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Histone H4 Lysine 91 Acetylation: Short Article A Core Domain Modification Associated with Chromatin Assembly

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Summary

The acetylation of the NH_2-terminal tail of histone H4 by type B histone acetyltransferases (HATs) is involved in the process of chromatin assembly. Histone H4 associated with a nuclear type B HAT complex contains modifications in its globular core domain as well. In particular, acetylation was found at lysine 91. A mutation that alters this residue, which lies in the interface between histone H3/H4 tetramers and H2A/H2B dimers, confers phenotypes consistent with defects in chromatin assembly such as sensitivity to DNA damaging agents and derepression and alteration of silent chromatin structure. In addition, this mutation destabilizes the histone octamer, leading to defects in chromatin structure. These results indicate an important role for histone modifications outside the NH_2-tail domains in the processes of chromatin assembly, DNA repair, and transcriptional silencing.

Introduction

Histone posttranslational modifications clearly play a critical role in the regulation of chromatin. In addition, it is likely that these modifications are important for the initial formation of this structure as well. The link between histone modification and chromatin assembly was first suggested by the observation that histones H3 and H4 are modified rapidly after their synthesis. For example, newly synthesized histone H4 is phosphorylated on serine 1 (Ruiz-Carillo et al., 1975). This is an evolutionarily conserved modification that appears to be cell cycle regulated, with peak levels seen at the G1/S transition and mitosis (Barber et al., 2004; Ruiz-Carillo et al., 1975).

The best-characterized modification of newly synthesized histones is the acetylation on specific lysine residues in the NH_2-terminal tails of histones H3 and H4 that occur rapidly after their synthesis. Once assembled into chromatin, new H3 and H4 molecules are deacetylated during chromatin maturation (reviewed in Annunziato and Hansen [2000]). One striking aspect of this acetylation is its evolutionary conservation. There are four lysine residues in the NH_2-terminal tail domain of histone H4 that are subject to reversible acetylation (positions 5, 8, 12, and 16).

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Supplemental Data

Supplemental Data include Supplemental Experimental Procedures, a Supplemental Reference, and one figure and are available with this article online at http://www.molecule.org/cgi/content/full/18/1/123/DC1/.

Accession Numbers

Microarray data can be obtained from NCBI under accession number GSE2349.

The acetylation state of newly synthesized H4 from a variety of evolutionarily diverse organisms has been determined. The same pattern, where lysines 5 and 12 are modified, has been observed in each case (Chicoine et al., 1986; Sobel et al., 1995). For histone H3, where the presence of NH_2-terminal acetylation on newly synthesized molecules has been conserved, the specific pattern of acetylation has not.

Although it was long thought that histone modifications were predominantly located in the NH_2-terminal tails, recent evidence indicates that the histone core domains are also highly modified (reviewed in Cosgrove et al. [2004] and Freitas et al. [2004]). The functional relevance of most of these modifications has not been determined, and it is unknown whether any play a role in the process of chromatin assembly. Yeast Hat1p and Hat2p, components of a type B HAT complex responsible for the acetylation of the NH_2-terminal tail of newly synthesized histone H4 during de novo chromatin assembly, were recently identified as components of a nuclear complex containing the histone chaperone Hif1p and histones H3 and H4 (Ai and Parthun, 2004). As expected, the histone H4 associated with this nuclear chromatin assembly complex is modified by acetylation in its NH_2-terminal tail domain. Surprisingly, a more detailed examination of the modification state of this population of histone H4 revealed the presence of modifications in the globular core domain as well. Intriguingly, one site of acetylation, lysine 91, localizes to a region of H4 that is important for the interaction of the H3/H4 tetramer with H2A/H2B dimers. Molecular genetic and biochemical evidence indicate that the acetylation of lysine 91 influences the process of chromatin assembly and may function to modulate the formation of histone octamers.

Results and Discussion

Nuclear Hat1p-Hat2p-Hif1p Complex Associates with H4 Molecules Containing Core Domain Modifications

Histone H4 molecules that copurified with the nuclear Hat1p-Hat2p-Hif1p complex were digested with chymotrypsin, and the resulting peptides were analyzed by mass spectrometry. The molecular weight of several peptides indicated the presence of posttranslational modifications outside the NH_2-terminal tail domain. Dimethylation was observed for a peptide that encompassed residues 50–61 (Figure 1A). This peptide contains an arginine at position 55 and a lysine at position 59. Intriguingly, although this modification cannot be definitively localized, dimethylation of H4 lysine 59 was recently detected in histones isolated from bovine thymus, and mutations in yeast that alter H4 lysine 59 disrupt transcriptional silencing at telomeres and the silent mating loci (Zhang et al., 2003). The acetylation of K 91 is supported by three peptides, two spanning H4 residues 91–100 and a third spanning residues 91–97 (Figure 1A). In each case, there is a mass shift indicative of addition of an acetyl group that can be unambiguously localized to K 91. Two of these peptides indicated that the acetylation of K 91 was associated with the presence of two methyl groups on arginine residues 92 and/or 95. This modification was first identified in bovine histones by peptide mass fingerprinting and later confirmed by electron capture dissociation (Zhang et al., 2003; Zhang and Freitas, 2004). Hence, H4 K 91 acetylation also appears to be a highly conserved modification.

Figure 1B highlights in pink the location of this residue in the crystal structure of the nucleosome (Luger et al., 1997). The left panel displays the full nucleosome where this site of modification appears buried in the interior of the structure. However, by looking at only the histone H3/H4 tetramer (right), it is apparent that this modification is in the region of histone H4 that is important for the docking of the H2A/H2B dimers with the H3/H4 tetramer (Akey and Luger, 2003; Santisteban et al., 1997).

Histone H4 Lysine 91 Is Critical for DNA Damage Repair

The observation that histone H4 associated with the Hat1p-Hat2p-Hif1p complex is acetylated on K 91 suggests that this modification occurs on molecules involved in the process of chromatin assembly. The striking location of H4 K 91 in the nucleosome structure led us to hypothesize that the acetylation of this residue might influence the interaction of H2A/H2B dimers with H3/H4 tetramers and, hence, regulate the formation of histone octamers during chromatin assembly. To test this idea, yeast strains containing an H4 K91A allele were tested for phenotypes often observed in strains containing mutations in chromatin assembly factors, such as sensitivity to DNA damaging agents and defective silent chromatin structure (Adams and Kamakaka, 1999).

Figures 1C and 1D show the viability of strains carrying the H4 K91A allele that have been challenged with a variety of DNA damaging agents. The H4 K91A mutation causes sensitivity to both double-strand (MMS, camptothecin, and hydroxyurea) and single-strand (UV radiation) DNA damage. It should be noted that the phenotypes observed in the presence of the H4 K91A allele are not the result of alterations in H4 protein levels (data not shown).

We took advantage of the DNA damage sensitivity of the H4 K 91 mutants to obtain genetic evidence as to the aspect of the DNA repair process that is affected by this novel site of modification. We combined mutations in histone H4 K 91 with mutations in factors that disrupt distinct aspects of the DNA repair process and then compared the DNA damage sensitivity of the single mutants with that of the double mutant combinations. Increased sensitivity of the double mutants relative to the single mutants would indicate that the two mutations affect different aspects of the repair process. Conversely, similar sensitivities in the single and double mutants would suggest that the two mutations were affecting the same part of the DNA repair pathway.

Figures 1E–1H summarize this analysis. The histone H4 K91A allele increased the MMS sensitivity of strains that had been deleted for either the *MEC1* or *MEC3* kinases that are critical for the DNA damage checkpoint response (Figure 1E). This indicated that the DNA damage sensitivity of the H4 K91A mutant is not solely the result of a defect in the DNA damage checkpoint. Also, the H4 K91A allele increased the DNA damage sensitivity of strains that were defective in either the nonhomologous end-joining (NHEJ) or recombinational repair pathways (Δ*yku70* and Δ*rad52* mutants, respectively), suggesting that histone H4 K 91 was not directly involved in the DNA repair process (Figures 1F and 1G). However, combining mutations in H4 K 91 with mutations in factors involved in chromatin assembly yielded a different result (Figure 1H). The H4 K91A allele did not increase the MMS sensitivity of a strain that lacked the *ASF1* histone chaperone (Tyler et al., 1999). In addition, mutants lacking the *CAC1* subunit of the yeast CAF-1 chromatin assembly complex are specifically sensitive to single-strand DNA damage (UV radiation), and this sensitivity is not accentuated by the H4 K91A allele (Kaufman et al., 1997). Similar results were obtained when the K91A allele was combined with mutations in *HIR2* and *HAT1* (data not shown). These genetic data strongly support the involvement of histone H4 K 91 in the assembly of chromatin that occurs after the repair of damaged DNA.

Histone H4 Lysine 91 Influences Silent Chromatin Structure

The effects of histone H4 K 91 mutations on silent chromatin formation were assayed by using strains containing the *URA3* reporter gene inserted near a telomere, the silent mating loci, or an rDNA repeat. From Figure 2A, it is clear that the K91A allele completely disrupts silent chromatin formation at the telomeres and at the silent mating locus HMR. This mutation has minor effects at HML and little or no impact on rDNA silencing.

To globally assess the effect of the K91A mutation on gene expression, we performed microarray analysis to identify genes that are misregulated. We compared the genome-wide expression levels in the wild-type (wt) and K91A mutant yeast cells and found that 242 genes were upregulated and 65 genes were downregulated by at least 2-fold in the K91A mutant. There was a striking tendency for genes upregulated by the H4 K91A allele to be located near telomeres (Figure 2B), with 20% of these loci within 20 Kb of the end of the chromosome. Beyond 100 Kb from the telomere, upregulated genes were evenly distributed. Moreover, genes upregulated by the K91A allele tended to be found in clusters (defined as three upregulated genes within 15 Kb). 14 clusters were identified of which ten were within 33 Kb of a chromosome end. The H4 K91A allele had a global impact on the expression of telomere-proximal genes, as roughly half of the open reading frames (ORFs) within 10 Kb and a quarter of the ORFs located between 20 and 30 Kb of a chromosome end were upregulated at least 1.5-fold (Figure 2C). In contrast, genes that were downregulated in the K91A mutant were significantly depleted within 30 kb of the chromosome ends but distributed evenly throughout the remainder of the genome (Figure 2D).

The effect of the H4 K91A allele on yeast chromatin structure was assessed by using chromatin immuno-precipitation (ChIP) to determine whether important indicators of yeast chromatin structure, such as Sir2p levels, histone H4 NH_2-terminal tail acetylation, and histone H3 K 79 methylation, are altered at silenced and nonsilenced loci (Figure 3A). As expected, in the presence of wt histone H4, Sir2p is found near telomeres and at HMR, but not at a nonsilenced gene (Figure 3B). Introduction of the H4 K91A allele resulted in a dramatic loss of Sir2p at both telomeres and HMR (Sir2p levels were unaffected, data not shown). Consistent with this result, levels of histone H4 NH_2-terminal tail acetylation, which are low in silenced regions of the genome, increased at telomeres and HMR in H4 K91A cells (Figure 3C).

The methylation of histone H3 K 79 was the first modification identified in the globular core domain of the histones (Feng et al., 2002; Lacoste et al., 2002; Ng et al., 2002; van Leeuwen et al., 2002). This modification is widespread in the genome but specifically excluded from silenced regions and appears to function by preventing the promiscuous association of silencing factors, such as the Sir proteins, with euchromatin (Im et al., 2003; Ng et al., 2003; van Leeuwen and Gottschling, 2002). Because mutations that alter H3 K 79 generate silencing phenotypes similar to those observed with the H4 K91A mutation, we were interested in determining whether the loss of H4 K 91 acetylation influenced H3 K 79 methylation (Ng et al., 2002; van Leeuwen et al., 2002). By Western blot analysis, we did not detect a difference in overall H3 K 79 methylation abundance in the H4 K91A mutant strain (data not shown). We performed ChIP analysis to determine whether the K91A mutation alters the distribution of H3 K 79 methylation. We found that, although there was little change in H3 K 79 methylation at the HMR locus, there was a significant increase in the levels of this modification at telomeres (Figure 3D). Taken together, these results indicate that loss of H4 K 91 acetylation causes a substantial alteration of telomeric silent chromatin structure in which upregulation of telomere-proximal genes is accompanied by the acquisition of euchromatin-like features.

We next investigated the distribution of H4 K 91 acetylation. To facilitate this analysis, an antibody was raised against a synthetic peptide containing acetylated K 91. Consistent with the original identification of H4 K 91 acetylation in mammalian cells, this antibody, which had been immunodepleted to remove antibody recognizing unacetylated K 91, specifically recognizes histone H4 isolated from HeLa cells but fails to recognize recombinant H4 produced in *E. coli* (Figure 3E). In addition, dot blot analysis of modified and unmodified peptides encompassing several sites of H4 acetylation indicated that this antibody was highly specific for the acetylation of H4 K 91 (Figure 3F). Anti-acetylated H4 K 91 antibodies were then used to determine the relative abundance of this modification at several sites in the genome by ChIP. As an added measure to ensure the specificity of the ChIP reaction, the antibody was

preincubated with lysate isolated from a strain containing the H4 K91A allele to block any crossreactivity against other histone modifications. The results of the ChIP experiments indicated that H4 K 91 acetylation was significantly enriched in active regions of the genome and is present at low levels at telomeres and the HMR locus (Figure 3G). Importantly, no ChIP signal was detected from H4 K91A cells. In addition, the ChIP signal was not affected by deletion of *HAT1*, indicating that Hat1p is not the primary HAT responsible for the acetylation of H4 K 91 and that the Hat1p-Hat2p-Hif1p complex is not solely responsible for the deposition of histone H4 containing this modification consistent with the results shown in Figure 1H (data not shown). These data suggest that it is unlikely that H4 K 91 acetylation acts directly in the recruitment or assembly of factors involved in silent chromatin formation. Rather, this modification may influence silencing through defects in chromatin assembly in regions of silent chromatin or through a mechanism similar to that proposed for histone H3 K 79 methylation in which the presence of H4 K 91 acetylation in active chromatin works to confine silencing factors to the proper regions of the genome (van Leeuwen and Gottschling, 2002).

Because a mutation at H4 K 91 caused a redistribution of H3 K 79 methylation, we tested whether the reciprocal relationship also existed. Figure 3H shows the levels of H4 K 91 acetylation at silenced and nonsilenced regions in wt and H3 K79A cells. In the presence of the H3 K79A allele, there was a clear increase in H4 K 91 acetylation at silenced loci. This indicated that the changes in silent chromatin structure resulting from the loss of H3 K 79 methylation included increased levels of acetylation on H4 K 91.

To further explore the relationship between H4 K 91 acetylation and H3 K 79 methylation, we analyzed strains containing either single or double mutations that alter these residues. With respect to silent chromatin, the H3 K79A and H4 K91A alleles caused similar defects in silencing at telomeres and HMR that were not exacerbated when the mutations were combined (Figure S1 available with this article online). At HML, the H3 K79A allele had a more pronounced influence on transcriptional silencing than the H4 K91A allele. When these alleles were present in the same cell, the level of silencing was similar to that seen in the single H4 K91A mutant, suggesting that the effect of the H4 K91A allele at HML was dominant. Finally, none of the H3 K79A/H4 K91A allele combinations had a significant effect on rDNA silencing. Taken together, these results indicate H3 K79A and H4 K91A alleles have similar effects on silent chromatin structure and suggest that they function in a common pathway to regulate chromatin structure.

In the context of DNA damage repair, H3 K 79 methylation and H4 K 91 acetylation appear to function independently. As seen in Figure S1, K79A/H4 K91A double mutants were clearly more sensitive to DNA double-strand breaks than either of the single mutants. This result is consistent with the involvement of the H3 K 79 methyltransferase Dot1p in meiotic checkpoint control, and the recent demonstration that H3 K 79 methylation in mammalian cells is involved in the recruitment of the DNA damage checkpoint protein 53BP1 (a homolog of yeast Rad9p) to sites of DNA double-strand breaks (Huyen et al., 2004; San-Segundo and Roeder, 2000).

H4 Lysine 91 Affects Dimer/Tetramer Interactions

We next sought direct evidence that H4 K 91 influences the association of H2A/H2B dimers with chromatin and might, therefore, play a role in modulating the formation of histone octamers. First, defects in octamer formation and chromatin assembly would likely result in global defects in chromatin structure. Therefore, the structure of chromatin isolated from wt and H4 K91A cells was probed by digestion with micrococcal nuclease (MNase). An equal quantity of nuclei from wt and H4 K91A cells was digested for varying lengths of time, and the products were analyzed by agarose gel electrophoresis (Figure 4A). It is apparent at each time point that chromatin from the H4 K91A cells is digested more rapidly than wt chromatin.

To compare the stability of the histone octamers in the wt and H4 K91A cells, chromatin from each was applied to a hydroxyapatite column. Chromatin binds tightly to hydroxyapatite through interactions between the resin and DNA. Based on experiments originally performed with avian erythrocyte chromatin, the histone octamers can then be dissociated into H2A/H2B dimers and H3/H4 tetramers through step elution with buffer containing 0.9M NaCl and 2.0 M NaCl, respectively (Simon and Felsenfeld, 1979). We reasoned that if the H4 K91A mutation destabilized the dimer/tetramer interaction, the H2A/H2B dimers would elute more easily from the hydroxyapatite column. As seen in Figure 4B, the peak of histone H2B eluted from wt chromatin in 0.9 M NaCl as expected. However, histone H2B from H4 K91A chromatin eluted between 0.7 and 0.8M NaCl, suggesting a weakened interaction between the H2A/H2B dimers and H3/H4 tetramers. Taken together, these results indicate that H4 K 91 is important for the stability of histone octamers and the proper formation of chromatin structure.

The combined biochemical, structural, and genetic data described above suggest a model for the role of H4 K 91 acetylation in the assembly of histone octamers. We hypothesize that newly synthesized histone H4 is acetylated on K 91 prior to deposition onto DNA and that this acetylation modulates the association of H3/H4 tetramers and H2A/H2B dimers. After assembly of the acetylated H3/H4 tetramers onto DNA, the acetyl moiety on K 91 is removed, allowing for the stable binding of H2A/H2B dimers and completion of the histone octamer. One prediction of this model is that regulation of the positive charge of K 91 by acetylation plays an important role in controlling the process of chromatin assembly. In fact, the importance of this positive charge is also highlighted by the fact that this residue forms a salt bridge with a glutamic acid residue in histone H2B (E75 in yeast H2B) (Cosgrove et al., 2004).

We replaced H4 K 91 with amino acids that mimic the constitutively acetylated or unacetylated state to test this model. Substitution with glutamine, which is reminiscent of acetyl-lysine, would be predicted to disrupt proper chromatin assembly and display phenotypes similar to the K91A allele. Conversely, replacing K 91 with arginine, which is constitutively positively charged, would restore the ability of a salt bridge to form between H4 and H2B and, therefore, bypass this form of regulation and allow for chromatin assembly to proceed. Figures 4C and 4D compare the telomeric silencing and DNA damage sensitivity phenotypes of the H4 K91Q and K91R alleles. As predicted, the K91Q allele displays phenotypes similar to the K91A allele, whereas the K91R mutant is not defective in either telomeric silencing or DNA damage repair. Hence, these results are entirely consistent with a model in which the charge on K 91, which can be regulated by acetylation, influences the formation or stability of histone octamers.

The histone H4 molecules that are associated with the nuclear Hat1p-Hat2p-Hif1p complex are likely to represent newly synthesized histones. The unexpected observation that these histones contain modifications in their core domains suggests that the evolutionarily conserved acetylations on the H4 NH_2-terminal tail are not the only modifications that play a role in the process of chromatin assembly. In fact, although mutations that change the sites of NH_2-terminal tail modification have only minor phenotypic consequences, alterations in H4 K 91 result in severe defects in DNA repair and silent chromatin formation (Ma et al., 1998; Megee et al., 1990). This suggests that core domain modifications may actually play a more important role in chromatin assembly than NH_2-terminal tail acetylation.

The position of histone H4 K 91 in the nucleosome structure suggests that modification of this site could influence other processes in addition to chromatin assembly. For example, there is evidence that transcription by RNA polymerase II can result in disruption of nucleosome structure through the displacement of H2A/H2B dimmers (reviewed in Sims et al. [2004]). By preventing the reassembly of histone octamers, acetylation of K 91 could serve as a mechanism by which actively transcribed chromatin is kept in an open configuration. Deacetylation of this residue would then allow for the reassembly of normal chromatin structure as part of the process

of transcriptional repression. The observation that K 91 acetylation is enriched in euchromatic regions of the genome is consistent with this possibility. Although a potential role for H4 K 91 acetylation in transcription remains speculative, it would provide an example of histone modifications regulating gene expression through specific structural alterations in chromatin rather than through modulating the binding of chromatin associated proteins.

Experimental Procedures

Yeast Strains and Plasmids

Yeast genetic manipulations were done according to standard methods (Adams et al., 1997). Histone alleles were generated as described (Kelly et al., 2000). *MEC3*, *YKU70*, *RAD52*, *ASF1*, and *CAC1* were disrupted in UCC1111. *MEC1* was deleted in MPY302 after PCR-mediated disruption of *SML1* by *URA3* (Kelly et al., 2000).

Telomeric Silencing and DNA Damage Assays

Telomeric silencing and DNA damage sensitivity assays were performed as described (Kelly et al., 2000; Qin and Parthun, 2002).

Microarray Analysis

Yeast cells were grown to an OD 600 of 0.7–0.8 at 30°C and total RNA isolated by RNeasy Kits (QIAGEN). Yeast Genome S98 (Affymetrix) genechips were used. Gene expression levels were estimated from GeneChip PM probe intensities by means of an enhanced version of the Li-Wong PM-only algorithm. Three independent replicates were used for statistical analysis.

ChIP Analysis

ChIP assays were performed as described (Strahl-Bolsinger et al., 1997). Antibodies used in this study included anti-Sir2 (Santa Cruz Biotech Inc), anti-dimethyl-histone H3 K-79 antibody (Upstate), and anti-acetyl-histone H4 antibody. Before use in ChIP assays, the anti-acetyl-K 91 histone H4 antibody was immunoblocked by using UCC1111/K91A cell lysate to deplete nonspecific interactions. PCR analysis was performed in the linear range, and PCR products were electrophoresed through 2% agarose gel, stained with Vistra green (Invitrogen), and fluorescence quantitated by using a Storm 860 (Molecular Dynamics).

Western and Dot Blot Assays

Western and dot blots were performed by using standard techniques. Detailed information is in the Supplemental Data.

MNase Digestion

The yeast nuclei were isolated as described (Ai and Parthun, 2004). 0.6 g of nuclei were digested with 20 Worthington Units of MNase (Sigma) for increasing periods of time. Reactions were stopped by adding 10 μl 0.5 M EDTA. DNA was isolated, electrophoresed in 1% agarose gel, and visualized by ethidium bromide staining.

Hydroxyapatite Chromatography

Nuclei were isolated from 3 liters of cells. Hydroxyapatite resolution of histone dimers and tetramers were essentially as described with minor modifications (Simon and Felsenfeld, 1979; Stein and Mitchell, 1988). A detailed description is presented in the Supplemental Data.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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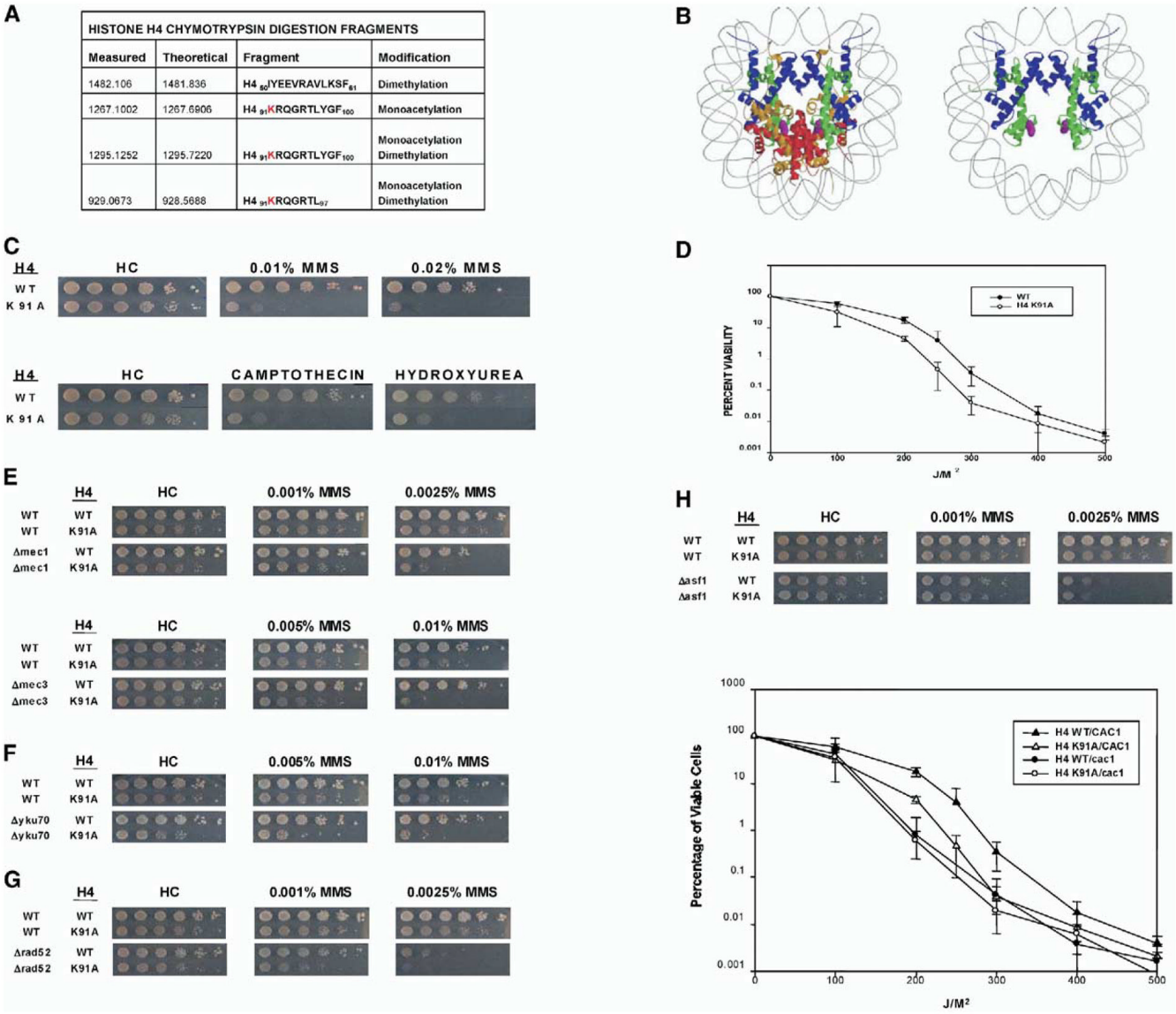


Figure 1. Histone H4 Associated with the Hat1p-Hat2p-Hif1p Complex Is Acetylated on Lysine 91, a Residue Important for DNA Damage Repair

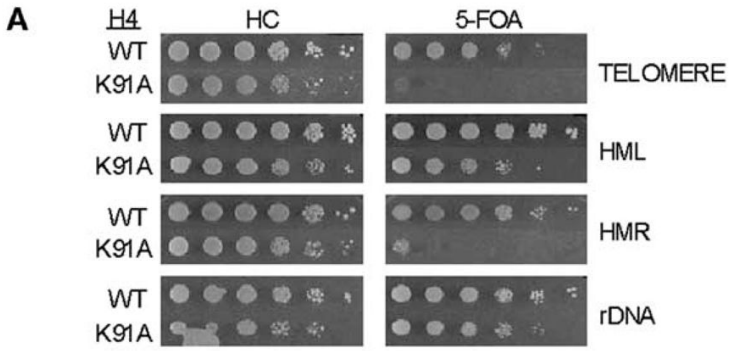
(A) Histone H4 resolved by SDS-PAGE was proteolytically digested, and the indicated modified peptides were identified by mass spectrometry.

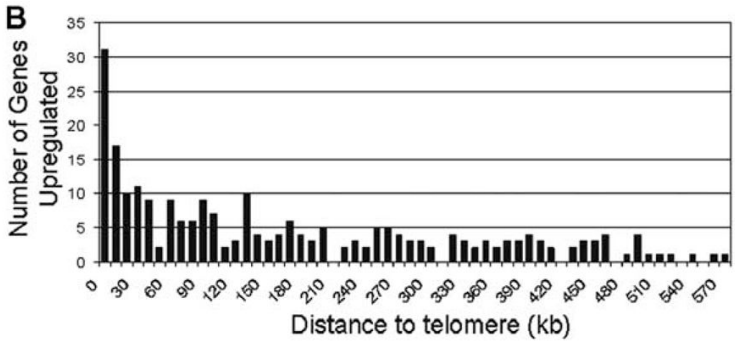
(B) Crystal structure of the nucleosome showing the full histone octamer (left) or the H3/H4 tetramer (right). Histone H4 K 91 is highlighted in pink. Structure was generated with MOLSCRIPT and RASTER 3D by using PDB code 1ID3.

(C) 10-fold serial dilutions of cells, containing the indicated allele of histone H4, were plated on synthetic complete plates (HC) ± the indicated DNA damaging agent.

(D) For the analysis of UV sensitivity, cells were plated on HC plates and then subjected to the indicated doses of UV radiation.

(E–H) The indicated histone H4 alleles were introduced into strains deleted for factors involved in the DNA damage checkpoint (E), NHEJ (F), recombinational repair (G), or chromatin assembly (H).





C

Distance from telomere (kb)	Fraction of ORFs upregulated > 1.5-fold	Fraction of ORFs upregulated >2-fold
0-10	0.49	0.23
10-20	0.27	0.123
20-30	0.23	0.08
30-40	0.16	0.08

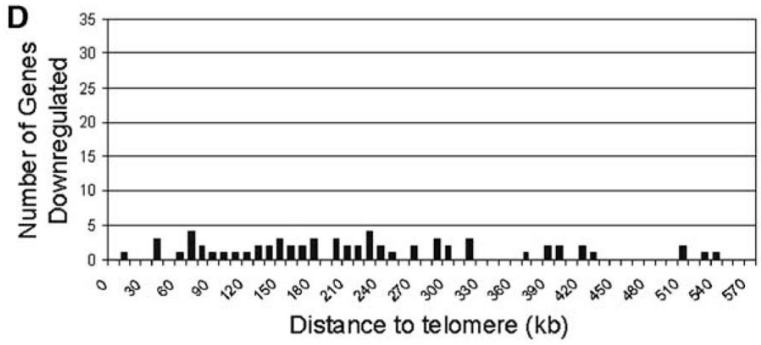


Figure 2. Histone H4 Lysine 91 Is Important for Gene Silencing

(A) The indicated alleles of histone H4 were transformed into strains carrying the *URA3* reporter gene at a telomere (UCC1111), the silent mating loci HML (UCC7266) and HMR (UCC7262), or the rDNA repeat (UCC1188) (van Leeuwen et al., 2002). Transcriptional silencing of *URA3* was assayed by comparing growth of cells on plates containing synthetic complete media (HC) ± 5-FOA (5-FOA).

(B) The number of genes that are upregulated in H4 K91A mutant, identified by microarray analysis, is plotted versus their distance to the telomere.

(C) The fraction of the total ORFs within each distance range from the telomere that are upregulated greater than 1.5- or 2-fold.

(D) The number of genes that are downregulated in H4 K91A mutant, identified by microarray analysis, is plotted versus their distance to the telomere.

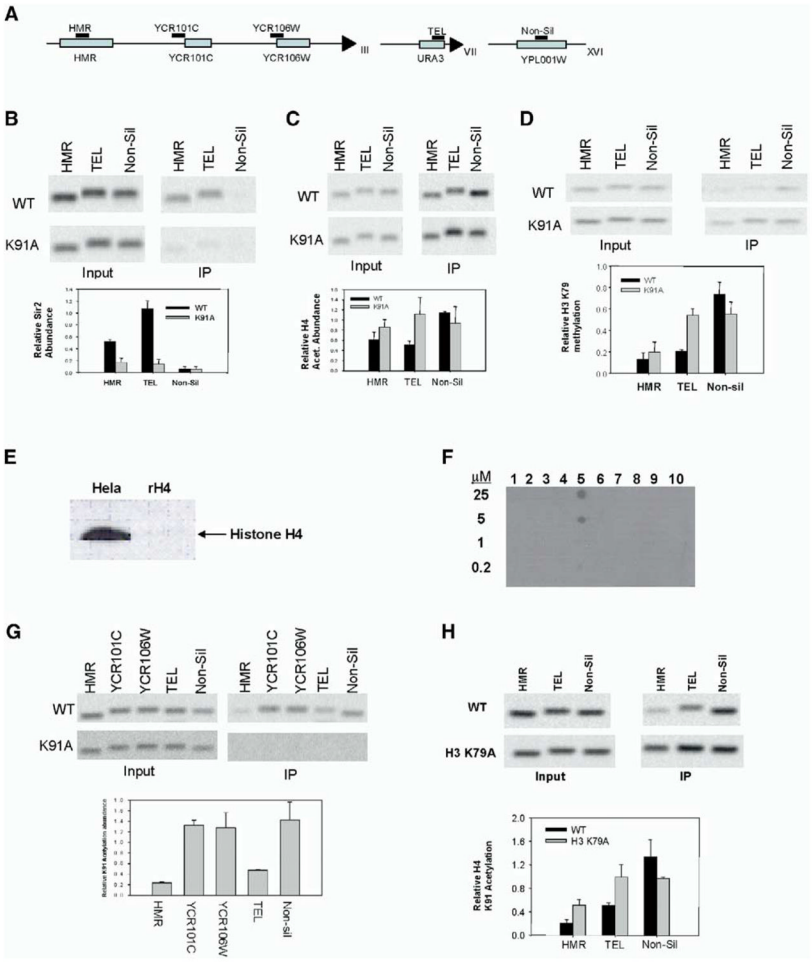


Figure 3. H4 K 91 Acetylation Is Important for Maintaining Silent Chromatin Structure and Is Enriched in Active Chromatin Regions

(A) Schematic representation of chromosomal regions assayed by ChIP.

(B–D) ChIP assays measuring the presence of Sir2p (B), acetylated histone H4 (C), and histone H3 lysine 79 methylation (D) at the indicated loci.

(E) A Western blot containing histones isolated from either sodium butyrate-treated HeLa cells or *E. coli*-expressing recombinant H4 was probed with immunodepleted anti-acetylated H4 K 91 antibodies.

(F) Either unmodified (even numbered lanes) or modified (odd numbered lanes) peptides encompassing sites of histone H4 acetylation (lanes 1 and 2, K 12; lanes 3 and 4, K 16; lanes 5 and 6, K 91; lanes 7 and 8, K 5; and lanes 9 and 10, K 8) were spotted in the concentrations indicated. Dot blots were then probed with immunodepleted anti-acetylated H4 K 91 antibodies.

(G) ChIP assays were performed with anti-acetylated H4 K 91 antibody to measure the relative abundance of this modification at the indicated loci.

(H) Abundance of acetyl-H4 K 91 at the indicated loci was determined in wild-type (wt) and H3 K79A cells by ChIP.

Bar graphs give the average of three independent trials with the SD represented by error bars.

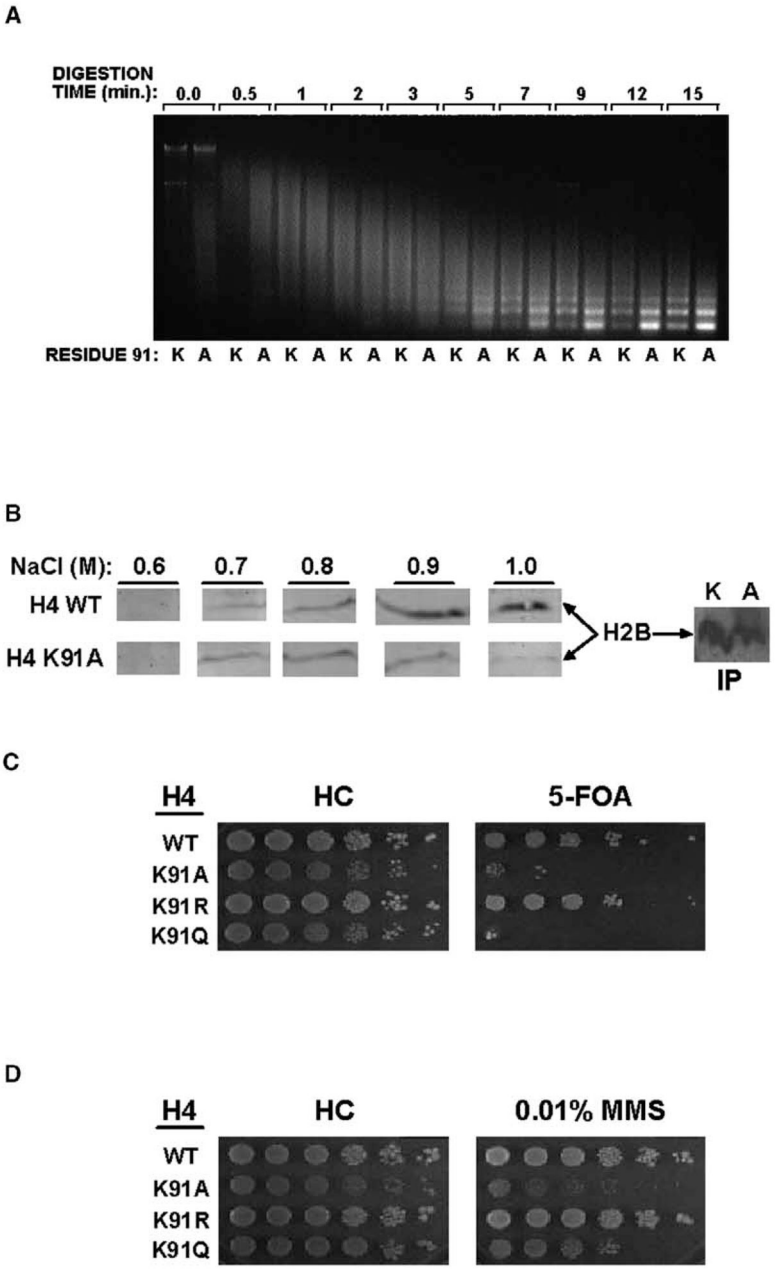


Figure 4. Histone H4 Lysine 91 Is Important for Dimer/Tetramer Interactions and the Proper Formation of Chromatin Structure

(A) Equal quantities of nuclei from wt and H4 K91A cells (lanes K and A, respectively) were digested with MNase for the indicated times. After deproteination, DNA was analyzed by agarose gel electrophoresis.

(B) An equal quantity of chromatin from wt and H4 K91A cells was applied to a hydroxyapatite column and step eluted with buffer containing the indicated concentrations of NaCl (lanes labeled IP indicate the input samples). Fractions were analyzed by Western blots probed with an antibody recognizing histone H2B. All lanes shown were from a single blot. This experiment was performed three times with identical results.

(C) Plasmids containing the indicated alleles of H4 were transformed into UCC1111 and telomeric silencing assayed as in Figure 2.

(D) Strains containing the indicated alleles of H4 were assayed for sensitivity to MMS as described in Figure 1.