MINIREVIEW / MINIREVIEW

How does the histone code work?[1]

Michael S. Cosgrove and Cynthia Wolberger

Abstract: Patterns of histone post-translational modifications correlate with distinct chromosomal states that regulate access to DNA, leading to the histone-code hypothesis. However, it is not clear how modification of flexible histone tails leads to changes in nucleosome dynamics and, thus, chromatin structure. The recent discovery that, like the flexible histone tails, the structured globular domain of the nucleosome core particle is also extensively modified adds a new and exciting dimension to the histone-code hypothesis, and calls for the re-examination of current models for the epigenetic regulation of chromatin structure. Here, we review these findings and other recent studies that suggest the structured globular domain of the nucleosome core particle plays a key role regulating chromatin dynamics.

Key words: histones, histone code, modifications, epigenetic, chromatin, nucleosome, dynamics, regulated nucleosome mobility, core, archaeal, combinatorial switch, histone octamer.

Résumé : Les patrons des modifications post-traductionnelles des histones sont corrélés avec des états chromosomiques distincts qui régulent l'accès à l'ADN, conduisant à l'hypothèse de l'existence d'un « Code Histone ». Il est cependant difficile de voir comment la modification des queues flexibles des histones conduit à des changements dans la dynamique du nucléosome et par conséquent dans la structure de la chromatine. La découverte récente que, comme les queues flexibles des histones, des domaines globulaires structurés des particules centrales du nucléosome soient aussi considérablement modifiés ajoute une dimension nouvelle et excitante à l'hypothèse du code histone, nous obligeant à réexaminer les modèles courants de régulation épigénétique de la structure de la chromatine. Nous passons ici en revue les découvertes et autres études récentes qui suggèrent que le domaine globulaire structuré des particules centrales des nucléosome puisse jouer un rôle clé dans la régulation de la dynamique de la chromatine.

Mots clés : histones, code histone, modifications, épigénétique, chromatine, nucléosome, dynamique, mobilité régulée des nucléosomes, centre, archée, commutateur combinatoire, octamère d'histone.

[Traduit par la Rédaction]

Introduction

Eukaryotic DNA is packaged into the nucleus with the help of a number of histone and nonhistone proteins that collectively make up the chromatin fiber. This highly dynamic fiber can exist in several states of compaction that regulate access to DNA for essential cellular processes, such as transcription, replication, repair, and recombination. At one extreme, chromatin adopts a condensed structure called heterochromatin, in which genes are less accessible and frequently transcriptionally silent (Owen-Hughes and Bruno 2004). Decondensed chromatin, called euchromatin, is much more accessible than heterochromatin, and contains the majority of actively expressed genes. Despite intense investigation, the molecular mechanisms that control the interconversion between chromatin states are not well understood.

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The nucleosome core particle

The fundamental repeating unit of chromatin is the nucleosome, a disc-shaped octamer that contains a histone H3/H4 heterotetramer flanked by 2 H2A/H2B heterodimers, around which 147 base pairs of genomic DNA are wrapped (Fig. 1). Each of the highly conserved histones contains a structured globular domain, which interacts with other histones and DNA within a nucleosome, and flexible tails, which protrude from the lateral surface of the histone octamer. The flexible tails are highly basic and are the substrates for numerous enzymes that introduce a diverse array of post-translational modifications, including acetylation, methylation, phosphorylation, and ubiquitylation. Because distinct histone post-translational modifications correlate with specific transcriptional states, a histone-code hypothesis

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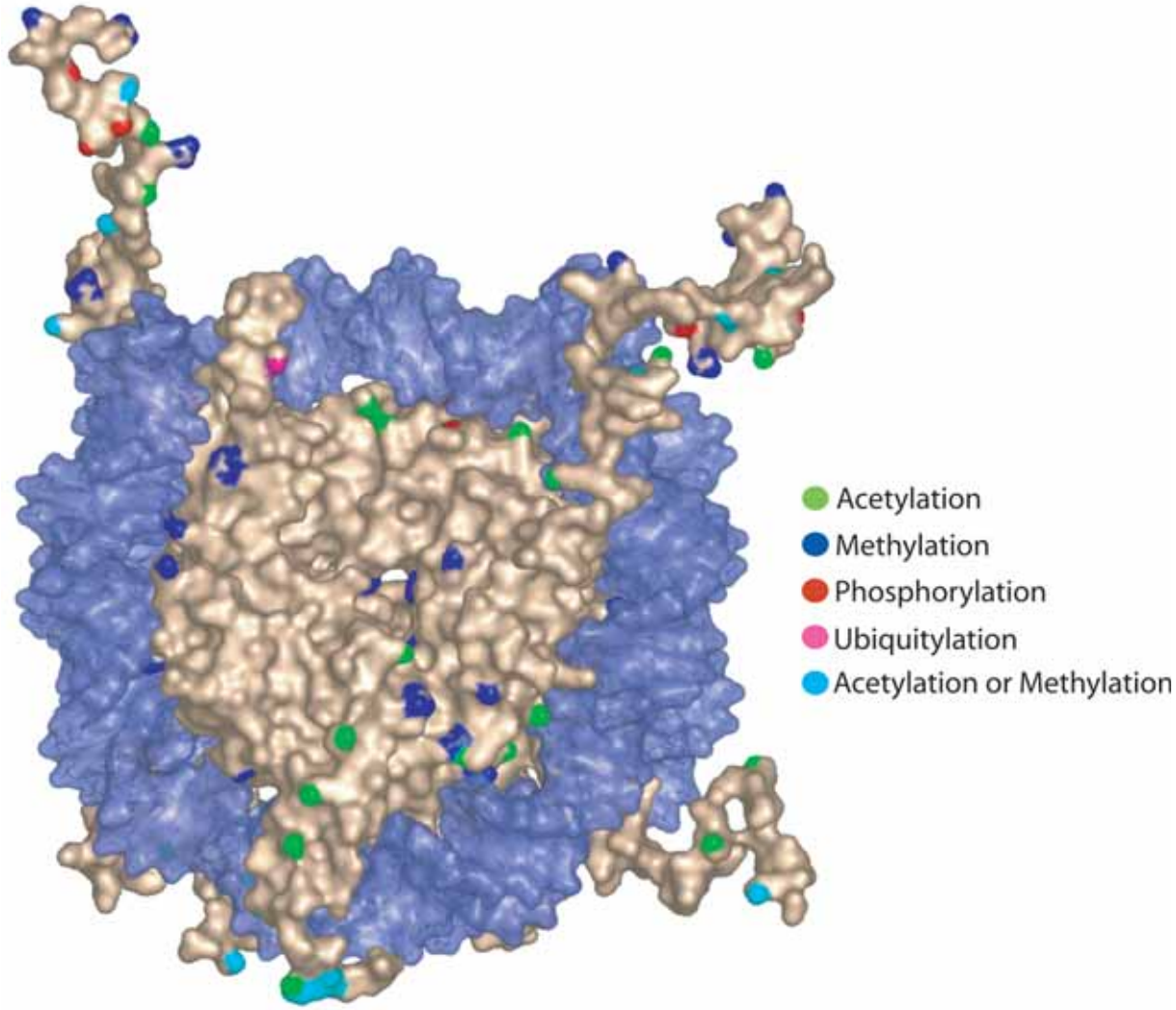
Fig. 1. Surface representation of the nucleosome core particle, viewed down the DNA superhelix axis (PDB code 1KX5) (Davey et al. 2002). DNA (light blue) is wrapped around the globular domain of the histone octamer colored in wheat. The positions of known histone modifications have been mapped (colored as indicated). The flexible histone tail domains are shown protruding radially from the lateral surface of the nucleosome core particle. Representations of the nucleosome were generated using PyMOL (Delano 2002).



has been proposed (Fischle et al. 2003*a*; Jenuwein and Allis 2001; Strahl and Allis 2000; Turner 2000). This hypothesis suggests that specific patterns of modifications are read like a molecular bar code to recruit the cellular machinery that brings about a distinct chromatin state. Although there is a wealth of evidence that supports the histone code in the recruitment of nonhistone proteins to the chromatin fiber (Jenuwein and Allis 2001), the molecular details by which these proteins regulate chromatin dynamics remain to be elucidated.

The flexible tails

A long-standing model suggests that histone modifications regulate the interaction between the highly basic histone tails and nucleosomal DNA or linker DNA (Angelov et al. 2001). Although some observations support this view (Ausio et al. 1989; Brower-Toland et al. 2005; Fletcher and Hansen 1995; Polach et al. 2000; Schwarz et al. 1996; Tse and Hansen 1997), the interpretation of these in vitro results are complicated by the common use of histone preparations derived from native sources, which have undefined post-translational modification states. A recent study by Dorigo et al. (2003) addressed this shortcoming by using recombinant modification-free histones to study the role of flexible histone tails in chromatin-fiber compaction. They found that deletion of all 4 histone tails (with the exception of residues 14–19 at the base of the H4 tail) did not affect the ability of reconstituted nucleosome arrays to fold into compact structures (Dorigo et al. 2003); this suggests that most of the tails are not required for 30-nm fiber formation. Deletion of the base of the histone H4 tail (residues 14–19) prevented complete compaction of nucleosome arrays, suggesting that this segment plays a role in chromatin-fiber compaction. It has been observed in the crystal structure of the nucleosome core particle that the base of the H4 tail makes interparticle contact with the histone H2A/H2B dimer of a neighboring molecule (Luger et al. 1997), suggesting that this section of the H4 tail stabilizes internucleosomal interactions. However, because the same crystal structure shows that the base

of the H4 tail also contacts the nucleosomal DNA, it is important to distinguish whether this effect is due to intra- or internucleosomal interactions. In support of the latter, it was recently shown that compacted nucleosome arrays could be stabilized by introducing interparticle disulfide crosslinks between the base of the histone H4 tail and the core domain of histone H2A (Dorigo et al. 2004).

The structured globular core

The structured globular domain of the histone octamer, once thought of only as a structural scaffold to guide DNA, is turning out to have a more dynamic and complex role in the regulation of chromatin structure. Early indications of the importance of the structured histone globular domain came from genetic screens in yeast, which identified numerous globular-domain amino-acid residues important for gene expression (Kruger et al. 1995; Park et al. 2002). The recent application of mass spectrometry to histone biology has led to the startling discovery that many of the same residues are targeted for post-translational modification (Cocklin and Wang 2003; Feng et al. 2002; Ng et al. 2002; van Leeuwen et al. 2002; Zhang et al. 2002, 2003; reviewed in Cosgrove et al. 2004 and Freitas et al. 2004). These new modifications dramatically expand the potential histone code and raise many questions about the roles of the flexible and structured parts of the nucleosome in chromatin regulation. To help make sense of this complexity, we recently proposed that histone post-translational modifications could be separated into 2 functional categories: class I and class II histone-code modifications (Cosgrove et al. 2004). Class I modifications function by regulating the recruitment of effector domains, such as the bromo and chromo domains, that target nonhistone proteins to DNA; whereas class II modifications regulate histone–DNA and histone–histone interactions, primarily by direct chemical interference.

Class I modifications

Class I histone modifications include all modifications that indirectly regulate chromatin structure through recruitment of chromatin-associated proteins. This includes the majority of modifications in the histone tails on which the histone code is based, and has been the subject of a number of excellent reviews (Berger 2002; Fischle et al. 2003*b*; Kouzarides 2002; Peterson and Laniel 2004). Class I modifications are important for the recruitment of a variety of proteins, such as histone-modifying enzymes, transcription factors, heterochromatin-associated proteins (i.e., HP1), and ATP-dependent nucleosome-remodeling enzymes. A common property of these proteins is that they often contain histone-modification recognition domains, such as the bromo and chromo domains, which recognize acetylated and methylated lysine residues, respectively, (Jenuwein and Allis 2001).

But how do these proteins regulate chromatin structure once they are recruited to the chromatin fiber? Several lines of evidence suggest that recruited proteins either stabilize or remodel specific chromatin states. For example, one model suggests that modifications at the nucleosomal level may set up defined chromosomal subdomains, which are then stabilized by heterochromatin-associated proteins (Jenuwein 2001). An example of this model is the methylation of histone H3 Lys 9 by SUV39H1 (Rea et al. 2000), which creates a high-affinity binding site for the chromodomain of heterochromatin protein HP1 (Bannister et al. 2001; Lachner et al. 2001). It is thought that HP1 dimerization brings together distant chromatin domains, thus stabilizing higher-order chromatin structures (Jenuwein 2001). Another possibility is that the binding of HP1 to H3 Lys 9–methylated nucleosomes in higher eukaryotes stabilizes heterochromatin in a manner analogous to linker histones, which have been shown to regulate nucleosome mobility (Ura et al. 1995, 1997) and stabilize the folding of nucleosomal arrays (Carruthers et al. 1998). However, the Suv39H1/HP1 mechanism is not universal; the budding yeast *Saccharomyces cerevisiae* lacks methylation of H3 Lys 9 and an obvious HP1 homologue, yet it still contains well-characterized heterochromatin (Jenuwein and Allis 2001).

The recruitment of proteins that remodel specific chromatin states is another mechanism for the regulation of chromatin structure. Remodeling can be accomplished by recruiting histone-modifying enzymes and ATP-dependent nucleosome-remodeling activities. Although histone-modification enzymes that target the flexible histone tails are being identified at a rapid pace (Peterson and Laniel 2004), it is still not clear how modifications in the flexible parts of the nucleosome regulates chromatin structure. On the other hand, the recruitment of ATP-dependent nucleosome-remodeling enzymes suggests a direct mechanism for overcoming the repressive nature of the nucleosome (Chang and Luse 1997; Fry and Peterson 2002; Soutoglou and Talianidis 2002). ATP-dependent nucleosome-remodeling enzymes use the energy of ATP hydrolysis to alter the physical properties of the nucleosome, so that nucleosomal DNA is made more accessible (Becker and Horz 2002). The classic view is that ATP-dependent nucleosome-remodeling enzymes are recruited by DNA-sequence-specific transcription factors to allow assembly of an active preinitiation complex, including RNA polymerase II, at the promoter (Lemon and Tjian 2000; Struhl 1999). However, recent studies suggest that several ATP-dependent nucleosome-remodeling enzymes can be recruited directly by specific class I histone-code modifications (Table 1). For example, it has recently been shown that the tandem bromodomains of the Rsc ATP-dependent nucleosome-remodeling complex specifically recognize acetylation of H3 Lys 14, which is required for gene activation (Kasten et al. 2004). In addition, the chromodomain of the Chd1 ATP-dependent nucleosome-remodeling complex was recently shown to recognize di- and trimethylation of histone H3 Lys 4, which in turn recruits the SAGA and SLIK histone-acetyltransferase complexes to promoters to promote gene activation (Pray-Grant et al. 2005). Acetylation of H3 Lys 14 and methylation of H3 Lys 4 are both histone modifications commonly associated with euchromatin (Jenuwein and Allis 2001).

Once ATP-dependent nucleosome remodelers are recruited to promoters, ATP hydrolysis is used to weaken histone–DNA contacts; this results in increased nucleosome mobility (nucleosome sliding or displacement) (Becker and Horz 2002) or histone-variant exchange (Mizuguchi et al. 2004). The mechanism that is used by these enzymes to weaken histone–DNA contacts is not well understood and is the subject of active debate (Langst and Becker 2004). However,

Table 1. Examples of ATP-dependent nucleosome-remodeling enzymes that are recruited by histone-tail post-translational modifications.

Complex	Effector domain	Histone modification[a]	Function	Reference
Swr1–Bdf1	bromo	H3-H4 tail	transcriptional activation	Krogan et al. 2003; Ladurner et al. 2003; Matangkasombut and Buratowski 2003
CHD–SAGA	chromo	H3 K4me	transcriptional activation	Pray-Grant et al. 2005
Rsc	bromo	H3 K134ac	transcriptional activation	Kasten et al. 2004
Nurd	chromo	H3 tail, H3 K9me?[b]	transcriptional repression	Nishioka et al. 2002; Zegerman et al. 2002
hSwi/Snf	bromo	H4 K8ac	transcriptional activation	Agalioti et al. 2002
BRAMA	????	H3 K4me H3 K9me H4 K20me	transcriptional activation	Beisel et al. 2002
Isw1p	????	H3 K4me2,3	transcription elongation/termination	Santos-Rosa et al. 2003
INO80	????	H2A S129ph	double-strand break repair	Morrison et al. 2004; van Attikum et al. 2004

[a]The Brno nomenclature for histone modifications is used (Turner 2005). Number of methyl groups for methyl lysines is indicated where known. ac, acetyl; me, methyl; ph, phosphoryl.

[b]Although it is known that the Mi-2 ATPase of the Nurd ATP-dependent nucleosome-remodeling complex contains a chromo domain, as of this writing, it has not been experimentally established that this chromodomain is required for histone H3 tail binding.

the recent discovery that many DNA-contacting residues of the nucleosome core are targeted for post-translational modification (Zhang et al. 2003) suggests a possible mechanism for the activity of ATP-dependent nucleosome remodelers. We recently proposed that the energy of ATP hydrolysis is used to lift or peel nucleosomal DNA off the lateral surface of the histone octamer, exposing core amino-acid residues for post-translational modification (Cosgrove et al. 2004). Although this remains to be demonstrated experimentally, the presence of histone modifications on the lateral surface of the nucleosome core suggests that nucleosome mobility can be regulated by post-translational modifications that alter histone–DNA contacts.

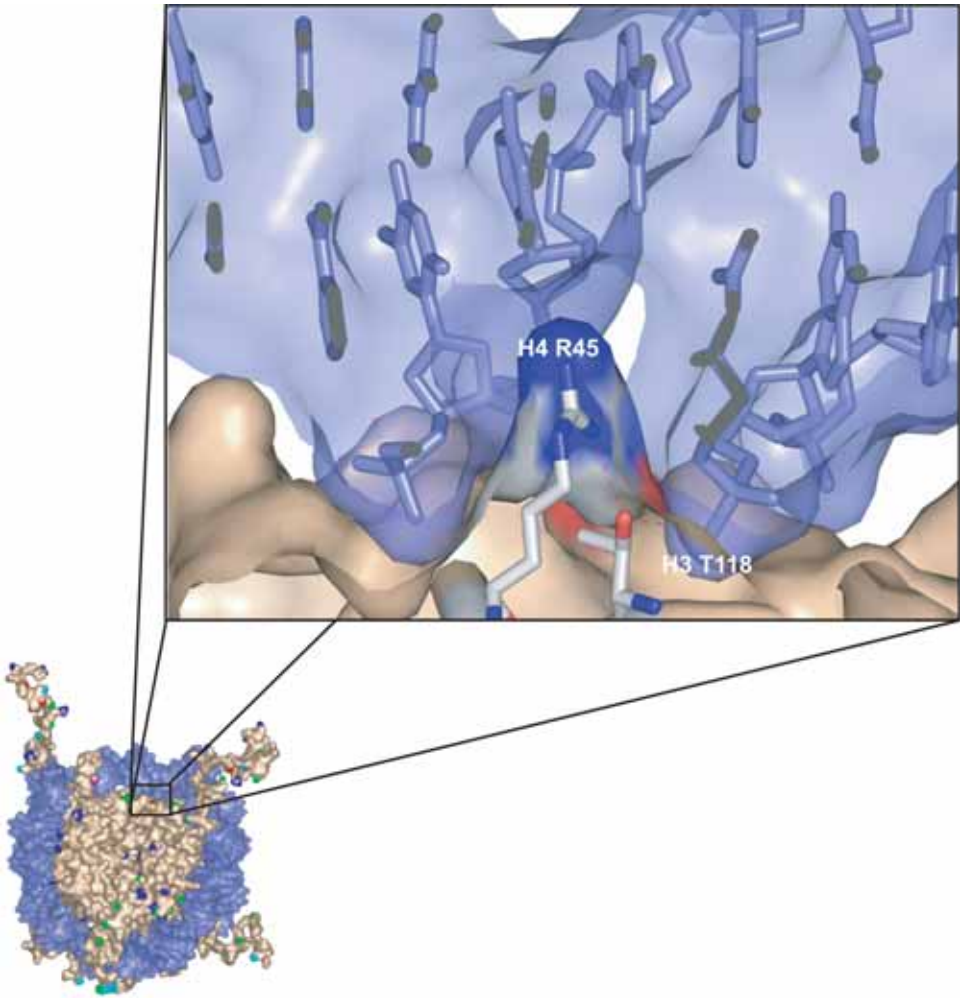
Class II histone modifications

Class II histone modifications are defined as modifications that function through direct chemical interference with histone–histone or histone–DNA interactions. Acetylation and phosphorylation are 2 examples of post-translational modifications that likely weaken histone–DNA contacts and promote increased nucleosome mobility. Conversely, removal of these modifications likely strengthens histone–DNA interactions and results in decreased nucleosome mobility. A proposal called the regulated nucleosome mobility model (Cosgrove et al. 2004) has been used to predict that mutation of lateral-surface amino-acid residues that disrupt histone–DNA interactions would increases nucleosome mobility and, consequently, gene transcription. Unbiased genetic screens in yeast have identified several such residues, called Sin mutations (Swi/Snf independent), that remove the need for the Swi/Snf ATP-dependent nucleosome-remodeling complex for gene expression (Kruger et al. 1995). Consistent with this model, recent biochemical and structural experiments have shown that Sin mutations significantly increase the thermal mobility of nucleosomes (Flaus et al. 2004), without significantly altering the 3-dimensional structure of the nucleosome core particle (Muthurajan et al. 2004). Conversely, the regulated nucleosome mobility model has been used to predict that mutation of lateral-surface residues that promote histone–DNA interactions will push the equilibrium of nucleosome mobility to a state of decreased mobility and, consequently, reduced gene expression. In support of this, it was recently shown that replacement of the lateral-surface H3 Lys 56 residue with arginine, which cannot be acetylated, results in the decreased expression of yeast histone and SUC2 genes (Xu et al. 2005). Xu et al. (2005) showed that histone H3 Lys 56 isacetylated by the Spt10 acetyltransferase and is required for histone gene expression. This suggests that H3 K56 acetylation may regulate histone gene expression in yeast by regulating nucleosome mobility. It remains to be determined if H3 K56 acetylation also occurs in mammals.

A striking example of how a post-translational modification affects a pair of lateral-surface Sin residues is shown in Fig. 2. The 3-dimensional structure of the nucleosome core particle shows H3 Thr 118 hydrogen bonded to the phosphate backbone of DNA and the arginine guanidinium of H4 Arg 45 (Luger et al. 1997). This residue, which is not known to be modified, is one of many arginines around the lateral surface of the nucleosome that protrudes into the minor groove of nucleosomal DNA to prevent its slippage over the lateral surface of the histone octamer. Modification of Thr 118, which has been shown to be phosphorylated in bulk histones from calf thymus (Zhang et al. 2003), likely repels the negatively charged phosphate backbone of DNA and alters the interaction of Arg 45 with the DNA minor groove. This might weaken histone–DNA contacts and contribute to an increased ability of the histone octamer to slide on the DNA in *cis* or to be completely displaced. In fact, this Arg–Th/Ser pair is repeated at least 5 times at various positions around the lateral surface of the histone octamer (Luger et al. 1997), possibly representing a combinatorial switching mechanism that generates varying degrees of nucleosome mobility.

Because the degree of nucleosome mobility likely depends on the precise number, position, and chemical makeup of lateral-surface post-translational modifications, there may

Fig. 2. Enlarged view of histone Sin residues H4 Arg 45 and H3 Thr 118. It is predicted that phosphorylation of Thr 118 will significantly alter the nucleosome structure in this region, resulting in weakened histone–DNA contacts and increased nucleosome mobility.



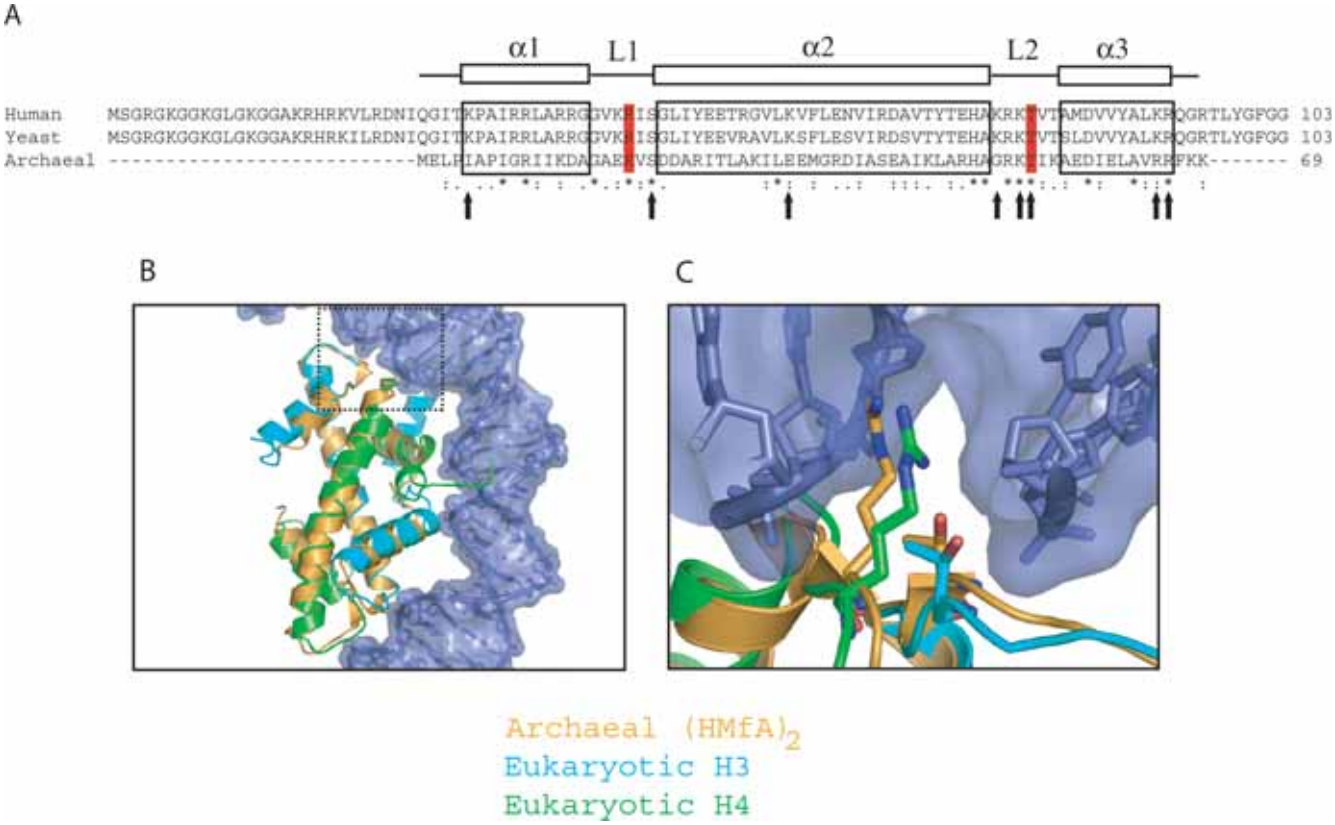
be class II histone modifications that result in variable nucleosome mobility. It is tempting to speculate that varying degrees of nucleosome mobility are responsible for regulating the interconversion between chromatin states. For example, it is likely that heterochromatic regionscontain regularly spaced nucleosomes with relatively low mobility, which facilitate the internucleosomal interactions required to fold the chromatin fiber into higher-order structures. Perhaps recruitment of heterochromatin proteins, such as linker histones and HP1, further modulates nucleosome mobility and allows the formation of even-higher-order chromatin structures. It is also likely that euchromatic regions contain nucleosomes with relatively high levels of mobility, whichprevent the internucleosomal interactions required for chromatin-fiber folding. Consistent with this model is the observation that a histone Sin mutation that increases the thermal mobility of nucleosomes on DNA (Flaus et al. 2004) prevents the folding of nucleosome arrays into the highly compact form representative of the 30-nm chromatin fiber (Horn et al. 2002).

The regulated nucleosome mobility model might apply to all species that use histones to compact chromatin, including simple eukaryotes, such as *S. cerevisiae*, and members of the archaeal domain of life. Notably, several Euryarchaeota encode homologues of eukaryotic histones that resemble the eukaryotic $[H3–H4]_2$ tetramer in structure and function (Fig. 3) (Reeve 2003; White and Bell 2002). A striking observation is that the archaeal histones lack the N- and C-terminal tails of eukaryotic histones, yet posses homologues of the same enzymes that are believed to regulate modifications of the tails in eukaryotes (e.g., Sir2). Could these archaeal enzymes modulate nucleosome mobility by post-translationally modifying lateral-surface residues on the archaeal tetramer? Indeed, several of the same DNA-interacting residues that are targeted for post-translational modification in higher eukaryotes are conserved in archaeal histones (Fig. 3). It remains to be determined whether these residues are modified in archaeal histones as they are in higher eukaryotes.

Class I or class II?

So far, the discussion has been limited to modifications that occur on the flexible histone tails or the lateral surface of the histone octamer, class I and class II histone code modifications, respectively. What about histone modifications that occur on the top and bottom of the nucleosome disc? Although it is not known which functional category these

Fig. 3. (A) ClustalW (Thompson et al. 1994) sequence alignment of human and yeast (*S. cerevisiae*) histone H4 with an archaeal homologue (HMf-2 from *Methanothermus fervidus*). The α1–α3 and L1–L2 loop secondary-structure elements are indicated at the top and by the boxed regions. Underneath the alignment: astericks (*), residues that are identical; colon (:), conservative substitutions; period (.), semiconservative substitutions. The arrows at the bottom indicate positions targeted for post-translational modification in either histone H3 or H4 in mammals (Zhang et al. 2003). The amino acids highlighted in red show the conservation of the Arg–Thr pair; it is predicted that this is part of a combinatorial switching mechanism that regulates nucleosome mobility. (B) Structural superposition of the archaeal HMfA dimer (PDB code 1B67) (Decanniere et al. 2000) with the eukaryotic histone H3/H4 dimer. (C) Magnified image of boxed region in (B), showing conservation of the Arg–Thr pair highlighted in red in (A).



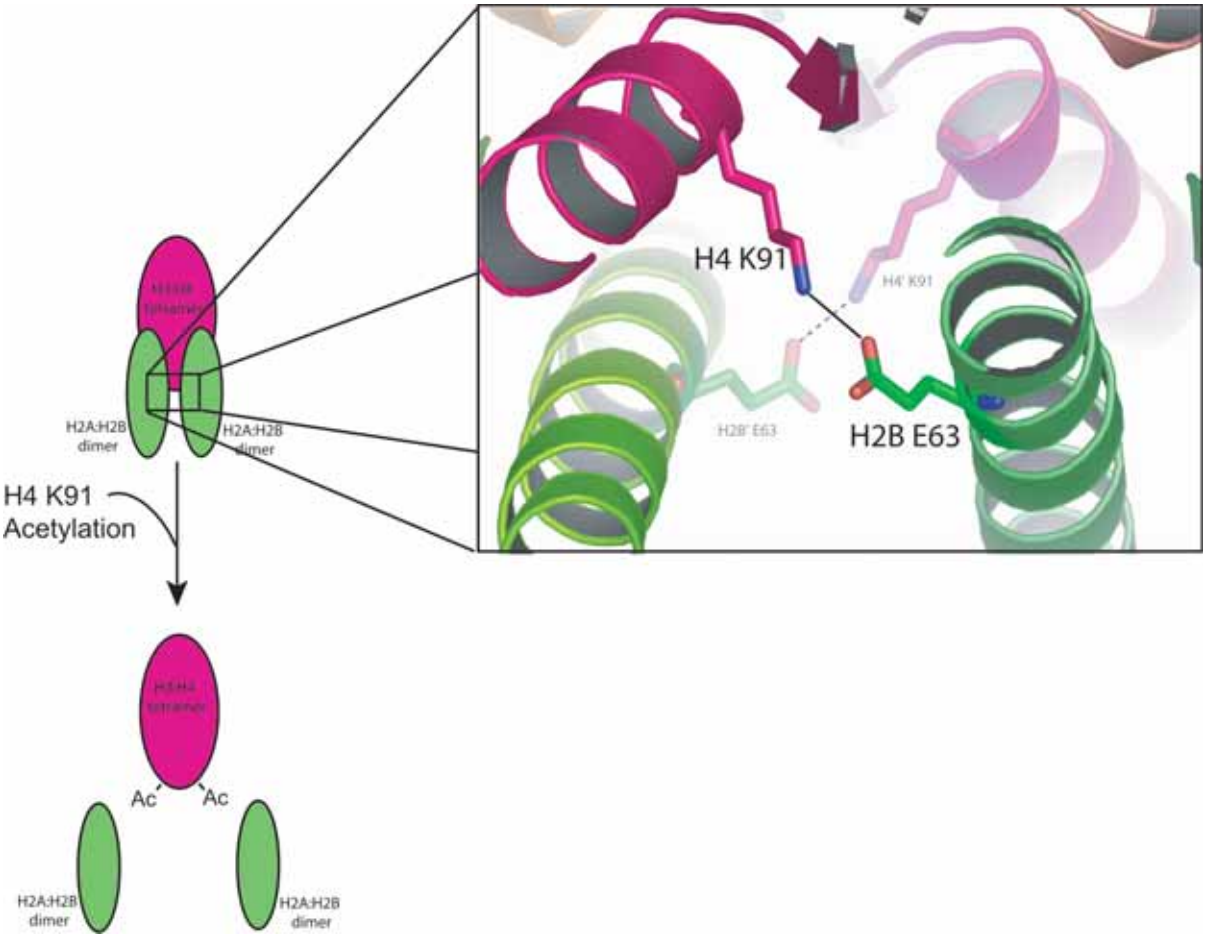
modifications fall into, there are several interesting observations that suggest some possibilities. One possibility is that these modifications function like class I modifications in the recruitment of nonhistone proteins. For example, methylation of H3 Lys 79 by the Dot1 methyltransferase has been shown genetically to regulate telomeric and homothallic mating (HM) loci silencing (Ng et al. 2002; van Leeuwen et al. 2002). This residue lies on a surface that has been identified, in a genetic screen for mutations in histones H3 and H4, as affecting all 3 types of silencing in yeast (Park et al. 2002). Deletion of the Dot1 gene and elimination of H3 Lys 79 methylation results in the redistribution of yeast Sir proteins at the expense of silenced loci (van Leeuwen et al. 2002), suggesting that Lys 79 methylation regulates the recruitment of Sir-protein complexes.

Other post-translational modifications on the top and bottom of the nucleosome core that can be considered class II modifications include those that might regulate inter- or intranucleosomal interactions. For example, although the function of acetylation of the 3 lysines on the nucleosome face of histone H2B (Lys 108, Lys 116, and Lys 120) is unknown, these residues line the ridge of a shallow groove, observed in an X-ray structure, to bind the base of the histone H4 N-terminal tail from an adjacent nucleosome (Luger et al. 1997). This suggests that acetylation at these sites plays a role in regulating internucleosomal interactions.

Modification of intranucleosomal interactions may be important for gene activation or histone-variant exchange. For example, in the unmodified form, H4 Lys 91 forms a salt bridge with H2B Glu 63 (Fig. 4), which might contribute to the stabilization of the histone octamer. H4 Lys 91 has been shown to be a target for acetylation, providing a potential mechanism for promoting H2A/H2B dimer release by disrupting this salt bridge and destabilizing the interaction of the H2A/H2B dimer with the nucleosome. This is supported by a recent study showing that the H4 K91A mutation results in destabilization of the histone octamer, which results in phenotypes in yeast that are consistent with defects in chromatin assembly (Ye et al. 2005).

The presence of several modified residues deep within the nucleosome center, where they appear inaccessible to modifying enzymes, suggests that some residues are modified before nucleosome assembly or after removal of 1 H2A/H2B dimer. Examples include residues H2A Lys 99 and H4 Arg 92. Interestingly, H2A Lys 99 is 1 of the 2 residues that vary in the structured-core domain of the human H2A variant H2A.X; it is replaced by glycine. Indeed, sequence comparisons of H2A.X homologues from organisms, ranging from

Fig. 4. Enlarged view of residues in the core of the nucleosome that stabilizes the histone octamer. Residue H4 Lys 91 forms a salt bridge with residue H2B Glu 63 of the opposite H2A/H2B dimer. It is predicted that acetylation of Lys 91 will disrupt this interaction, leading to destabilization of the histone octamer. We propose that this is a mechanism that catalyzes H2A/H2B removal from the nucleosome.



protist to mammals, show that this position is never lysine (Redon et al. 2002). Histone H2A is deposited into nucleosomes during DNA replication, whereas H2A.X is enriched at DNA double-strand breaks (Malik and Henikoff 2003). Could it be that that methylation of H2A K99 by nucleosomal-assembly complexes is an epigenetic mark that targets histone H2A deposition to nucleosomes during DNA replication? Likewise, could the H2A.X variant with a glycine at position 99 be recognized by a different nucleosome-assembly complex, one that deposits H2A.X to nucleosomes for DNA repair? It is hoped that these questions will be addressed in future studies.

Conclusion

The recent discovery that the structured globular domain of the nucleosome core particle can be as extensively modified as the flexible histone tails (Zhang et al. 2003) is a significant milestone in chromatin biology. This discovery raises several questions that underscore the importance of looking again at the role of the structured nucleosome core domain in the regulation of chromatin structure. What are the enzymes that target residues in the nucleosome core? How do they gain access? What is the functional significance of each modification? Are the modifications conserved throughout evolution? This discovery opens several potential lines of investigation that are likely to yield insights into the mysterious and dynamic nature of chromatin.

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