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Regulatory mechanisms governing chromatin organization and function

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Abstract

Nucleosomes, the basic structures used to package genetic information into chromatin, are subject to a diverse array of chemical modifications. A large number of these marks serve as interaction hubs for many nuclear proteins and provide critical structural features for protein recruitment. Dynamic deposition and removal of chromatin modifications by regulatory proteins ensure their correct deposition to the genome, which is essential for DNA replication, transcription, chromatin compaction, or DNA damage repair. The spatiotemporal regulation and maintenance of chromatin marks relies on coordinated activities of writer, eraser, and reader enzymes and often depends on complex multicomponent regulatory circuits. In recent years, the field has made enormous advances in uncovering the mechanisms that regulate chromatin modifications. Here, we discuss well-established and emerging concepts in chromatin biology ranging from cooperativity and multivalent interactions to regulatory feedback loops and increased local concentration of chromatin-modifying enzymes.

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Keywords

Chromatin, Histone modifications, DNA methylation, Epigenetics.

Readout of chromatin modifications through cooperative multivalent interactions

Nucleosomes serve as interaction hubs for many nuclear proteins and provide critical structural features for protein recruitment. Histones acquire a large variety of post-translational modifications (PTMs) that modulate and diversify interactions between regulatory proteins and the genome [1]. The proteins that write, erase or read these modifications play central roles in shaping the chromatin landscape in response to extracellular signals, while mechanisms that maintain their presence on chromatin ensure memory and inheritance of chromatin modifications during cell division [2]. At the time of writing, 22 types of histone modifications have been described, including acetylation, citrullination, methylation, phosphorylation, and ubiquitination (Figure 1a). With eight modifiable amino acid residues at about 138 positions on five canonical histone variants, more than 550 possible histone modifications have been reported [3]. Several of these PTMs can co-exist on the same nucleosome resulting in an immense theoretical number of combinatorial possibilities. The majority of the PTMs found on histones can be classified into two groups: modifications in the globular domain and modifications located on the flexible amino-terminal tails of histones extruding from the nucleosome. PTMs in the globular domains of histones can directly affect transcription and nucleosome structure [4]. In contrast, the modifications on histone tails create a diverse array of potential interaction signals for proteins containing reader domains that specifically recognize histone PTMs (Figure 1b) [5,6]. In addition, it has become increasingly clear that nucleosome-binding proteins can target multiple nucleosome features such as nucleosomal or extranucleosomal DNA, the "acidic patch", a negatively charged surface, present on each side of the histone-core, histone tails, and histone PTMs [7]. Extending the possibilities of chromatin readout, DNA methylation is often recognized in a sequence-dependent and -independent context [8,9]. The combinatorial readout of multiple marks and nucleosome features by reader proteins enhances binding through cooperative interactions, resulting in longer residence time on chromatin [10–12]. More importantly, it possibly contributes to context-dependent targeting of regulatory proteins to particular genomic sites on the basis of readout of multiple marks (Figure 2a).

Signal reinforcement and memory through self-propagation of histone modifications

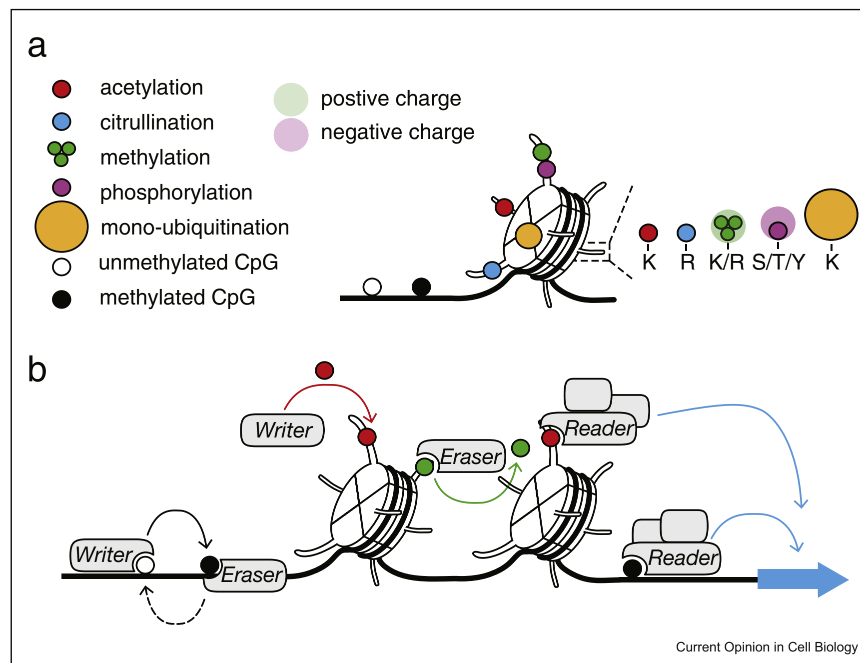
Histone modifications can stimulate chromatin-modifying enzymes, resulting in their activity on

adjacent nucleosomes to reinforce and propagate chromatin states through a positive feedback loop and in absence of the initiating signal (Figure 2b). For example, two well-described histone modifications associated with repressed chromatin states create binding sites for other factors that compact chromatin and form large chromatin domains of constitutive (marked by H3K9me3) or facultative (marked by H3K27me3) heterochromatin. These histone PTMs and the compacted chromatin structure can spread hundreds of kilobases across the genome and are stably inherited after DNA replication [13–15]. The enzymes that deposit H3K9me3 and H3K27me3 rely on a self-propagating mechanism with a central "write-and-read" function. In this self-propagating mechanism, the enzyme or complex that deposits the PTM can also recognize the installed PTM, inducing an allosteric switch that stimulates the enzyme to deposit the mark on neighbouring histone tails, creating a positive feedback loop [16–19]. The trimethylation of H3K9 by Suv39h-family proteins exemplifies this mechanism. The chromodomain of Suv39h1 binds to H3K9me3, resulting in allosteric activation of its carboxy-terminal SET methyltransferase domain and subsequent H3K9 methylation [17,19].

Besides the allosteric regulation of histone lysine methyltransferases (KMT), automethylation of KMTs has emerged as a novel regulatory mechanism to regulate their enzymatic activity. Several KMTs display an autoinhibited conformation that prevents aberrant enzymatic activity. Analogous to the activation of many protein kinases by autophosphorylation, new studies have identified that intramolecular automethylation of specific lysines at an internal loop enhances the H3K27 methylation (H3K27me) activity of EZH2 in the Polycomb Repressive Complex 2 (PRC2) [20,21]. The H3K9 methyltransferase Clr4 of the fission yeast *Schizosaccharomyces pombe* and its human homolog SUV39H2 also show automethylation of distinct lysines in a flexible regulatory loop, resulting in a conformational switch that enhances the H3K9me activity of Clr4/SUV39H2 [22]. These studies suggest that automethylation-induced activation of lysine methyltransferases might be a broadly conserved mechanism that reinforces the deposition of modifications.

The positive feedback loops that arise from the "write-and-read" properties of lysine methyltransferases must be tightly regulated to prevent unwanted spreading of histone modifications. How does the cell counteract the uncontrolled spreading of histone marks? Histone demethylation by specific chromatin modification erasers and histone exchange are two regulatory mechanisms identified over the last years, primarily resulting in the clearance of histone modifications [23]. An emerging mechanism to counteract the uncontrolled

Figure 1



Schematic of exemplary histone and DNA modifications on mammalian chromatin (**a**) Chromatin is subject to a diverse array of chemical modifications with different molecular properties (molecular size, charge, and stability). Nucleosomes are enzymatically modified at the surface, the lateral side, and the flexible histone tails that extrude from the nucleosome (**b**) Specialized writer, eraser, and reader proteins play central roles in the placement, erasure, and reading of these modifications. All chromatin modifications known to date are reversible and create a highly dynamic platform for protein recruitment to regulate access to the genetic information.

spread of histone modifications is the suppression of chromatin writers through protein inhibitors. Recent studies discovered a protein called Enhancer of Zeste Homologs Inhibitory Protein (EZHIP) containing a flexible and conserved histone-like sequence sufficient to inhibit EZH2 activity and reduce the spread of H3K27me3 in cancer and germ cells [24,25]. These findings raise the question of whether similar factors exist with the ability to inhibit other chromatin writers.

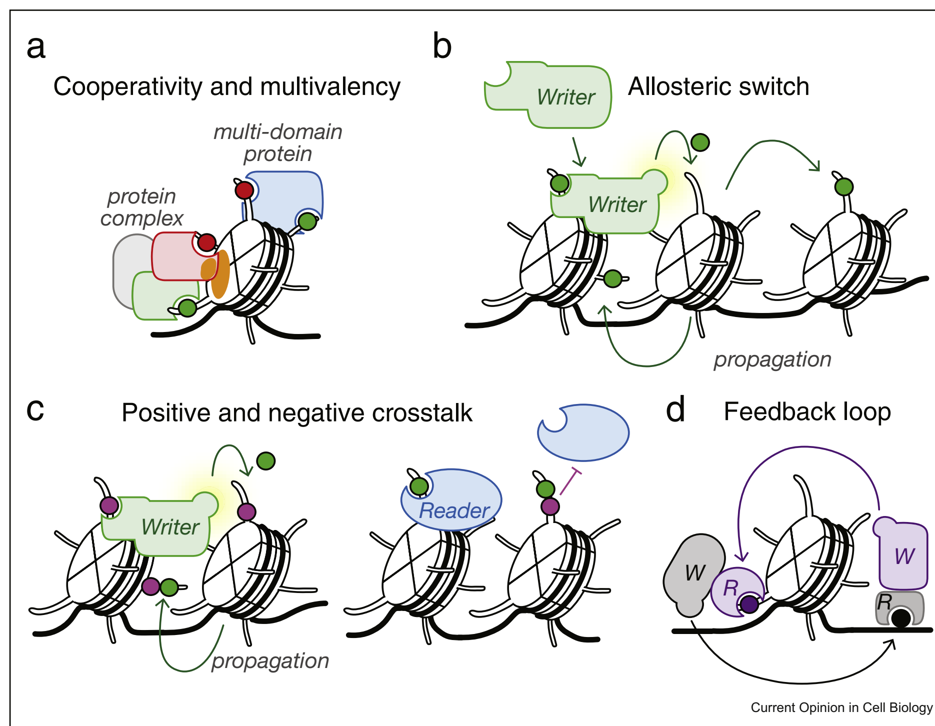
Regulatory feedback loops between different chromatin pathways

Similar to the autostimulatory mechanisms described above, some modifications can influence the recognition or deposition of other modifications. This interdependence of chromatin marks, frequently called “crosstalk”, is a widespread regulatory mechanism in eukaryotic chromatin pathways [1,26,27]. Two main regulatory outputs can emerge from this crosstalk: a stimulatory and an inhibitory outcome. The stimulatory scenario involves an initial modification that triggers increased writing activity for other modifications, allowing the reinforcement of a particular chromatin state containing two or more modifications on histones or DNA.

Positive feedback

Many chromatin pathways exhibit positive reinforcement between different chromatin modifications (Figure 2d). The conserved interplay between the repressive modifications H3K9 and DNA methylation is a recurring example found in mammalian cells, fungi, and plants [28]. The presence of H3K9 methylation or direct association of the DNA methyltransferases (DNMTs) with H3K9 methyltransferases and H3K9me3 readers such as G9a or MPP8 is crucial in targeting *de novo* DNA methylation at heterochromatic regions to reinforce a repressed chromatin state in mammalian cells [29,30]. The machinery that maintains DNA methylation further depends on positive crosstalk between H3K9 and DNA methylation and histone ubiquitination. Symmetrical CpG methylation depends on the activity of the methylation maintenance enzyme

Figure 2



Regulatory principles operating on chromatin (**a**) Cooperativity and multivalent interactions: the combinatorial readout of multiple marks and nucleosome features by distinct sets of reader modules in effector complexes enhances binding through cooperative interactions and contributes to the context-dependent recognition of particular genomic sites (**b**) Allosteric regulation of effector proteins: several enzymes that deposit repressive histone PTMs rely on a self-propagating mechanism with a central “write-and-read” leitmotif. Binding to PTMs induces a conformational switch that results in increased enzymatic activity (**c**) Crosstalk between histone modifications. Stimulatory effect (left): a chemical modification triggering the activity of a methyltransferase (green). Inhibitory effect (right): example of a phosphorylation mark (purple) that interferes with the readout of a neighbouring methyl mark (green) (**d**) A positive feedback loop involving several chromatin modifications: Readers of H3K9me3 recruits a de novo DNA-methyltransferase leading to the methylation of CpG (mCpG) sites at H3K9me3 genomic regions. Consequently, readers of mCpG sites can recruit repressive effector complexes to deposit H3K9me3 allowing the reinforcement of a repressed chromatin state.

DNMT1 in concert with the multidomain protein ubiquitin-like, containing PHD and RING finger domains 1 (UHRF1). UHRF1 recognizes hemimethylated DNA at replication forks through its SET- and RING-associated (SRA) domain and H3K9me3-containing nucleosomes via its tandem TUDOR-PHD (TTD-PHD) domain. UHRF1 then mono-ubiquitinates H3K18 and H3K23, and together with its ubiquitin-like (UBL) domain, creates signals for the recruitment of DNMT1 to replication forks [31]. DNMT1 binding to ubiquitinated H3 through its replication foci targeting sequence (RFTS) domain induces a conformational change in DNMT1 that triggers its activation and ensures maintenance of symmetrical DNA methylation at CpG sites [32].

A similar positive reinforcement between different modifications has been described for Polycomb group (PcG) proteins, which are essential for developmental gene regulation from insects to vertebrates. Genetic and biochemical studies revealed two core PcG repressive complexes, PRC1 and PRC2, with distinct enzymatic activities, accessory subunits, targeting mechanisms, but related functions. PRC1 catalyses the mono-ubiquitination of histone H2A at lysine 119 (H2AK119ub1) through the E3 ubiquitin ligases RING1A/B subunits. The core complex of PRC2 catalyses the mono-, di- and tri-methylation of histone H3 at lysine 27 (H3K27me1/2/3) through the methyltransferase EZH1/2. Both PRC1 and PRC2 exist in different arrangements with distinct subunit compositions that potentially influence the functional properties of the complexes. The synergistic activity of PRC1 and PRC2 results in the formation of transcriptionally silent Polycomb domains, characterized by compacted chromatin with H2AK119ub and H3K27me3 marks. PcG proteins are evolutionary conserved from flies to humans, but their recruitment mechanisms to target sites diverged significantly. Early studies proposed a hierarchical recruitment model in which first PRC2 is recruited to target genes leading to H3K27me3 deposition. Subsequent binding of H3K27me3-reader containing PRC1 complexes (canonical PRC1 complexes) results in chromatin compaction [33]. Recent studies have established a second parallel pathway capable of recruiting PRC2.2 complexes via recognition of variant PRC1-mediated H2AK119ub1 [34,35]. A new Cryo-EM structure provides additional support for a model where the H2AK119ub1 signal initiates the recruitment of PRC2.2 and stimulates the placement of H3K27me3 via allosteric activation of EZH2. PRC2.2 subunits JARID2 and AEBP2 each recognize one of the two ubiquitin moieties on the symmetrically modified nucleosome providing additional anchoring interactions with the nucleosome. EZH2 is further stabilized through interactions with the H3 tail and the nucleosomal DNA on the substrate nucleosome. Together with ubiquitin-dependent anchoring, these interactions result in the stabilization of the catalytic site and increased enzymatic activity of EZH2 [36]. PRC2 further propagates H3K27me3 on neighbouring unmodified nucleosomes in a “write-and-read” mechanism. Previous genetic and structural studies revealed how the readout of H3K27me3 through PRC2 subunit EED allosterically activates the SET domain of EZH2 [16,18,37]. Finally, H3K27me3 is read by CBX subunits of canonical PRC1 complexes, leading to chromatin compaction and reinforcement of a repressed chromatin state [38,39].

Negative feedback

The second theme involves the inhibitory effect of one modification over another. For example, neighbouring PTMs on the same histone tail can alter the binding of readers toward their target modifications, resulting in the repulsion of an effector protein from chromatin and allowing the rapid off-switch of a distinct function. A well-described and evolutionary conserved example is the inhibition of PRC2 activity by H3K4me3 or H3K36me2/3 on the H3 tail of the substrate nucleosome [40]. Recent structural studies suggest that inhibition of EZH2 activity is caused through the loss of electrostatic interactions between tri-methylated H3K36 and the phosphate backbone of nucleosomal DNA, which normally help stabilize H3K27 into the active site [36,41]. This negative feedback is also reflected by the genome-wide anticorrelation between H3K27me3 and H3K36me3 [42].

Negatively charged modifications on chromatin can mediate similar inhibitory effects. Sixteen phosphorylation sites of serine, threonine, or tyrosine residues on histone tails occur beside or nearby lysine residues that can be methylated. Phosphorylation of histone tails can block the binding of readers towards adjacent methylated lysine residues (Figure 2c). These so-called phospho/methyl switches provide a binary mechanism to regulate the readout of histone marks [43]. The first observation of a phospho/methyl switch describes the phosphorylation of H3S10 by Aurora B-type kinases during mitosis, which releases the H3K9me3 reader heterochromatin protein 1 (HP1) from heterochromatin, even though the H3K9me3 mark persists [44,45]. Since then, similar phospho/methyl switches have been confirmed in various chromatin-related pathways [46].

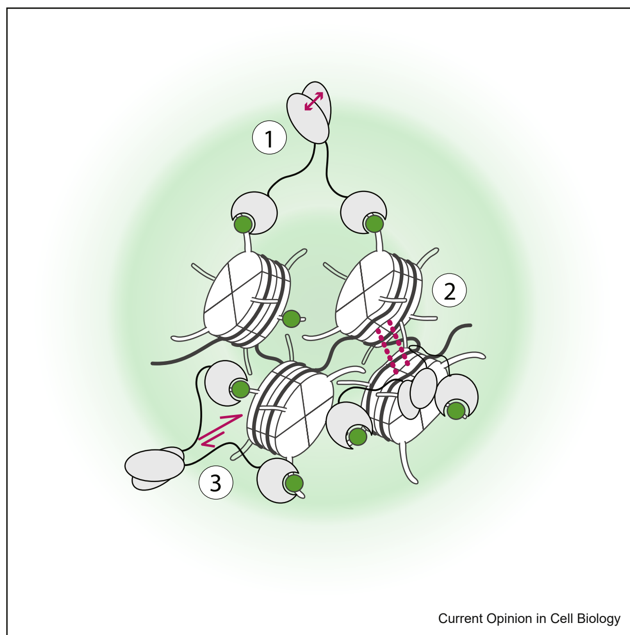
Phospho/methyl switches also function upon signal-induced phosphorylation of histone 3 during interphase. Histone phosphorylation induces initiation or elongation of transcription by transcriptional coactivators [47,48]. Recently, Armache et al. showed that the unique serine 31 residue on the histone variant H3.3 is phosphorylated (H3.3S31ph) in a stimulation-dependent manner along rapidly induced genes in mouse macrophages. H3.3S31ph ejects the prebound

ZMYND11 elongation corepressor and augments the activity of SETD2, a H3K36me3 methyltransferase associated with the active transcription machinery, to induce the transcription of stimulation-induced genes [49]. These results illustrate how a single mark (H3.3S31ph) causes an inhibitory effect while simultaneously triggering an activatory feedback loop enabling cells to rapidly respond to environmental cues (Figure 2c).

High local concentration of chromatin regulators for concerted action in space and time

High local abundance of binding sites created by DNA methylation or histone modifications allow the concentrated localization of enzymatic activity to a confined space, resulting in increased regulatory activity. Akin to increased effective molarity in chemistry, the high concentration of chromatin regulators at specific genomic sites is a central theme in many chromatin pathways that regulate access to DNA such as transcription, replication, and repair. In DNA repair for example, an initial genomic lesion is recognized by specialized proteins resulting in the deposition of acetylation, poly-ADP-ribosylation, phosphorylation, and ubiquitination of adjacent nucleosomes. These modifications serve as secondary signals to recruit repair factors and to amplify signalling by the DNA damage response (DDR) pathway [50,51]. Heterochromatin represents another well-studied example where a high local concentration of chromatin regulators establishes a stable repressive domain. Positive feedback loops between H3K9me3 and DNA methylation create a high density of binding sites recruiting various specialized reader proteins to heterochromatic regions [52,53]. In turn, several readers recruit additional repressive complexes, further sustaining the high density of repressive marks [30,54]. Chromatin compaction, a characteristic of repressed heterochromatin, is established through HP1 proteins, likely operating through multiple mechanisms (Figure 3). First, HP1 proteins contain a reader domain and a dimerization domain separated by a flexible linker, allowing HP1 proteins to bridge neighbouring H3K9me3-marked nucleosomes via self-dimerization [10,55]. Second, the fission yeast HP1 homolog Swi6 reshapes the nucleosome core to promote internucleosomal interactions [56]. Third, HP1-dimerization reduces the off-rate of the protein from chromatin [10,57], further stabilizing the interactions mentioned above.

Figure 3



High local abundance of binding sites created by H3K9me3 allows HP1-mediated heterochromatin compaction. HP1 binds to H3K9me3 via its chromodomain and bridges two neighbouring histone tails on the same or adjacent nucleosomes through self-dimerization using its chromoshadow domain (1). Also, HP1 contacts the nucleosome core and remodels histone–histone contacts allowing internucleosomal interactions (2). Finally, multiple H3K9me3 marks along heterochromatin and HP1-dimerization increase the residence time of HP1 on chromatin, further stabilizing the interactions mentioned above (3).

Several chromatin modifications and their associated processes appear to occupy distinct spatial territories in the nucleus, resulting in increased proximity between factors involved in the same regulatory pathway. Recent observations have suggested that this behaviour is driven by phase–separation properties of chromatin regulators, such as for HP1 [58,59], PRC1 member CBX2 [60], the acetylation reader and transcriptional coactivator BRD4 [61], 53BP1 in the DNA damage response [62], and *in-vitro* reconstituted chromatin [63]. The role of phase separation in driving compartmentalization of chromatin is currently vigorously debated [64–66]. However, the phase–separation properties observed in numerous chromatin-associated proteins might serve as an additional regulatory layer. In addition to modification readout, it helps increase the local concentration of effector proteins and confine modifications with their functions to specific genomic and subnuclear territories. In the future, it will be imperative to design more physiological experiments to study the phase separation properties of endogenous proteins and demonstrate essential functions of phase separation on chromatin-related processes [67].

Conclusions and future perspectives

Chromatin regulators play essential roles in regulating genome function and are among the most frequently mutated proteins in cancers, hinting to relevant gene–regulatory pathways and providing potential therapeutic targets. Despite decades of progress in the field,

many important questions remain regarding the molecular coordination between different regulatory layers that control the deposition of chromatin modifications to the genome and how these influence genome function. A significant challenge in the field remains to understand how the chemical language on chromatin defines the local protein interactome of the genome [53]. Detailed understanding of how modifications influence the spatiotemporal recruitment of effectors to designated genomic sites should help us obtain a clearer view on the hierarchy of events that lead from individual modifications to recruitment of effectors, crosstalk to other pathways, and finally establishment of robust and cell-type-specific regulatory circuits. Furthermore, disentangling the direct developmental roles of chromatin regulators, which is often hampered by complex mutant phenotypes, is imperative to understand how multicellular organisms arise. As recently exemplified, more refined perturbation of gene activity with combined single-cell readout will undoubtedly bring the insights needed [68]. In the years to come, we will witness a more mechanistic understanding of how chromatin contributes to developmental processes as single-cell epigenomics and single-cell proteomics technologies will mature in the future.

Conflict of interest statement

Nothing declared.

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