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REVIEW

Regulation of nucleosome dynamics by histone modifications

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Chromatin is a dynamic structure that must respond to myriad stimuli to regulate access to DNA, and chemical modification of histones is a major means by which the cell modulates nucleosome mobility and turnover. Histone modifications are linked to essentially every cellular process requiring DNA access, including transcription, replication and repair. Here we consider properties of the major types of histone modification in the context of their associated biological processes to view them in light of the cellular mechanisms that regulate nucleosome dynamics.

Eukaryotic genomes are tightly wound around octamers of core histone proteins to form nucleosomes, the basic unit of chromatin. Nucleosomes must be densely packed to achieve the 10,000–20,000-fold compaction[1] necessary to fit a genome into the small volume of the nucleus but must also allow proteins involved in transcription, replication and repair to access DNA. The properties of nucleosomes can be altered in several ways, including the replacement of canonical core histones with specialized variants[2], repositioning or eviction of histones from DNA by ATP-dependent chromatin remodeling enzymes[3] and, the topic of this Review, covalent modification of histones. Histone modifications have been of great interest ever since the discovery that histones which are associated with highly transcribed genes are hyperacetylated[4]. In the nearly 50 years since that seminal discovery, well over 100 distinct histone modifications have been described, with more being discovered at a rapid pace. These range from the well known, such as lysine methylation, lysine acetylation and serine/threonine phosphorylation, to more exotic modifications such as crotonylation[5,6].

With the advent of genome-wide chromatin immunoprecipitation (ChIP)-based techniques such as ChIP with tiled microarray analysis (ChIP-chip) or high-throughput sequencing (ChIP-seq), mapping of global patterns of histone modifications has become commonplace and has been performed in many organisms[7–11]. One insight that has emerged from such studies is the association of particular modifications with distinct types of *cis*-regulatory elements. Promoters are generally marked with high levels of H3K4me3 regardless of their transcriptional state. Putative enhancers tend to be marked with H3K4me1 alone or in combination with H3K27ac or H3K27me3, depending on the transcriptional activity of putatively regulated genes[12,13]. Although these marks have been informative for the large-scale identification of regulatory elements, a simple question remains: why are they there? Indeed, how and why certain histone modifications are established at specific genomic loci remains unclear. Here we examine the known properties of key histone modifications and the biological processes to which they are linked to place the modifications in the context of nucleosome dynamics—that is, processes in which nucleosomes are translocated, unwrapped, evicted or replaced.

DNA access through histone acetylation

Histone acetylation, discovered in 1961, was the first described histone modification[14]. Early studies revealed the association of hyperacetylated histones with actively transcribed genes, indicating a role for histone acetylation in facilitating transcription[4,15]. Indeed, the chemistry of histone acetylation suggests a mechanism by which it might facilitate gene expression. Acetylation neutralizes the positive charge of lysine residues, weakening charge-dependent interactions between a histone and nucleosomal DNA, linker DNA or adjacent histones, and thus increasing the accessibility of DNA to the transcription machinery. *In vitro*, tetra-acetylation of the histone H4 tail substantially reduces its affinity for DNA[16]. The histone H4 tail charge neutralization model was supported *in vivo* by the finding that changing the position of a lysine on the tail did not alter its effect on the phenotype[17]. Later, the charge neutralization model was tested by microarray analysis of yeast strains harboring all possible combinations of lysine-to-arginine mutations at positions 5, 8, 12 and 16 of the histone H4 tail to mimic the positively charged, unacetylated state of lysines at these positions[18]. It was hypothesized that if charge effects are responsible for the regulation of transcription by these lysine residues, then similar sets of genes should be dysregulated by all four single lysine-to-arginine mutants and by mutants containing all combinations of two or three lysine-to-arginine mutations. Indeed, the single mutants and those containing lysine-to-arginine mutations in combination at positions 5, 8 and 12 affected nearly identical sets of genes. Only strains encoding histone H4 with the K16R mutation showed distinct transcriptional effects, with ~10% of affected genes showing effects discordant with the other mutations (**Fig. 1**). Similarly, transcriptional analysis of strains harboring various combinations of lysine-to-arginine mutations at positions 4, 9, 14, 18, 23 and 27 of the histone H3 tail revealed that these residues have generally redundant roles in transcription[19]. Overall, these results suggested that the cumulative charge neutralization imparted to histones by multiple lysine acetylations influences transcriptional outcomes, rather than the acetylation of specific lysines.

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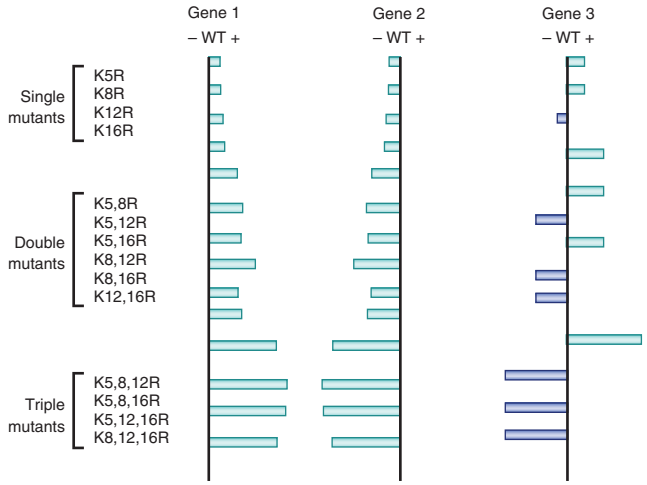

Figure 1 Cumulative charge neutralization as the predominant mechanism of transcriptional regulation by lysine acetylation. Schematic of gene expression changes relative to the wild type (WT) induced by substitution of one, two or three histone H4 tail lysine residues with arginine[18]. Genes 1 and 2 display monotonic increases and decreases in expression, regardless of the residue(s) changed. Gene 3 displays Lys16-specific transcriptional effects, leading to discordance with the effects of Lys5, Lys8 and/or Lys12 substitution.

Histone lysine acetylation also functions in other cellular processes that require DNA access. Before DNA replication, chromatin regulates the accessibility of DNA to replication factors and modulates the firing and efficiency of replication origins, with a nucleosome-depleted, DNase-hypersensitive chromatin configuration conducive to proper origin firing[20]. It has recently been shown that histone acetylation is associated with productive origin activation[21], suggesting that charge neutralization of lysines is important not only for proper transcription but also for efficient DNA replication by relaxing histone-DNA contacts. Histone acetylation also occurs at DNA double-strand breaks[22] and may therefore be used to increase DNA access for repair factors.

Establishing, removing and binding histone acetylation. Histone acetylation is highly dynamic, with half-lives on the order of a few minutes for many acetylation events[23]. Lysine residues are acetylated by lysine acetyltransferases, which generally have low substrate specificity[24], and are deacetylated by lysine deacetylases. Both enzymes are associated with sites of active transcription[25], consistent with a role for rapid acetylation and deacetylation cycles to facilitate the mobilization and restoration of nucleosomes during polymerase transit[26]. That is, acetylation of nucleosomes at promoters and within gene bodies facilitates efficient polymerase transit by loosening histone-DNA contacts, with subsequent deacetylation tightening histone-DNA contacts to promote chromatin reassembly after a round of transcription.

Acetylated lysine residues are recognized by a specific protein module, the bromodomain, which was first described in the context of the *Drosophila melanogaster* brahma chromatin-remodeling enzyme[27]. Bromodomains are found in a wide range of chromatin-associated proteins, including lysine acetyltransferases and chromatin remodeling proteins[28]. Histone acetylation may also influence chromatin remodeling. It was initially observed through genetic experiments in *D. melanogaster* that overexpression of the H4K16 acetyltransferase MOF exacerbated phenotypes associated with loss of the ISWI chromatin remodeler, suggesting that H4K16ac interferes with ISWI remodeling activity[29]. Indeed, *in vitro* experiments with the *D. melanogaster* ISWI chromatin remodeler indicated that its remodeling activity is inhibited by H4K12ac and H4K16ac[29,30] by interfering with the conformation of the adjacent histone H4 tail basic patch (Arg17-His18-Arg19) necessary for ISWI remodeling[31]. The influence of H4K16ac on ISWI remodeling may help explain why acetylation of this residue has effects on transcription that are distinct from those mediated by acetylation of H4K5, H4K8 and H4K12 (ref. 18).

Lysine acylation: a general strategy for DNA access? In addition to acetylation, a variety of less well understood histone lysine coenzyme A–dependent acylations have recently been described: crotonylation, formylation, succinylation, malonylation, propionylation and butyrylation[6,32]. Similar to acetylation, these acylations neutralize the positive charge of lysine, ostensibly weakening histone-DNA contacts. Although additional work is needed to ascertain the biological relevance of this extended family of histone lysine acylations, it may be that lysine acylation is a general means to facilitate DNA access for processes such as transcription, replication and repair.

Histone methylations: modulators of nucleosome stability?

Mono-, di- or trimethylation of a lysine residue does not affect its positive charge, and so the effect of methylation on nucleosome dynamics is thought to be less direct than that of acetylation. Histones can also be mono- or dimethylated on arginines[33], but much less is known about the effects of histone arginine methylation on nucleosome dynamics. Here we consider histone lysine methylations associated with activation (H3K4me and H3K36me) and repression (H3K9 and H3K27) of transcription.

Histone methylations as regulatory modules. Enrichment of H3K4me3 at promoters has been described in many eukaryotes[34] and depletion of H3K4 methyltransferase complexes causes drastic reductions in global H3K4me3 amounts. Impairment of these complexes, however, results in minimal transcriptional effects[35,36], raising the possibility that direct transcriptional regulation is not the primary function of H3K4me3. Similarly, enrichment of H3K36me2/3 over gene bodies is highly conserved[37], but loss of the H3K36 methyltransferase Set2 has only minor effects on transcription[35]. What, then, are the roles of these histone modifications so often associated with active transcription? It has been suggested that H3K4me3 and H3K36me3 may function as regulatory modules in some contexts. *In vitro*, H3K4me3 inhibits trimethylation of H3K27 by the *D. melanogaster* and human variants of Polycomb repressive complex 2 (PRC2)[38]. *In vivo*, the Trithorax complex antagonizes Polycomb silencing through the establishment of H3K4me3 (ref. 39), and Trithorax-mediated H3K4me3 may thus have an antirepressor function through inhibition of H3K27me3 methylation.

Another example of histone methylation serving as a regulatory module can be found in the conserved Rpd3S lysine deacetylase complex. This complex contains the chromodomain protein Eaf3, which associates with H3K36me3 in gene bodies[40,41]. In yeast, deletion of Eaf3 or the H3K36 methyltransferase Set2 increases histone acetylation in gene bodies, leading to the suggestion that H3K36me3 is responsible for Rpd3S recruitment to gene bodies[42]. However, it was later shown that loss of H3K36me3 or the Eaf3 chromodomain does not affect Rpd3S localization[43], strongly suggesting that H3K36me3 regulates the catalytic activity of Rpd3S instead.

Methylation-mediated nucleosome stability. In the case of H3K9me3 and H3K27me3, which are associated with heterochromatin formation

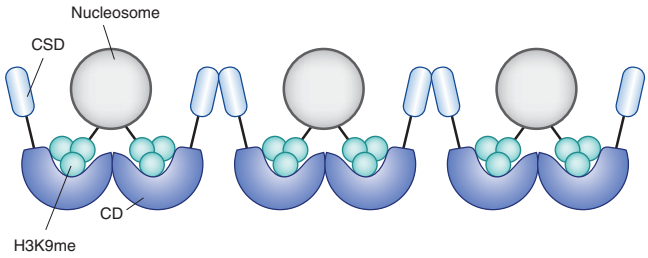

Figure 2 Swi6-mediated chromatin stabilization. Swi6 molecules dimerize via their chromodomains (CD) to recognize H3K9-trimethylated histone tails in a single nucleosome. These dimers then contact adjacent dimers via their chromo-shadow domains (CSD) to stabilize nucleosomes and promote heterochromatin spreading. Model is adapted from ref. 46.

and Polycomb silencing, respectively, methylation increases the affinity of certain protein modules for histone residues. Methylated lysines are bound by many domains, including Tudor, chromo, PWWP, MBT and PHD[44]. Notable examples of histone methylation increasing binding affinity include the chromodomains of HP1 and Polycomb. *In vitro*, the HP1 chromodomain binds H3K9me, whereas the Polycomb chromodomain binds H3K27me, with more methyl groups increasing the affinity of each chromodomain for its preferred residue[45]. By enhancing the affinity of certain proteins for histone tails, histone methylation also appears to enhance nucleosome stability. *In vitro*, Swi6 (the *Schizosaccharomyces pombe* ortholog of HP1) molecules dimerize via their chromodomains to recognize pairs of H3K9me3-modified tails in a single nucleosome and subsequently bridge adjacent H3K9me3-modified histone tails to stabilize nucleosomes and promote heterochromatin formation[46]. Perhaps the *in vivo* situation is similar, with oligomers of Swi6 enhancing nucleosome stability to promote heterochromatin formation (**Fig. 2**).

H3K36me3 also has a role in stabilizing nucleosomes. It was initially observed that H3K36me3, established cotranscriptionally by Set2, was involved in suppressing cryptic transcription in gene bodies[40,41]. Subsequent work demonstrated that the prevention of cryptic transcription by H3K36me3 was due to suppression of nucleosome turnover[47]. It is now known that there are two mechanisms facilitated by H3K36me3 that may enhance nucleosome stability: histone deacetylation and chromatin remodeling. H3K36me3 regulates the activity of the Rpd3S complex, which prevents histone turnover by deacetylating histones[47]. H3K36me3 also enhances the affinity of the repressive Isw1b chromatin remodeling complex for nucleosomes[48,49]. Together, histone deacetylation and repressive chromatin remodeling, both facilitated by the presence of H3K36me3, suppress nucleosome turnover to prevent spurious initiation of transcription in gene bodies.

Distinct methylation states. An interesting aspect of histone lysine methylation is the potential for distinct modification states on a single residue. A single lysine residue may be unmodified or attain a mono-, di- or trimethylated state. Several general and specific factors may influence the methylation state of a given lysine residue. Broadly speaking, it may be that the degree of accessibility to its modifying enzyme(s) could influence the establishment of distinct methylation states. For instance, other histone modifications could modulate the accessibility of a particular residue such that it is more or less available to a modifying enzyme. One such example may be the well-known *trans*-histone pathway by which H2BK123ub1 promotes H3K4me3 and H3K79me3 (ref. 50). A simple explanation for these findings is that histone ubiquitylation induces substantial alterations in nucleosome or chromatin conformation that increase the exposure of H3K4 and H3K79 to their modifying enzymes (**Fig. 3a**). Similarly, the duration of exposure of a residue to its modifying enzyme may influence its final modification state. Many histone methyltransferases are proposed to act in a processive fashion—that is, a single methyltransferase performs multiple rounds of methylation on a single residue[51,52]. Thus, trimethylation takes more time than dimethylation, which in turn takes more time than monomethylation. If a processive methyltransferase is only transiently bound to a site of action, it may only have time to monomethylate its target residue, whereas at a more stable site of chromatin association it would have sufficient time to process to trimethylation (**Fig. 3b**).

DNA access through histone phosphorylation

Phosphorylation is the chemical means by which the majority of signals are transduced in a cell. Phosphorylation imparts a negative charge to its modified residue, and this fact suggests that histone phosphorylation has a similar role to acetylation in modulating nucleosome dynamics[53]. As the phosphates of the DNA backbone are negatively charged, the addition of phosphates to histones would create charge repulsion between histones and DNA, potentially loosening the association of DNA with histones. Indeed, phosphorylated histones are less effective at inhibiting DNase I digestion of chromatin than are unphosphorylated histones[54]. In a specific example of histone phosphorylation affecting histone-DNA contacts, phosphorylation of threonine 118 on the histone H3 core interferes with nucleosome wrapping, increases remodeling and makes chromatin more accessible to DNase I *in vitro*[55].

Histones are phosphorylated in a wide variety of biological contexts. Phosphorylation of the histone H2A variant histone H2A.X at serine 139 in humans (various residues in other organisms, such as histone H2A Ser129 in yeast), known as histone γH2A.X, is a critical component of the cellular response to DNA double-strand breaks. After phosphorylation by the ATM or ATR kinase, histone γH2A.X

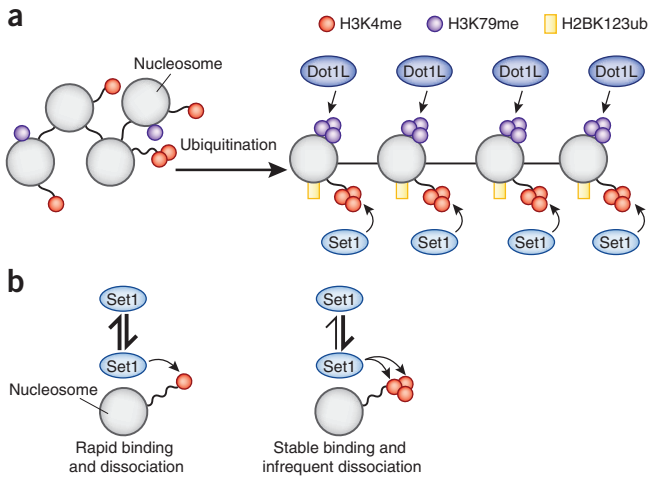

Figure 3 Models for the establishment of distinct methylation states. (**a**) Residue accessibility model. Non-ubiquitylated nucleosomes contain low levels of H3K4me and H3K79me because of poor accessibility of these residues to their modifying enzymes (Set1 and Dot1L). Ubiquitylation induces conformational changes in chromatin that promote increased accessibility of these residues, leading to efficient deposition of H3K4me3 and H3K79me3. (**b**) Duration of residue exposure model. When Set1 transiently associates with its site of action (left), it cannot process beyond H3K4me1 before it dissociates. When Set1 more stably associates with its cognate site (right), it can process from unmethylated H3K4 to H3K4me3 before it dissociates from chromatin.

recruits the DNA repair machinery to double-strand breaks[56]. It may also be possible that histone γH2A.X increases the accessibility of DNA to repair factors via charge repulsion. Notably, phosphorylation of *Drosophila* histone H2A.Z (formerly known as histone H2Av[57]) in the context of DNA damage (histone γH2A.Z.X) stimulates histone γH2A.Z.X acetylation by Tip60 complex, increasing the exchange of histone γH2A.Z.X for unphosphorylated histone H2A.Z.X[58]. Histone phosphorylation also appears to have roles during development. After fertilization in the sea urchin, sperm histones are heavily phosphorylated and then are lost from chromatin, presumably owing to weakened histone-DNA contacts[59].

Histone phosphorylation also alters the affinity of chromatin-binding proteins for their targets. Phosphorylation of residues adjacent to a methylated lysine reduces the affinity of the lysine residue's cognate methyl-binding protein(s). One such example is HP1, which as discussed above binds with high affinity to H3K9me3 (ref. 45). Binding of HP1 chromodomains to peptides harboring H3K9me3 is severely impaired by H3S10 phosphorylation (H3S10p), and the co-occurrence of H3K9me3 and H3S10p coincides with the release of HP1 from mitotic chromatin[60,61]. It is unclear how H3S10p reduces the affinity of HP1 for H3K9me3, though the mechanism may involve charge repulsion. It appears that this may represent a paradigm for the regulation of chromatin-binding protein affinity, as several lysine-serine pairs occur in histone tails, and at least one such pair (histone H3 Lys27 and Ser28) has been found to operate in a manner similar to that of histone H3 Lys9 and Ser10 (ref. 62).

ADP ribosylation

All four core histones, as well as linker histone H1, are known to be mono–ADP-ribosylated and poly–ADP-ribosylated in a variety of biological contexts[63]. ADP ribosylation imparts a negative charge to its modified residues, suggesting that this modification creates charge repulsion between histones and DNA, similar to phosphorylation. Accordingly, electron microscopy and sedimentation analysis has revealed that poly–ADP-ribosylated chromatin adopts a more relaxed structure[64]. Studies of histone ADP ribosylation involvement in biological processes are consistent with the loosening of chromatin. During heat shock, poly–ADP ribosylation over heat-shock gene bodies is greatly increased and is linked to nucleosome eviction[65]. Histones are also poly–ADP-ribosylated upon induction of DNA damage[64], potentially suggesting that this modification is used by the cell as a means to enhance access of repair proteins to DNA.

Glycosylation

Modification of histones by β-N-acetylglucosamine (OGlcNAc) was discovered relatively recently[66,67]. Histones H2A, H2B and H4 may harbor this modification on several threonine and serine residues. Total histone O-GlcNAcylation increases with heat shock and is correlated with decreased sensitivity of chromatin to micrococcal nuclease digestion, suggesting that this modification might be involved in transcriptional repression[66]. However, it is unclear how histone O-GlcNAcylation might lead to decreased chromatin accessibility. It is also interesting that a recent report has linked O-GlcNAcylation of H2BS112 to H2BK120ub1 and active transcription[68]. Perhaps, then, O-GlcNAcylation of this particular residue promotes accessibility of H2BK120 to the ubiquitylation machinery. Overall, histone O-GlcNAcylation is poorly understood and awaits further study.

How large modifications impact nucleosome dynamics

Most histone modifications consist of small chemical moieties; in contrast, ubiquitylation is the addition of a 76 amino-acid globular domain to a single lysine side chain. Histone monoubiquitylation events appear to have diverse effects on nucleosome dynamics. In particular, monoubiquitylated histone H2B (H2BK123ub1 in yeast and H2BK120ub1 in human) seems to have many context-dependent effects on chromatin. H2BK123ub1 functions in gene bodies to promote transcriptional elongation and chromatin reassembly after transcription as well as at promoters by helping to establish a repressive chromatin architecture[69–71]. Consistent with a role in chromatin assembly, H2BK123ub1 has been found to stabilize nucleosomes *in vivo*[70,72]. However, *in vitro,* histone H2B ubiquitylation inhibits chromatin compaction and increases internucleosomal distance on synthetic nucleosome arrays[73]. Histone H2B ubiquitylation is also induced at double-strand breaks and is necessary for proper recruitment of repair proteins[74], suggesting that it may help induce an accessible chromatin conformation at sites of DNA damage. The other well-characterized histone monoubiquitylation event, H2AK119ub1, appears to have a role in Polycomb-mediated transcriptional repression[75], perhaps via inhibition of transcriptional elongation[76]. Such inhibition contrasts with the promotion of transcriptional elongation by H2BK123ub1 and suggests a context dependence of histone ubiquitylation functions.

Similar to ubiquitylation, sumoylation is a ligation of a large (~100 amino acid) domain to a single lysine side chain. All four core histones are known to be sumoylated *in vivo*[77,78], and histone sumoylation is generally associated with transcriptional repression, although it is not clear how this effect is mediated. The complexity of effects mediated by histone ubiquitylation also suggests that the impact of sumoylation on nucleosome dynamics is likely to be more extensive than is currently recognized.

How do these large modifications exert such diverse effects? In the case of H2BK123ub1, it may be that its ability to inhibit chromatin compaction is relevant *in vivo* and increases nucleosome turnover to enable efficient transcriptional elongation and binding of repair proteins. Its promotion of nucleosome stability may be the means by which it promotes chromatin reassembly after transcription and helps maintain a repressive promoter architecture. Alteration of nucleosome conformation by ubiquitylation may increase the access of modifiable residues to their cognate modifying enzymes, as loss of H2BK123ub1 is linked to reduced methylation of histone Lys4 and Lys79 via an unknown mechanism[50,79].

Propagation of histone modifications

Maintenance of histone modifications through the cell cycle is important for the propagation of chromatin states, but the dynamic nature of chromatin poses a substantial challenge in this regard. DNA replication necessitates the incorporation of newly synthesized histones into replicated DNA, and outside of replication histones are frequently turned over at active regulatory elements and transcribed genes. Here we discuss cellular strategies for the maintenance of histone modifications in the context of replication-independent and replication-dependent nucleosome loss.

Nucleosome turnover. Regulated nucleosome turnover is increasingly recognized as a means of modulating gene expression and delimiting chromatin states[80]. Genome-wide profiling of the histone H3 variant histone H3.3, which is deposited in a replication-independent manner, revealed its association with the transcribed regions of active genes, promoters and binding sites for regulatory factors in many eukaryotes[81]. Studies measuring the incorporation of tagged histones in yeast also showed that histone turnover is high at promoters and chromatin boundary elements but is low in gene

bodies[82,83]. Measurement of histone turnover by covalent attachment of tags to capture histones and identify turnover (CATCH-IT), a metabolic labeling technique, has confirmed the association of histone H3.3 with regions of rapid nucleosome turnover, including promoters and sites of regulatory factor binding[84]. Each cycle of nucleosome turnover effectively erases histone modifications from a particular location; how, then, are histone modifications maintained in the face of ongoing nucleosome turnover?

Sites of high nucleosome turnover as measured by analysis of histone H3.3 enrichment, tagged histone incorporation and CATCH-IT are generally highly enriched for histone modifications associated with active transcription, such as acetylation and H3K4 methylation[11,83,85,86], and nucleosomal histone H3.3 itself is more highly enriched for these marks than nucleosomal histone H3.1 (refs. 87–89). Notably, soluble histone H3.3 is also more highly enriched for these modifications than soluble histone H3.1 is[88]. Mechanistically speaking, for instance, histone acetylation may help to destabilize nucleosomes by loosening histone-DNA contacts, allowing histone H3.3–containing nucleosomes to be more readily exchanged. Consistent with this hypothesis, nucleosomes that are preferentially lost after histone H3 depletion tend to be acetylated[90]. Conversely, H3K36me3 suppresses histone turnover in gene bodies, and also attenuates interactions between soluble, acetylated nucleosomes and histone chaperones[47].

As mentioned above, histones are heavily modified before deposition, and this might serve as one pathway by which marks are maintained during nucleosome turnover. Another possibility is that modifying enzymes remain bound to sites of turnover, ready to modify their cognate residues upon deposition of new histones. One such example may be Suv39h, the H3K9 methyltransferase that is recruited by HP1 (ref. 91), which in turn binds to methylated H3K9 (ref. 92). To maintain H3K9me3 despite nucleosome turnover, the ATRX ATPase is thought to act at regions of the genome where a nucleosome has been lost[93]. Interaction of the ATRX ATPase with chromatin is enhanced by its ADD domain, which binds with the highest affinity and specificity to histone tails containing both H3K4me0 and H3K9me3 (ref. 93). The ATRX ATPase recruits the histone H3.3–specific DAXX histone chaperone complex, which incorporates a new histone H3.3–containing nucleosome[94]. The previously established local population of Suv39h then methylates the newly deposited nucleosome so that the methylation is identical to that of the lost nucleosome (**Fig. 4**). In this scenario, H3K9me3 not only promotes its own continuity by maintaining high local concentrations of Suv39h but also serves as a key component in the dynamic process of histone replacement by stabilizing the association of the ATRX ATPase with neighboring nucleosomes. Although soluble (preassembly) histone H3.3 is acetylated, it contains very little methylation[88], suggesting that the continued association of modifying enzymes with chromatin is required for propagating the full modification complement of a particular nucleosome. Deposition of an unmethylated nucleosome would allow its modification to be guided by the chromatin context, thus enabling the propagation of modifications.

DNA replication. The assembly of chromatin after DNA replication presents a particular challenge to the maintenance of histone modifications. During replication, parental (histone H3.1–H4)$_2$ tetramers segregate to one of the daughter strands, forming nucleosomes with old or new histone H2A–H2B dimers[95]. Thus, half of the nucleosomes in the newly synthesized daughter strands would, in principle, have 'naive', unmodified (histone H3.1–H4)$_2$ tetramers. It has been postulated that the random distribution of parental (histone H3.1–H4)$_2$ tetramers to one strand or the other provides a half-dose of histone modifications to each strand, which could then be used as a template for the same modification of newly deposited neighboring nucleosomes after replication[96]. In the cases of these modifications, newly deposited nucleosomes could be modified during the passage of the replication fork. Indeed, several histone-modifying enzymes associate with chromatin and/or the PCNA replication clamp during replication, including HDACs, the H3K9 methyltransferases SETDB1 and G9a, and the H4K20 methyltransferase Set8 (refs. 97,98). Histone-modifying enzymes might remain associated with chromatin during replication, allowing newly deposited histones to be efficiently modified after passage of the replication fork[99,100].

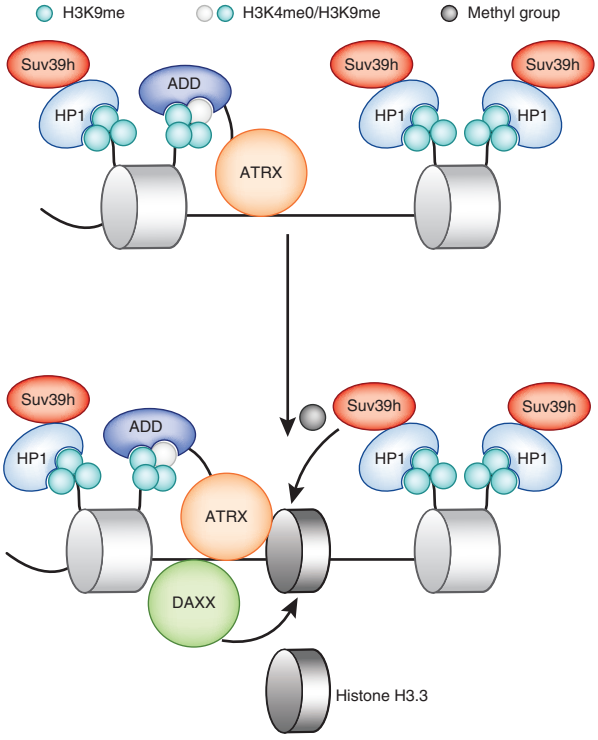

Figure 4 Model for maintenance of a histone modification through replication-independent nucleosome turnover. ATRX binds a site where a nucleosome has been lost (top); its interaction with chromatin is stabilized via the binding of its ADD domain to a histone tail bearing H3K9me3 without concurrent H3K4 methylation. HP1 binding to H3K9me3-marked nucleosomes around this site increases the local concentration of Suv39h. ATRX recruits the H3.3-specific DAXX histone chaperone complex and facilitates deposition of a new, histone H3.3–containing nucleosome (bottom). The high local concentration of Suv39h then facilitates H3K9 methylation of the deposited nucleosome, ensuring continuity of the mark.

Lessons from genome-wide histone modification patterns

Well over 100 histone modifications have been described, and it is likely that other forms and sites of modification will be uncovered. The large number of known and potential histone modifications has led to the suggestion that there would be a high degree of combinatorial complexity in modification patterns *in vivo*. However, a recurring theme in studies mapping the genomic distributions of histone modifications by ChIP-chip and ChIP-seq is the small number of major histone modification patterns identified. For instance, just two principal components accounted for ~81% of the variance in the patterns

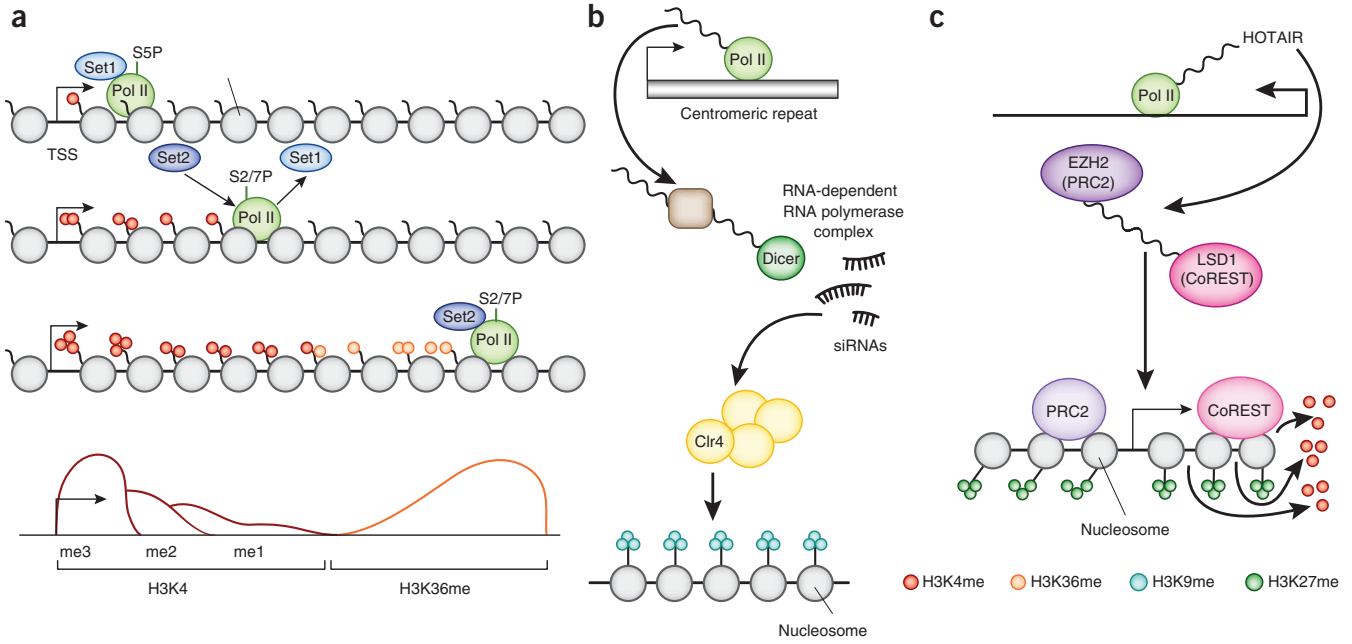

Figure 5 Histone modifications as consequences of dynamic chromatin processes. (**a**) During transcription, Set1 interacts with initiating, Ser5-phosphorylated (S5P) Pol II and methylates H3K4 proximal to promoters (top). With the onset of transcriptional elongation, Pol II is predominantly Ser2/7-phosphorylated (S2/7P), leading to dissociation of Set1 (middle). Set2 associates with Ser2/7-phosphorylated Pol II, which then deposits H3K36me throughout the gene body concurrent to transcriptional elongation (bottom schematic). Below the schematics is a diagram of the distributions of H3K4me and H3K36me in genes. (**b**) In siRNA-dependent targeting, Pol II transcribes centromeric repeats, which are transcribed by RNA-dependent RNA polymerase and processed by Dicer to generate siRNAs, which target the Clr4 methyltransferase complex to sites of future heterochromatin to establish H3K9me. (**c**) In lincRNA-dependent targeting, Pol II transcribes a lincRNA (HOTAIR), which associates with components of the PRC2 H3K27 trimethyltransferase complex (EZH2) and the CoREST H3K4 demethylase complex (LSD1). HOTAIR then targets these complexes to promoters to remove H3K4me3 and establish H3K27me3, contributing to a repressive chromatin state.

of 12 histone marks mapped at single-nucleosome resolution in yeast[8], far fewer than the possible 2^{12} (4,096) potential combinations. In human CD4$^+$ T cells, over 50% of the possible variance in a data set consisting of 41 histone modification ChIP-seq experiments[101] could be accounted for with just three principal components, as opposed to the 2^{41} (~2.2×10^{12}) possible groups. Similar results have been observed in *D. melanogaster*[11], *Caenorhabditis elegans*[86], *Arabidopsis thaliana*[7] and in other human cell lines[102]. Furthermore, complex patterns of histone modifications can be correlated to simple measurements of histone turnover, DNase I hypersensitivity and binding of ATP-dependent chromatin remodelers and transcription factors[103]. The picture that emerges from such studies is that although a few histone modifications have specific roles in transcription and other dynamic processes, histone modifications in general act in a cumulatively simple manner. Analysis of the chromatin state around binding sites for 119 chromatin-binding proteins across several dozen cell lines revealed a limited number of histone modification patterns, each containing multiple acetylations and methylations[104]. The limited number of modification states associated with the binding of such diverse factors suggests that histone modifications act in concert to promote general outcomes of protein-DNA association, such as nucleosome turnover and positioning.

Perspective

There is increasing evidence that histone modifications modulate dynamic processes that affect nucleosomes. For instance, H3K4me2/3, found near gene promoters, and H3K36me2/3, found in gene bodies, are associated with active transcription. However, any role in transcriptional regulation must be subtle because loss of Set1 and Set2, which catalyze H3K4me2/3 and H3K36me2/3, respectively, causes relatively few changes in gene expression[35]. H3K4me2/3 and H3K36me2/3 are established cotranscriptionally by Set1 and Set2, respectively, and the association of Set1 and Set2 with polymerase II (Pol II) depends on initiation- and elongation-related phosphorylation of the Pol II C-terminal domain[105]. Moreover, chemical inhibition of RNA Pol II elongation results in decreased H3K4me3 and H3K36me2/3 (refs. 106,107). These observations strongly suggest that H3K4me2/3 and H3K36me2/3 are components of the mechanisms that modulate nucleosome stability during Pol II transit (**Fig. 5a**).

Modifications associated with gene silencing can help to reduce nucleosome mobility, for example, in the case of H3K9me3, which when bound by HP1 favors heterochromatin formation. In *S. pombe*, H3K9me3 deposition is dependent on the transcription of centromeric repeats by RNA Pol II to generate transcripts that are processed into small interfering RNA (siRNA) and used to target the H3K9 methyltransferase Clr4 to regions of future heterochromatin[108] (**Fig. 5b**). In this case, H3K9me3 is the result of both transcription and the targeting activity of noncoding RNA, and, although it is necessary for HP1-mediated nucleosome bridging to complete heterochromatin formation, cannot be properly targeted without siRNA. Long noncoding RNAs have also been shown to be necessary for targeting histone-modifying activities[109] (**Fig. 5c**). In these examples, histone methylation is the end result of transcription of long noncoding RNAs and the subsequent nucleation and targeting of histone modifying completes. These studies point to a view of histone modifications as cogs in dynamic chromatin processes, wherein histone modifications reinforce changes in nucleosome occupancy, positioning or composition mediated by processes

such as transcriptional elongation, chromatin remodeling and the targeting actions of noncoding RNAs[80,110].

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