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Regulated nucleosome mobility and the histone code

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Post-translational modifications of the histone tails are correlated with distinct chromatin states that regulate access to DNA. Recent proteomic analyses have revealed several new modifications in the globular nucleosome core, many of which lie at the histone-DNA interface. We interpret these modifications in light of previously published data and propose a new and testable model for how cells implement the histone code by modulating nucleosome dynamics.

The organization of eukaryotic DNA into chromatin regulates whether proteins that mediate many essential cellular processes can gain access to specific genomic regions. Because the position of nucleosomes on DNA and the organization of chromatin into higher-order structures can affect the binding of proteins to DNA, eukaryotes have evolved a complex array of enzymes that modify chromatin. Central mechanisms for regulating chromatin activity include reorganization of nucleosome position by ATP-dependent nucleosome-remodeling factors and covalent modification of histone proteins[1]. Nucleosome-remodeling enzymes harness the energy of ATP hydrolysis to alter the position of nucleosomes on DNA, leading to either activation or repression of transcription. Histone-modifying enzymes introduce a complex array of post-translational modifications that can either activate or repress transcription, depending on the type of chemical modification and its location in the histone octamer. Previous studies have pointed to the flexible N-terminal histone tails as targets of numerous functionally important modifications. This has led to the proposal that particular patterns of histone modifications form a 'histone code' that alters the structure of higher-order chromatin and helps recruit effector molecules[2,3]. However, the mechanism by which these modifications alter transcriptional activity, either directly or via the binding of effectors, has remained unclear. Various observations have suggested a connection between nucleosome remodeling and covalent histone modifications, but no clear mechanism has emerged that explains how these processes may work in concert to regulate chromatin activity.

Recent proteomics analyses have revealed a large number of previously unknown histone modifications[4–6], many of which have been mapped by Freitas *et al.*[7] onto the nucleosome structure. Surprisingly, many newly identified modifications lie in the structured globular histone core, some of which are in a position to interfere with the binding of DNA to the nucleosome lateral surface. This contrasts markedly with previously identified modifications, most of which are not in a position to interfere with the wrapping of DNA about the histone octamer. These observations require rethinking about how modification of residues at the histone-DNA interface functions in the context of chromatin. We review here these recent findings and present a further analysis of the core histone modifications, focusing primarily on the modifications on the nucleosome lateral surface that are likely to regulate histone-DNA interactions. These data lead us to propose a new model for the mechanism and regulation of chromatin activity, which is controlled by factors that regulate the equilibrium between nucleosomes with low mobility and those with high mobility. This model provides a basis for understanding how post-translational modification enzymes and ATP-dependent nucleosome-remodeling complexes work in concert to catalyze nucleosome mobility. In addition, it provides a new and testable framework for understanding how the histone code is implemented.

Mechanisms for regulating chromatin activity

Nature has evolved a vital mechanism to regulate the delicate balance between organizing chromatin within the limited volume of the nucleus and allowing macromolecular complexes access to DNA. Central in this process is the nucleosome, which consists of a 147-base pair segment of genomic DNA wrapped around the lateral surface of a disc-shaped octamer comprising histone proteins H2A, H2B, H3 and H4. Protruding from the globular portion of the octamer are the N-terminal tails of the histone proteins, which are unstructured in the context of a single nucleosome but are thought to take part in formation of higher-order chromatin organization by mediating interactions with nucleosomes and other chromatin proteins[8]. The histone core binds the DNA backbone at 14 superhelix locations, making >120 direct atomic interactions[9]. Despite this, nucleosomes are highly dynamic and can slide along DNA over substantial distances *in vitro*[10]. The considerable energy barrier for sliding *in vivo* is overcome by the input of energy in the form of ATP hydrolysis by ATP-dependent nucleosome-remodeling complexes[11,12]. These enzymes are multiprotein complexes containing a central nucleic acid–stimulated ATPase belonging to the Swi2/Snf2 superfamily[13]. Nucleosome-remodeling complexes are thought to weaken histone-DNA contacts, generating a stably remodeled nucleosome that has increased mobility[14] and whose DNA is more accessible to interacting proteins[11]. However, the molecular mechanism by which nucleosome-remodeling complexes weaken histone-DNA contacts has remained elusive.

Several models have been proposed to explain catalyzed nucleosome mobility. The 'twisting' model predicts that histone-DNA contacts at the entry sites of the nucleosome are disrupted by a

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Table 1 Summary of known and new histone modifications in the globular nucleosome core domain

Histone	Residue	Mod.[a]	Nucleosome location (SHL)[b]	Secondary structure	Function unmodified	Mutation	Phenotype	Reference
H3	Arg52	M	L 6.0		Indirect (H_2O-mediated) DNA binding			
	Arg53	M	L 6.0		Unknown, >5 Å from DNA	R53K in CENP-A		46
	{Lys56}	M	L		Indirect DNA binding			
	Lys79	M	T/B 3.0	Loop 1	Unknown, methylated by DOT1	K79E/R/A K79T in CENP-A	Lrs	34,58
	Lys115	A	L 0	Loop 2	Indirect DNA binding	K115G in CENP-A		60
	Thr118	P	L 0.5	Loop 2	DNA binding, H4 Arg46 binding	T118I	Sin increases nucleosome sliding, 5× bias in one direction	39
	Lys122	A/M	L 0	α3	Indirect DNA binding			
H4	Lys31	A	L 0.5	α1	Indirect DNA binding			
	Ser47	P	L 0.5	Loop 1	Indirect DNA binding	S47C	Sin, little effect on sliding	39,61
	Lys59	M	T/B 1.5	α2	Salt bridge with H4 Glu63, surface-exposed	K59A K59R	Loss of silencing Support silencing at HML	6
	Lys77	A	L 3.5	Loop 2	Indirect DNA binding			
	Lys79	A/M	L 2.5	Loop 2	DNA binding	K79M	Lrs	34
	Lys91	A	C	α3	Forms salt bridge with H2B Glu63			
	Arg92	M	C	α3	Unknown			
H2A	Lys36	A	L 4.5	α1	Unknown	K36R in H2A.Z variant		46
	{Lys74}	M	L 5.5	Loop 2	Unknown			
	{Lys75}	M	L 5.5	Loop 2	DNA binding			
	{Arg77}	M	L 5.5	Loop 2	Inserts into minor groove			
	Lys99[c]	M	C		Unknown	K99G in H2A.X variant		46
	Lys119	A/U	C-T 0		Unknown			
H2B	{Lys31}	M	L		Unknown			
	Ser33	P	L 4.5		DNA binding, cap of helix dipole		Transcriptional activation	62
	Lys40	M	L 4.5	α1	Indirect DNA binding			
	{Arg76}	M	C	α2	Unknown			
	Lys82	A	L 2.5	Loop 2	Charge neutralizes negative helix dipole			
	{Arg83}	M	L 3.5	Loop 2	DNA binding			
	{Arg89}	M	T/B 3.5	α3	Unknown			
	Arg96	M	T/B 3.5	α3	Unknown			
	Lys105	A	T/B 4.5		Unknown			
	Lys113	A	T/B 4.5		Unknown			
	Lys117	A/U	T/B 4.5		Unknown	K123R (yeast)	Loss of H3 Lys4 and Lys79 methylation and silencing at telomeres	63–65

Curly brackets indicate residues that are possibly modified, but whose assignment was ambiguous[6].

[a]Mod., modification. M, methylation; A, acetylation; P, phosphorylation; U, ubiquitylation. [b]Nucleosome location: C, center; L, lateral surface; T/B, top/bottom; C-T, C-terminal tail; and superhelix location (SHL). [c]H2A Lys99 in *B. taurus* is H2A Arg99 in *X. laevis*.

catalyzed distortion of DNA that is propagated over the surface of the histone octamer, resulting in nucleosome relocation. However, the observation that nicked DNA does not interfere with nucleosome sliding is inconsistent with this model[15]. The 'loop recapture' model proposes that linker DNA lying in the path of the histone movement is pulled into the nucleosome to replace a looped-out DNA segment[16]. Propagation of this loop through the nucleosome could then lead to nucleosome sliding. However, it has not been demonstrated how this model can be used to explain the generation of stably remodeled nucleosomes, which retain increased DNA accessibility and elevated rates of nucleosome sliding even after depletion of the nucleosome-remodeling enzyme and in the absence of further ATP hydrolysis[17–22].

Post-translational modification of histone proteins can alter chromatin activity by creating binding sites for protein domains that recognize specific histone modifications. Bromodomains, which recognize acetyl-lysine, and chromodomains, which recognize methyl-lysine, are two types of effector domains commonly found in many complexes that interact with chromatin, including nucleosome-remodeling enzymes and many transcription factors[23,24]. Acetylation and methylation are therefore chemical marks that can trigger recruitment of proteins that act specifically on chromatin or on DNA.

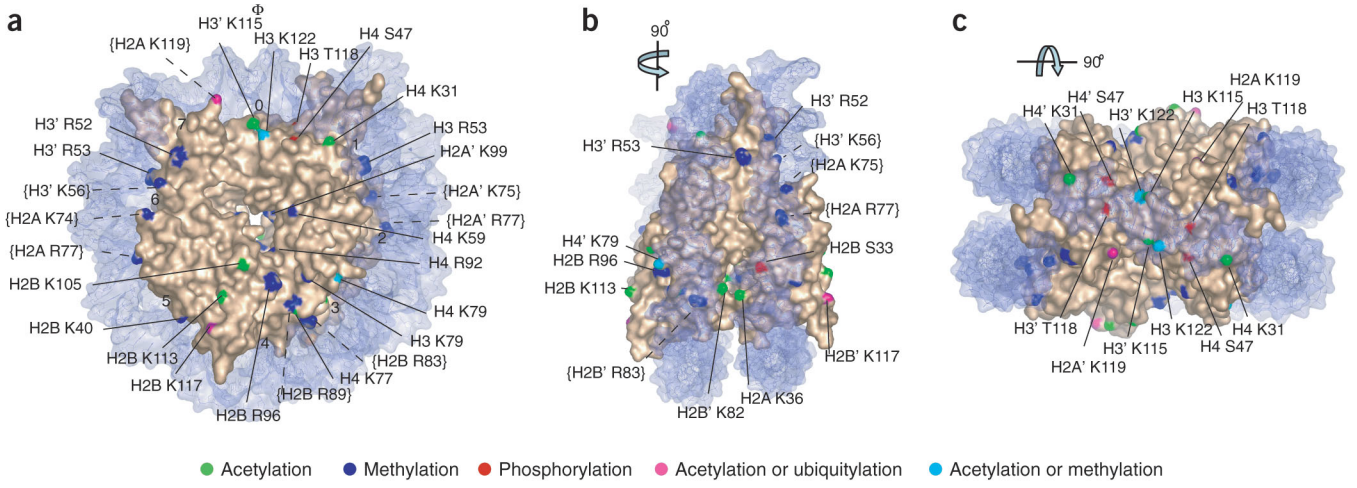

Figure 1 Annotated map of previously known and newly identified histone modifications on the surface of the *X. laevis* nucleosome core particle[26]. (**a**) Surface representation of the vertebrate nucleosome core particle (without flexible tails) viewed down the DNA superhelix axis. The functional groups of modified residues are colored according to the type of post-translational modification, with acetylation in green, phosphorylation in red, methylation in blue, sites that can be either acetylated or ubiquitylated in purple, and sites that can be either acetylated or methylated in light blue. The DNA superhelix is transparent light blue, with superhelix locations (0–7) indicated. Ambiguous sites of modification are indicated by curly brackets and dashed lines. (**b**) View as in **a** but rotated 90° around the molecular dyad axis (Φ). (**c**) View as in **a** but rotated 90° around the horizontal axis looking down at the top of the molecule in **a**. Representations of nucleosomes were generated using PyMOL (http://www.pymol.org).

Phosphorylation of tyrosine/serine/threonine and ubiquitylation of lysine can similarly be recognized by specialized domains. The dense clustering of many of these modifications in the N-terminal tails of histone proteins has led to the suggestion that particular combinations of modifications may be specifically recognized[3], although the details of this combinatorial readout mechanism remain to be elucidated.

Lateral surface histone modifications

Several recent studies have used mass spectrometry to identify a large number of new histone modifications located primarily in the globular core of the histone octamer[4–6]. Although most previously known modifications are located in the flexible N-terminal tails of the histone proteins, the location of these new modifications in the well-structured histone core made it possible to map their precise location on the nucleosome. Freitas *et al.*[7] modeled the position of these modifications onto the 3.1-Å structure of the yeast nucleosome[25] and noted the presence of many modifications at the protein-DNA interface. We mapped the same set of modifications onto the crystal structure of the *Xenopus laevis* nucleosome core particle determined at a resolution of 1.9 Å (ref. 26). In the *X. laevis* nucleosome, 96% of the core histone residues are identical to those in *Bos taurus*, the species studied by Zhang *et al*[6]. Our modeling treated only those residues identical between the two species. Of the new modifications, 27 were unambiguously mapped to the structured nucleosome core domain (**Table 1**). Twelve of the modified residues map to the top, bottom or center of the nucleosome core, and do not contact DNA (**Fig. 1a**). The remaining 15 modified residues map to the nucleosome lateral surface, and lie at the protein-DNA interface. The positions of the modified residues on the nucleosome lateral surface form a striking 'railroad track'–like pattern of serine and threonine phosphorylation, lysine and arginine methylation, and lysine acetylation that follows the path of the DNA around the histone octamer (**Fig. 1b,c**). Many of these residues are either directly involved in histone-DNA interactions or are located near the DNA, suggesting that these modifications could directly modulate the association of the histones with DNA[7].

The observed lateral surface modifications are expected to alter the free energy of histone-DNA interactions. This suggests that different combinations of covalent modifications on the nucleosome lateral surface could affect the relative rates of nucleosome mobility (**Fig. 2**). Stable modifications, such as lysine methylation, may be a mechanism for establishing differential basal rates of nucleosome mobility depending on the type of chromatin and the number and position of methyl marks. Further control of nucleosome mobility can be achieved by reversible acetylation and phosphorylation. The recent discovery of methyl-arginine citrullination adds another potential mechanism for altering interactions with nucleosomal DNA[27,28]. Because the DNA would block any histone-modifying enzymes from targeting these side chains in the context of the nucleosome, either the modifications must occur in the absence of DNA, or there must be a mechanism for temporarily exposing the nucleosome lateral surface so that modifying enzymes could gain access to the target residues.

New model for chromatin dynamics

The presence of histone modifications on the nucleosome lateral surface indicates that the modifications could play a direct role in modulating histone-DNA interactions within the nucleosome, and could determine the stability of nucleosome positioning and the ease with which a nucleosome can be translocated along the DNA. We propose here a new model for modulating chromatin dynamics, the 'regulated nucleosome mobility' model, in which changes in the affinity of the histone octamer for DNA result in changes in the equilibrium between mobile and relatively stationary nucleosomes (**Fig. 2**). We suggest that this equilibrium may be regulated by the concerted action of macromolecular nucleosome-remodeling machines and histone-modifying enzymes that modify histone side chains at the histone-DNA interface in either the presence or absence of nucleosomal DNA. These macromolecular complexes include ATP-dependent nucleosome-remodeling enzymes, which catalyze either nucleosome movement or histone variant exchange, and nucleosome assembly and disassembly machines (**Fig. 3**). By modulating the energy of histone-DNA interactions, the accessibility of genomic DNA and its packaging into higher-order chromatin can be regulated.

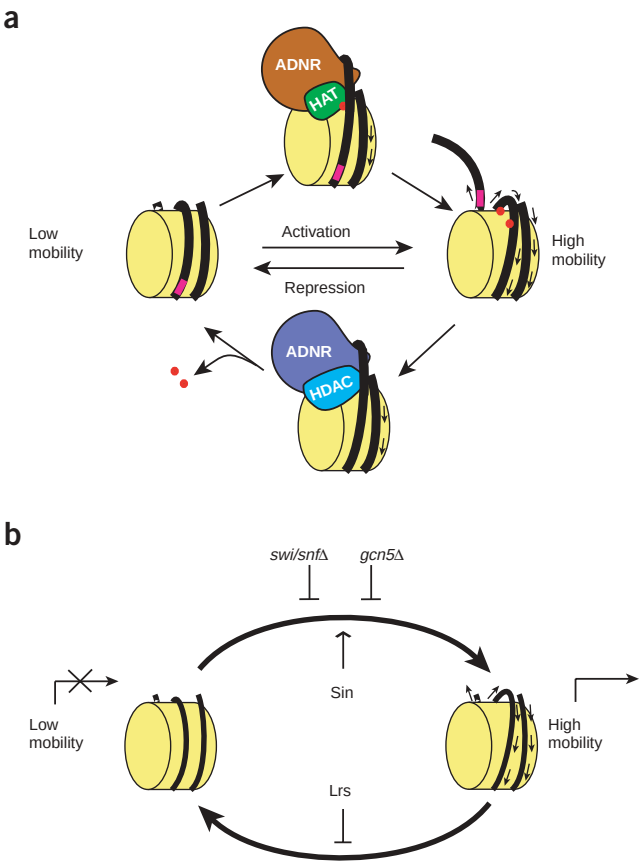

Figure 2 The regulated nucleosome mobility model. (**a**) Chromatin is regulated by factors that control the equilibrium between nucleosomes with low versus high mobility. Proposed intermediates are shown on the pathway of transcriptional activation and repression, catalyzed by the concerted action of ATP-dependent nucleosome-remodeling factors (ADNR) and covalent histone-modification enzymes. Activation can be achieved by histone modifications that weaken histone-DNA contacts, such as acetylation by HATs, resulting in increased nucleosome mobility. Repression is achieved by histone modifications that restore histone DNA contacts, such as deacetylation by HDACs, resulting in decreased nucleosome mobility. (**b**) Uncoupling regulation of nucleosome mobility by *GCN5* or Swi/Snf knockouts blocks gene expression by preventing the switch from nucleosomes with low mobility to nucleosomes with high mobility. Mutation of histone-DNA contacts (Sin mutations) relieves repression in *gcn5*Δ and *swi/snf*Δ strains by weakening histone-DNA contacts, resulting in increased nucleosome mobility and access to DNA. Mutations that prevent restoration of histone-DNA contacts (Lrs mutations) uncouple control of nucleosome mobility by deacetylation, preventing the switch from nucleosomes with high mobility to low mobility, resulting in the loss of gene silencing.

The regulated nucleosome mobility model can be used to explain the mechanism of catalyzed nucleosome mobility by ATP-dependent nucleosome-remodeling enzymes. We suggest that one role of nucleosome-remodeling enzymes is to use the energy of ATP hydrolysis to expose potential sites for post-translational modification on the nucleosome lateral surface that otherwise would be occluded by superhelical DNA. One or more of these exposed side chains could then be chemically modified by histone-modifying enzymes, thereby interfering with optimal histone-DNA contacts and allowing the histone octamer to slide along the DNA in a stably remodeled state. Mobile nucleosomes could then be more readily displaced by proteins and enzymes that must access the DNA for DNA replication, repair, recombinsation and transcription. A nucleosome could be returned to a less mobile state by removing reversible modifications such as acetylation and phosphorylation, again with the aid of ATP-dependent nucleosome-remodeling enzymes to expose the histone core to histone deacetylases or phosphatases. This model therefore readily explains how ATP-dependent nucleosome-remodeling enzymes can both activate and repress transcription.

The proposed model provides a mechanistic link between nucleosome remodeling and covalent histone modifications and explains why several ATP-dependent nucleosome remodeling complexes associate with histone-modifying enzymes (**Table 2**)[1]. A functional link between nucleosome remodeling and histone modifications was first demonstrated by genetic studies in yeast[29,30]. Mutations in subunits of the Swi/Snf nucleosome-remodeling complex exhibit synthetic lethality with mutations in components of the *GCN5*-dependent histone acetyltransferase (HAT) complexes, SAGA and Ada, suggesting that these complexes interact functionally in yeast. Several subunits of the Swi/Snf complex were subsequently shown to be shared with a Gcn5p-containing HAT complex[30], and the *GCN5* bromodomain was shown to be required for the stable association of Swi/Snf to promoters *in vivo*[31]. Although isolated Gcn5p can acetylate the flexible histone N-terminal tails[32], this modification is not sufficient for nucleosome remodeling *in vivo*[31]. In addition, the Swi/Snf complex can generate the stably remodeled state in tailless nucleosomes even after depletion of Swi/Snf[33]. This is consistent with our model, in which the associated Gcn5p HAT acetylates the core nucleosome lateral surface residues, reducing the energy of interaction with DNA and allowing increased nucleosome mobility once the enzyme complex is removed.

Experimental support for regulated nucleosome mobility

The regulated nucleosome mobility model is strongly supported by the results of unbiased genetic screens in yeast[34,35] (**Table 1**). Freitas *et al.*[7] pointed out that several modified residues coincide with side chains in histones H3 and H4 that are sites of 'Sin' mutations[35], which relieve the requirement for the Swi/Snf nucleosome-remodeling complex for gene expression. Several of these mutations are located in

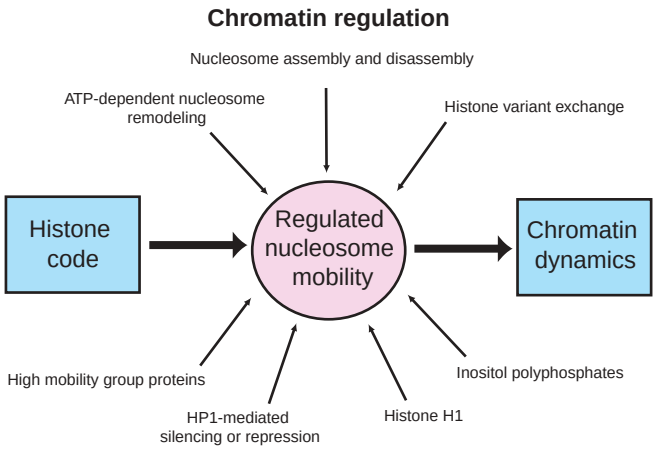

Figure 3 Regulated nucleosome mobility, a missing link in the regulation of chromatin dynamics. Regulated nucleosome mobility suggests how the histone code is implemented. Cellular factors that may affect nucleosome mobility are indicated.

Table 2 Examples of ATP-dependent nucleosome-remodeling complexes and associated modification enzymes

Complex	Central ATPase	Histone-modification enzyme	Recognition domains	Reference
Activation complexes				
Swi/Snf	Snf2	SAGA-Gcn5	Bromo	29
hSwi/Snf	Brg1	P300/CBP	Bromo	66
NURF	ISWI	P300/pcaf	Sant/Bromo	67
INO80	ISWI-related	NuA4		68
RSC	Sth1	NuA4	Bromo	69
Repression complexes				
NuRD	Mi-2	HDAC1	Chromo/MDB	1,70
Isw2p	ISWI	Sin3-RPD3	Sant	71
hSwi/Snf	Brg1	Sin3-RPD3	Bromo	72
hSwi/Snf	hBrm	Sin3-RPD3	Bromo	72
NoRC	Snf2H	HDAC1	Sant	73

specialized loop regions (L1-L2 loops[36]) that make many direct contacts with DNA at superhelix location 0.5 (**Fig. 1**). Interestingly, the same Sin mutations also relieve the *GCN5* requirement for gene expression[30,37]. The dual effects of Sin mutations are readily explained by the proposed model, which predicts that Swi/Snf exposes histone lateral surface residues and enables Gcn5p to acetylate side chains at the histone-DNA interface. This is expected to reduce the affinity of nucleosomal DNA for the histone octamer, thereby increasing nucleosome mobility (**Fig. 2**). Mutations in either Swi/Snf components or *GCN5* would decrease acetylation of lysine residues on the nucleosome lateral surface, resulting in reduced nucleosome mobility and decreased transcriptional expression. In contrast, histone Sin mutations are expected to weaken histone-DNA contacts, resulting in greater nucleosome mobility without the requirement for *GCN5* and Swi/Snf (**Fig. 2b**). This model is further supported by recent studies showing that Sin mutations indeed weaken histone-DNA contacts[38] and increase nucleosome sliding[38,39]. It remains to be shown whether these 'Sin' residues are indeed modified in yeast nucleosomes as they are in mammals.

The regulated nucleosome mobility model predicts that deacetylation of histone lateral surface residues will restore histone-DNA contacts, increasing the affinity of nucleosomal DNA for the histone octamer and decreasing nucleosome mobility. Consistent with this, mutations in the Sin3p–RPD3 histone deacetylase complex can also relieve the requirement for the Swi/Snf nucleosome-remodeling complex[40], as well as for the Gcn5p histone acetyltransferase[30,37]. The Sin3p–RPD3 histone deacetylase complex is associated with several ATP-dependent nucleosome-remodeling complexes (**Table 2**), consistent with its proposed role in regulating nucleosome mobility by deacetylating lateral surface residues.

Recent data suggest that heterochromatic genes can also be regulated by changes in nucleosome mobility. In a screen for histone H3 and H4 point mutations that result in the loss of ribosomal DNA silencing (Lrs mutations), Park *et al.*[34] identified several additional lateral surface residues important for histone-DNA interactions. Recent studies[5,6] show some of these to be targets of post-translational modifications (**Table 1**). Like Sin mutations, Lrs residues cluster in one of the L1-L2 loop regions of the histone octamer that form numerous direct interactions with DNA at superhelix location 3.0 (**Fig. 1**). These residues interact with DNA in a manner analogous to that of Sin-L1-L2 loop residues at superhelix location 0.5 (ref. 34), which is the location of the Sin mutations that have increased nucleosome mobility[38,39]. This suggests that covalent modification of Lrs residues may also alter the affinity of the histone octamer for DNA, thereby regulating nucleosome mobility.

Chromatin dynamics and nucleosome mobility

The regulated nucleosome mobility model is consistent with a critical role for nucleosome dynamics in the regulation of higher-order chromatin. Varying degrees of nucleosome mobility are most likely necessary to achieve the positioning and spacing of nucleosomes needed to form higher-order chromatin. High nucleosome mobility will be required for destabilization of the chromatin fiber, thereby increasing DNA accessibility, whereas low nucleosome mobility will be required to stabilize the chromatin fiber, with a concomitant decrease in DNA accessibility. This model is strongly supported by the observation that histone Sin mutations that increase nucleosome mobility[38,39] also prevent the formation of higher-order chromatin fibers from nucleosome arrays[41].

A fundamental feature of the regulated nucleosome mobility model is that DNA accessibility can be controlled by any factor that directly or indirectly affects the affinity of nucleosomal DNA for the histone octamer (**Fig. 3**). This is supported by the observation that nonhistone components such as inositol polyphosphates[42,43] and high mobility group–like proteins[44] can regulate transcriptional activation by altering nucleosome mobility. Indeed, high mobility group–like proteins have been shown to partially relieve the requirement for Swi/Snf[44] and GCN5 (ref. 37). Several studies have shown that the recombinant ATPase subunit alone can induce nucleosome remodeling *in vitro*, albeit at much lower rates than with the full complex purified from native sources[11]. It may be that, whereas temporary breaks in histone DNA contacts can allow some nucleosome mobility, adding modifications at the nucleosome lateral surface helps perpetuate nucleosome mobility.

Post-translational modifications that disrupt histone-DNA or histone-histone contacts may also be important for catalyzing histone variant exchange (**Fig. 3**). Replacement of histone subunits with specialized histone variants is also catalyzed by ATP-dependent nucleosome-remodeling complexes that contain histone-modification enzymes[45,46]. Similarly, regulated nucleosome mobility may also be modulated by nucleosome assembly machines, which have recently been linked to histone-modifying complexes[47]. The decreased DNA affinity that results from modifying DNA-contacting residues could readily facilitate each of these processes.

The histone code and regulated nucleosome mobility

Much of the recent research in the chromatin field has justifiably focused on post-translational modifications in the flexible N-terminal histone tails, which are highly correlated with distinct chromosomal states. For example, hyperacetylation of histone tails and H3 Lys4 methylation is associated with transcriptionally active euchromatin, whereas hypoacetylation and H3 Lys9 methylation is associated with heterochromatin[48]. These observations gave rise to the histone code hypothesis, which posits that histone N-terminal tail modifications alter chromatin structure either directly, by influencing histone-DNA or histone-histone interactions, or indirectly through the recruitment of proteins that recognize the chemical marks in the histone tails[2]. However, recent experiments indicate that interactions between histone tails and DNA may be more limited in impact than previously thought, as tailless nucleosome arrays still exhibit substantial compaction behavior in analytical ultracentrifugation experiments[49]. This suggests that post-translational modifications of the histone tails may serve as an intricate and dynamic signaling platform that indirectly regulates

higher-order chromatin structure by an as yet undetermined mechanism. The recent discovery of several post-translational modifications on the nucleosome lateral surface[4–6] adds a new dimension to the problem that must be accounted for by any model for the regulation of chromatin activity.

The regulated nucleosome mobility model suggests a mechanism for how the histone code is implemented (**Fig. 3**). This model predicts that an important function of histone N-terminal tail modifications may be to recruit effector proteins and nucleosome-remodeling activities that ultimately lead to changes in nucleosome mobility. This is supported by observations that the ISWI nucleosome-remodeling complexes, NURF, CHRAC and ISWI, require the histone H4 N-terminal tail to induce nucleosome sliding[50–52]. Our model also provides a probable explanation for why the central ATPase subunit of various Swi2/Snf2 family members contains histone modification recognition motifs, such as the 'bromo,' 'chromo,' 'sant' and 'slide' domains[53]. For example, the Swi/Snf and Gcn5p bromodomains are required for the stable association of Swi/Snf with acetylated promoters[31,54]. Additionally, the tandem bromodomains of the RSC nucleosome-remodeling complex recognize acetylated H3 Lys14, which is required for gene activation[55]. Furthermore, the NuRD ATP-dependent nucleosome-remodeling complex, which contains HDAC activity and a pair of chromodomains, can be pulled down from HeLa cell nuclear extracts by peptides containing the heterochromatic H3 Lys9 methylation mark, but not by peptides containing euchromatic H3 Lys4 methylation mark[56], consistent with its role in transcriptional repression[57].

The histone code model should therefore be modified to account for two types of functional outcomes resulting from covalent histone modification. We propose naming them class I and class II histone code modifications, where class I modifications function primarily in recruitment, and class II modifications function primarily by direct chemical interference. Class I histone code modifications are located primarily in the histone tails, and are involved in the recruitment of chromatin-stabilizing proteins or ATP-dependent chromatin-remodeling factors. Class II histone code modifications are located primarily in the nucleosome lateral surface, and include all modifications that alter direct interactions between the histone octamer and DNA. Modifications that lie elsewhere on the nucleosome may fall in either of these functional classes. Those that have been mapped to the top/bottom or center of the nucleosome are likely to have important roles in chromatin compaction by regulating internucleosome interactions[25,34,36,58], or by recruiting effector domains that may also result in regulated nucleosome mobility.

The regulated nucleosome mobility model also suggests a mechanism for other epigenetic phenomena, such as transcriptional silencing by DNA methylation. We propose that DNA methylation may be another epigenetic mark that functions analogously to class I histone modifications in the recruitment of chromatin-remodeling complexes that ultimately regulate nucleosome mobility. This is supported by the observation that the transcriptionally repressive NuRD chromatin-remodeling complex contains a component that specifically recognizes methylated DNA, called a methyl-binding domain (MBD)[57]. Silencing by DNA methylation may be the result of the recruitment of NuRD, which would allow deacetylation of nucleosome lateral surface residues by NuRD's associated histone deacetylase activity, resulting in reduced nucleosome mobility.

Summary and outlook

The regulated nucleosome mobility model presented here outlines a straightforward mechanism for regulating transcription and other processes through the control of nucleosome mobility. This model accounts for many previously published experimental observations, unifies previously unconnected observations, and provides testable hypotheses. A key feature is the proposal that a relatively simple mechanism can explain the seemingly complex action of ATP-dependent nucleosome-remodeling complexes. By separating the DNA from the lateral surface of the histone core, nucleosome-remodeling enzymes can expose side chains at or near the protein-DNA interface to post-translational modification enzymes, which in turn can facilitate an increase or decrease in nucleosome mobility. An attractive feature of this mechanism is that it would require much less ATP hydrolysis than current models for catalyzed nucleosome mobility[53], which depend on continued ATP hydrolysis to 'pump' DNA over the nucleosome surface. The concerted action of ATP-dependent nucleosome-remodeling factors and histone lateral surface modification enzymes would provide a much more energetically efficient way to generate accessible DNA for virtually all essential cellular processes that require DNA as a substrate.

The regulated nucleosome model is strongly supported by histone lateral surface mutations that may mimic situations in which histone-DNA contacts are prevented. The model therefore predicts that mutations designed to mimic the deacetylated state should lead to increased gene silencing or repression. Lys→Arg mutations could accomplish this by uncoupling regulation of nucleosome mobility by reversible acetylation, as arginine cannot be acetylated. Lys→Gln mutations at the same position (which may mimic acetylated lysine) would be predicted to show the opposite phenotypes. These predictions can be readily tested in yeast gene-silencing assays. It will also be important to distinguish the role of specific modifications in generating different types of nucleosome mobility. For example, does the position of the modification determine whether DNA will partially unwrap from the nucleosome[59], or slide in *cis* to a new location? Is a greater number of modifications required for the *trans* displacement of the histone octamer from DNA? Are certain modifications required for sliding in a particular direction, as suggested by some Sin mutations[39]? Will core modifications that disrupt histone-histone or histone-DNA interactions help catalyze histone variant exchange? The discovery of these new modifications in the nucleosome globular core opens a new and exciting chapter in the study of chromatin dynamics, and may provide new insights into the molecular basis of genetic and epigenetic diseases.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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