of *HTA1* and *HTB1* contains a Flag-tagged *HTB1* gene. Therefore, an antibody against the Flag epitope was used to detect H2B and its monoubiquitination form.

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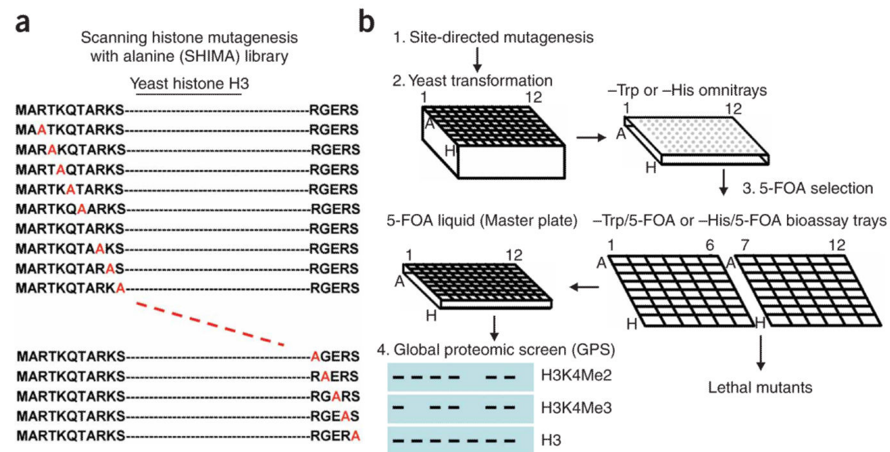

Figure 1.

Schematic representation of the experimental procedure. (**a**) Scanning histone mutagenesis with alanine (SHIMA). A library of alanine mutants at all residues of the four core histones, except the wild-type alanine residues in yeast *S. cerevisiae*, was systematically generated. (**b**) High-throughput yeast transformation and global proteomic screen (GPS) of *S. cerevisiae* histone mutants. The plasmids containing alanine point mutations within histone genes were generated by site-directed mutagenesis (Methods). The entire collection of histone alanine mutant libraries in *S. cerevisiae* was generated by transforming yeast shuffle strains (YBL 574 for H3 and H4, 4131 for H2A and H2B) and selection for the transformants, followed by a second selection on 0.1% (w/v) 5-FOA to remove wild-type histones. The collection of histone mutants was further analyzed by GPS to identify the amino acid residues required for proper H3 methylation.

H3 (HHT2) plate1

	1	2	3	4	5	6	7	8	9	10	11	12
A	R2A	T11A	L20A	T32A	R40A	R49A	S57A	L65A	E73A	L82A	L92A	V101A
B	T3A	G12A	S22A	G33A	Y41A	E50A	T58A	P66A	I74A	R83A	Q93A	S102A
C	K4A	G13A	K23A	G34A	K42A	I51A	E59A	F67A	Q76A	F84A	E94A	L103A
D	Q5A	K14A	R26A	V35A	P43A	R52A	L60A	Q68A	D77A	Q85A	S95A	F104A
E	T6A	P16A	K27A	K36A	G44A	R53A	L61A	R69A	F78A	S86A	V96A	E105A
F	R8A	R17A	S28A	K37A	T45A	F54A	I62A	L70A	K79A	S87A	E97A	D106A
G	K9A	K18A	P30A	P38A	V46A	Q55A	R63A	V71A	T80A	I89A	Y99A	T107A
H	S10A	Q19A	S31A	H39A	L48A	K56A	K64A	R72A	D81A	G90A	L100A	N108A

H3 (HHT2) plate 2

	L1	L2	L3
1	L109A	Q120A	R129A
2	I112A	K121A	L130A
3	H113A	K122A	R131A
4	K115A	D123A	G132A
5	R116A	I124A	E133A
6	V117A	K125A	R134A
7	T118A	L126A	S135A
8	I119A	R128A	

H4 (HHF2) plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1A	G9A	H18A	I26A	R35A	K44A	E52A	F61A	S69A	R78A	V86A	R95A
B	G2A	L10A	R19A	Q27A	R36A	R45A	E53A	L62A	V70A	K79A	V87A	T96A
C	R3A	G11A	K20A	G28A	L37A	I46A	V54A	E63A	T71A	T80A	Y88A	L97A
D	G4A	K12A	I21A	I29A	R39A	S47A	R55A	S64A	Y72A	V81A	L90A	Y98A
E	K5A	G13A	L22A	T30A	R40A	G48A	V57A	V65A	T73A	T82A	K91A	G99A
F	G6A	G14A	R23A	K31A	G41A	L49A	L58A	I66A	E74A	S83A	R92A	F100A
G	G7A	K16A	D24A	P32A	G42A	I50A	K59A	R67A	H75A	L84A	Q93A	G101A
H	K8A	R17A	N25A	I34A	V43A	Y51A	S60A	D68A	K77A	D85A	G94A	G102A

H2A (HTA1) plate 1

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1A	S10A	G23A	V31A	N39A	P49A	Y58A	N69A	I79A	I88A	K96A	Q105A
B	G2A	K13A	L24A	H32A	Y40A	V50A	L59A	R72A	I80A	R89A	L97A	G106A
C	G3A	S15A	T25A	R33A	Q42A	Y51A	E62A	D73A	P81A	N90A	L98A	G107A
D	K4A	Q16A	F26A	L34A	R43A	L52A	I63A	N74A	R82A	D91A	G99A	V108A
E	G5A	S17A	P27A	L35A	I44A	T53A	L64A	K75A	H83A	D92A	N100A	L109A
F	G6A	R18A	V28A	R36A	G45A	V55A	E65A	K76A	L84A	E93A	V101A	P100A
G	K7A	S19A	G29A	R37A	S46A	L56A	L66A	T77A	Q85A	L94A	T102A	N111A
H	G9A	K21A	R30A	G38A	G47A	E57A	G68A	R78A	L86A	N95A	I103A	I112A

H2A (HTA1) plate 2

	L1	L2
1	H113A	S121A
2	Q114A	K123A
3	N115A	T125A
4	L116A	K126A
5	L117A	S128A
6	P118A	Q129A
7	K119A	E130A
8	K120A	L131A

H2B (HTB1) plate 1

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1A	P13A	S24A	R32A	S41A	K49A	I57A	L65A	F73A	L83A	S93A	L103A
B	K3A	E15A	T25A	S33A	S42A	Q50A	S58A	N66A	E74A	Y86A	R95A	I104A
C	E5A	K16A	S26A	K34A	Y43A	T51A	Q59A	S67A	R75A	N87A	E96A	L105A
D	K6A	K17A	T27A	R36A	I44A	H52A	K60A	F68A	I76A	K88A	I97A	P106A
E	K7A	P18A	D28A	K37A	Y45A	P53A	S61A	V69A	T78A	K89A	Q98A	G107A
F	P8A	K21A	G29A	E38A	K46A	D54A	M62A	N70A	E79A	S90A	T99A	E108A
G	S10A	K22A	K30A	T39A	V47A	T55A	S63A	D71A	S81A	T91A	V101A	L109A
H	K11A	T23A	K31A	Y40A	L48A	G56A	I64A	I72A	K82A	I92A	R102A	K111A

H2B (HTB1) plate 2

	L1	L2
1	H112A	T122A
2	V114A	K123A
3	S115A	Y124A
4	E116A	S125A
5	G117A	S126A
6	T118A	S127A
7	R119A	T128A

Figure 2.

Key to the organization of the SHIMA library. Each mutant histone is stored in the indicated cell block.

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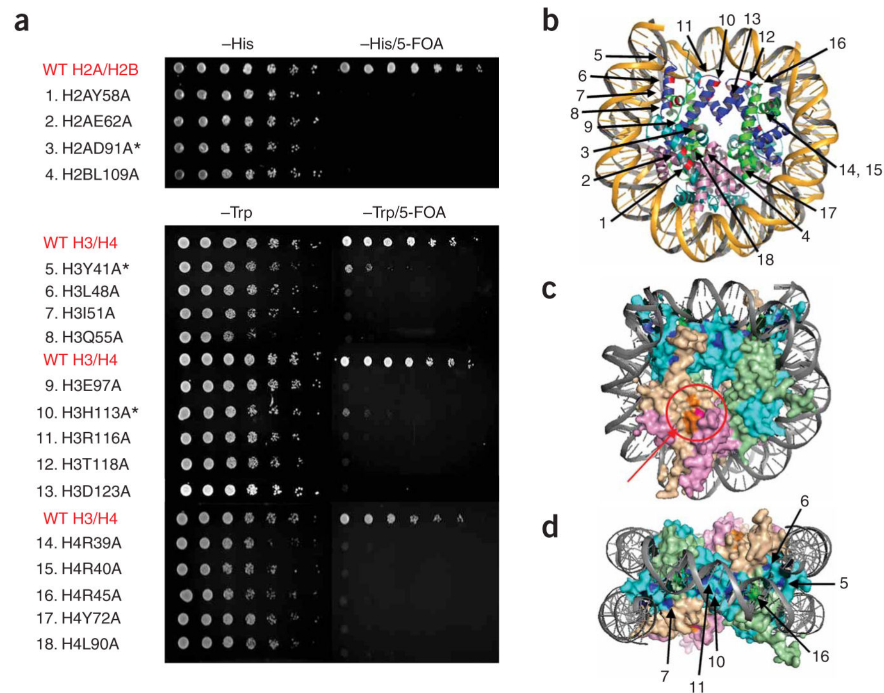

Figure 3.

Identification of amino acid mutations essential for viability under normal growth conditions. (**a**) Viability of histone alanine mutants. Strains expressing histones containing each single alanine mutation in the presence of wild-type histones (either *HTA1* and *HTB1*, or *HHT2* and *HHF2*) were plated with an initial OD_{600} of 0.5, followed by a four-fold serial dilution onto SC-Trp in the absence or presence of 5-FOA. * indicates that these mutants started to form revertants after 5 days of incubation at 30 °C; however, the appearance of the colonies of these mutants was repeatedly delayed and the number of colonies was extremely low. (**b**) Mapping of lethal residues on the nucleosome crystal structure as determined previously60. The locations of lethal residues are shown in red and numbers indicate the corresponding histone mutants shown in Figure 2a. Representation of nucleosome was generated using PyMOL. (**c,d**) Mapping of lethal residues in the surface of the yeast nucleosome on the crystal structure of the nucleosomes as determined previously (PDB 1ID3)60. H2A, H2B, H3 and H4 lethal residues are shown in orange, pink, blue and green, respectively. Red circles show H2A and H2B lethal residues. Representations of nucleosomes were generated using PyMOL (http://pymol.sourceforge.net/).

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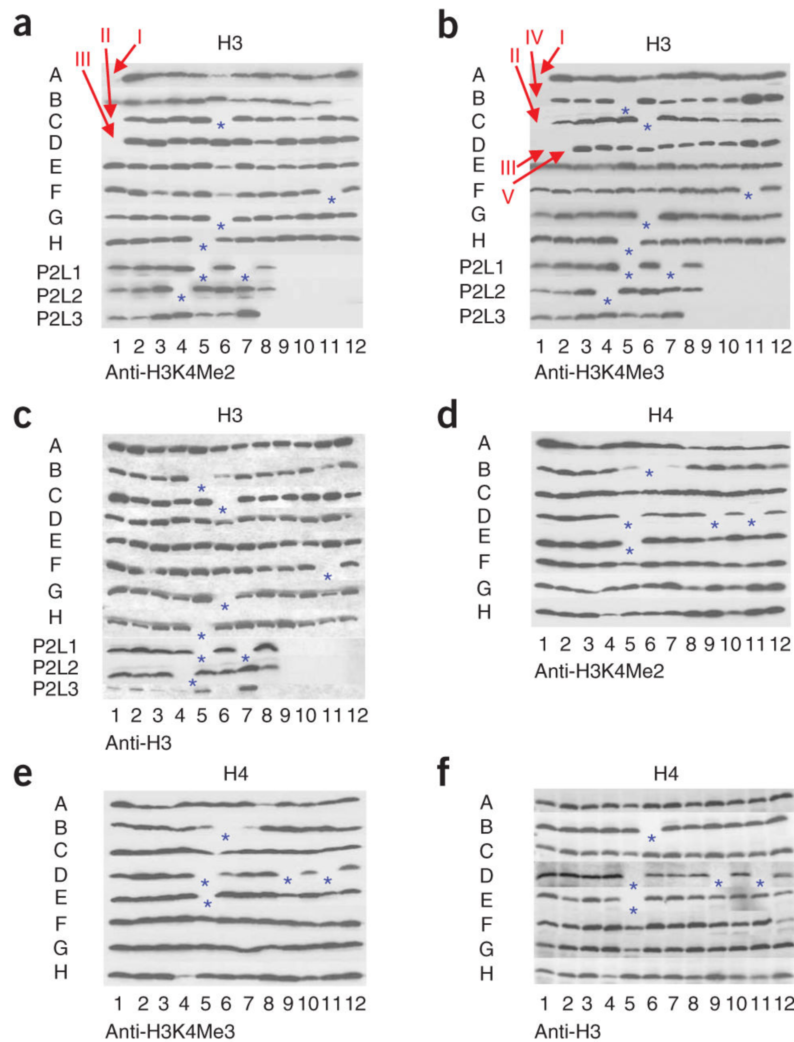

Figure 4.

GPS analyses to defining amino acid residues of histone H3 and H4 required for proper H3K4 methylation. Cell extracts prepared from the entire histone-mutant collection (**a-c** from H3 mutants, **d-f** from H4 mutants) were subjected to SDS-PAGE and western blot analysis and tested for the presence of dimethylated lysine 4 of histone H3 (H3K4Me2) and trimethylated lysine 4 of histone H3 (H3K4Me3) as indicated below panel. As the loading control, an antibody to histone H3 was also used. Red arrows indicate the possible hits, and blue asterisks (*) indicate the positions of empty wells (lethal residues). For the key to the organization of the strains within each plate, see Figure 2. Positions I, II, III, IV and V indicate histone H3 Arg2, Lys4, Gln5, Thr3 and Lys14, respectively. P2 indicates plate 2.

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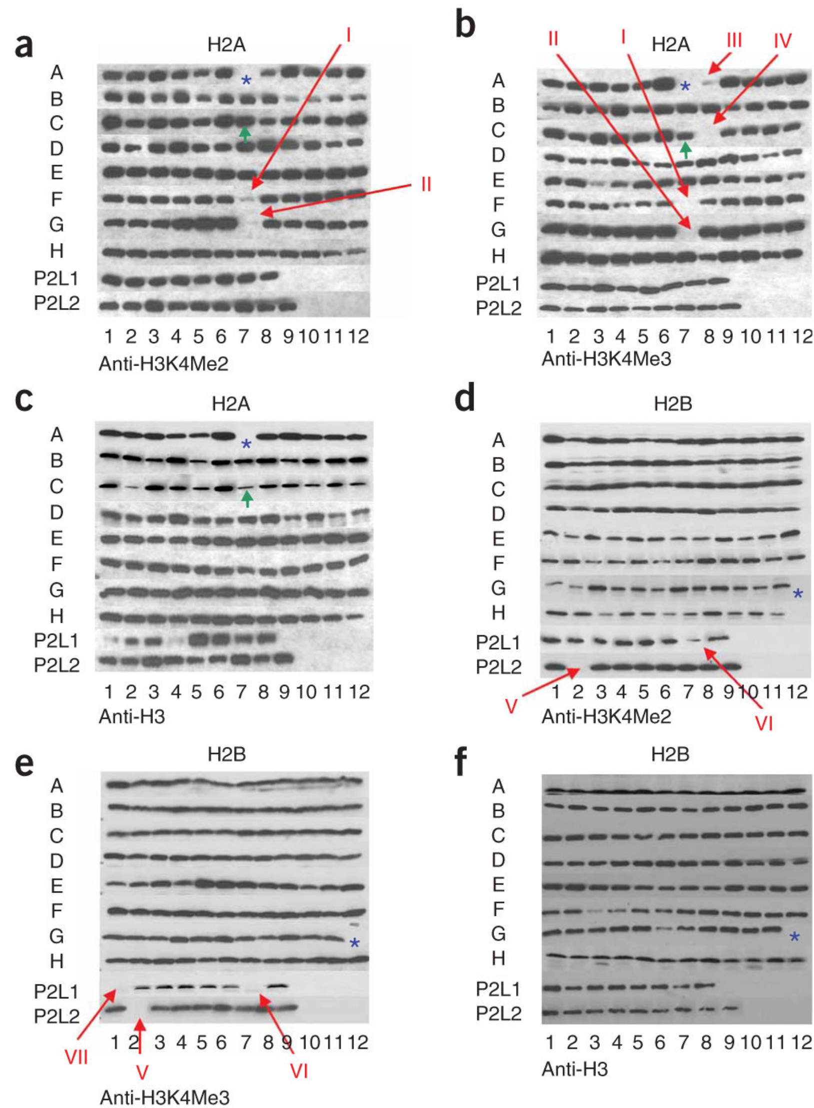

Figure 5.

GPS analyses in defining amino acid residues of histones H2A and H2B required for proper H3K4 methylation. Cell extracts prepared from the entire histone-mutant collection (**a-c** from H2A mutants, **d-f** from H2B mutants) were subjected to SDS-PAGE and western blot analysis, and tested for the presence of dimethylated lysine 4 of histone H3 (H3K4Me2) and trimethylated lysine 4 of histone H3 (H3K4Me3) as indicated under panel. As the loading control, an antibody to histone H3 was also used. Red arrows indicate the possible hits, and blue asterisks (*) indicate the positions of empty wells (lethal residues), green arrows (C8) indicate the positions where the wild-type was used in place of a lethal residue-bearing strain as a positive control for growth in these regions. For the key to the organization of the strains within each plate, please see Figure 2. Positions I, II, III and IV indicate histone H2A Glu65, Leu66, Asn69 and Asp73, respectively. Positions V, VI and VII indicate histone H2B Lys123, Arg119 and His112, respectively. P2 indicates plate 2.

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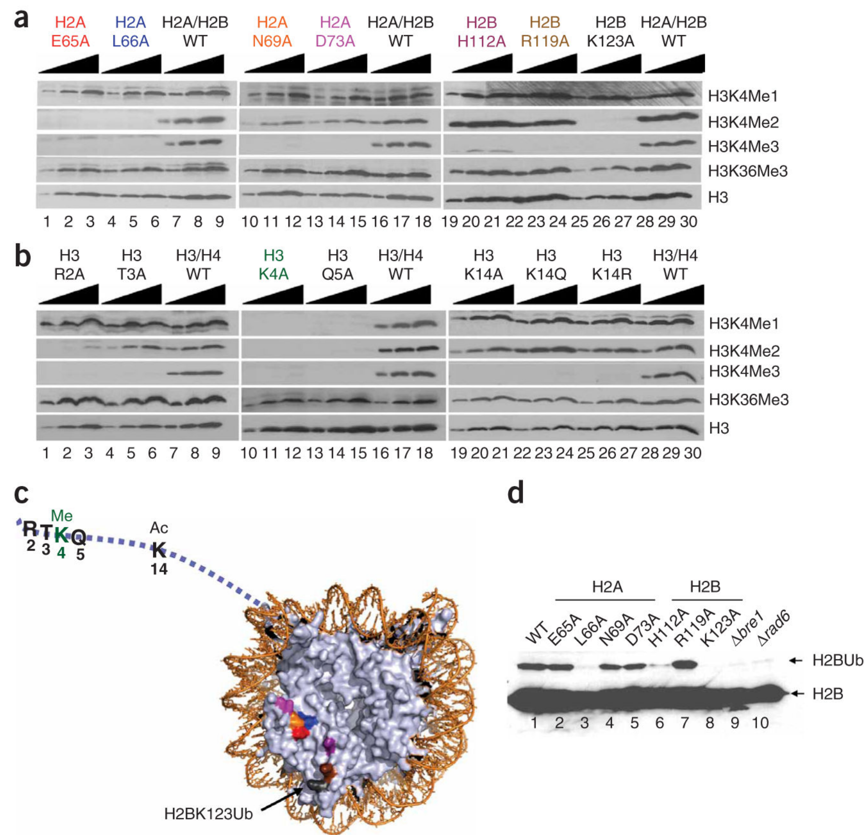

Figure 6.

Identification of a nucleosomal patch regulating the H3K4 methylation pattern. (**a,b**) Titration analyses of histone mutants identified as defective in proper H3K4 methylation. Titration analysis was performed to confirm the positive hits obtained from GPS analysis. Extracts were analyzed by SDS-PAGE followed by western blot analysis, and testing for the presence of monomethylated lysine 4 of histone H3 (H3K4Me1), dimethylated lysine 4 of histone H3 (H3K4Me2) and trimethylated lysine 4 of histone H3 (H3K4Me3). As a positive control, extracts from the strain carrying a plasmid containing wild-type histone (either **HTA1** and **HTB1** or **HHT2** and **HHF2**) were loaded onto each individual gel. (**c**) The locations of residues required for normal levels of H3K4 methylation were mapped onto nucleosomes of the crystal structure (PDB 1ID3)60. Ac, acetylation; Ub, monoubiquitination. (**d**) Identification of histone amino acid residues required for H2B monoubiquitination. Extracts from H2A and H2B mutants identified as defective in Lys4 methylation were tested for the presence of H2B monoubiquitination.