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Emerging roles for chromatin as a signal integration and storage platform

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Abstract

Cells of a multicellular organism, all containing nearly identical genetic information, respond to differentiation cues in variable ways. In addition, cells are plastic, able to execute their specialized function while maintaining the ability to adapt to environmental changes. This is achieved through multiple mechanisms, including the direct regulation of chromatin-based processes in response to stimuli. How signal transduction pathways directly communicate with chromatin to change the epigenetic landscape is poorly understood. The preponderance of covalent modifications on histone tails coupled with a relatively small number of functional outputs raises the possibility that chromatin acts as a site of signal integration and storage.

Introduction

How do organisms, and cells within organisms, respond to their environment? In mammals, information conveyed to sensory organs is transmitted to the central nervous system, where the information is processed and converted to a physiological response. At the cellular level, signalling cascades initiated at the cell surface relay a message via effector proteins, ultimately culminating in the nucleus where transcription factors are targeted to induce or shut down a particular gene expression signature. This event may be transient in nature, for example when a cell needs to respond acutely to an event and then return to its previous steady state. However, the development, health and/or adaptation of an organism often require that an ephemeral environmental signal can be converted into a longer-lived phenotypic change. Epigenetic mechanisms, which allow heritable changes in gene expression without altering the genetic sequence, are one powerful way to turn a transient signalling event into a long-lived change in organism performance or function.

Multiple outcomes of a signalling event are possible. When a change in gene expression is required, DNA sequence-specific transcription factors are spurred into action. They can be regulated by several mechanisms, including: altered complex association with co-activators that stimulate transcription or increase DNA-sequence specificity¹; shuttling into or out of the nucleus driven by changes in post-translational modifications² and thereby protein interactions; and ubiquitin-mediated proteasomal degradation³. This paradigm for regulation and activation of transcription factors by signalling cascades initiated at the cell membrane or in the cytoplasm is well established, and demonstrates the importance of crosstalk between different cellular compartments. Transcription factors that bind specific

DNA sequences work in concert with chromatin regulators to orchestrate a change in transcriptional programme. Indeed, chromatin-based mechanisms, in which histones and DNA are modified and interpreted by ‘**writer/eraser**’ and ‘**reader**’ molecules respectively, are vital for transcriptional control.

Multiple different chemical modifications, identified through mass spectrometry and candidate approaches, heavily decorate the histone tails ⁴. This constellation of various modifications, which together regulate transcriptional outcomes, are often referred to as the ‘histone code’ ⁵.

The abundance of modifications raises the question of whether histone tails act as a site of signal integration ⁶ where multiple modifications or ‘inputs’ can convene over time and result in only a few directed outputs, that is, as a multiple-inputs-single-output system. In addition, how do signalling cascades communicate with writer, eraser and reader molecules? Stimuli-induced epigenetic alterations can persist long after the initiating signal is gone. Indeed, epigenetic changes can remain throughout the lifetime of an organism and even be transmitted to subsequent generations⁷. Given these potentially long-term consequences, it is imperative that histone modifications are tightly regulated but so far little is known about how this occurs. Exciting new studies have highlighted that this can be mediated both by direct, signalling-mediated regulation of chromatin-modifying enzymes as well as signal-induced nuclear complex assembly. It is also becoming clear that signalling events target proteins with histone tail-like sequences, dubbed ‘histone mimics’. These are scattered throughout chromatin-regulatory proteins and the post-translational modification of some can promote binding of known ‘reader’ proteins. One possibility, therefore, is that histone ‘mimic’ sequences might allow a single signalling event to coordinate changes on chromatin by co-modifying not only histones but also their regulators.

The idea that signalling might have direct effects on chromatin was originally postulated over ten years ago, whereby similar mechanisms of signal transduction that affect receptor tyrosine kinases could also function at chromatin ⁸. In this review, we focus on the new understanding being gained into the molecular basis of signalling to chromatin. We highlight ways in which signalling pathways, with a focus on kinase cascades, can directly regulate nucleosomes and chromatin-modifying enzymes, eliciting effects on chromatin modification, chromatin remodelling and deposition of **histone variants**. We also discuss the many influences that histone mimics can have on chromatin function, and speculate that transduction of cellular signals to histone mimics may be coordinated with signal transduction to nucleosomes. We outline the exciting questions that these studies have raised about the intersection between cell signalling and epigenetic regulation. Readers are referred elsewhere for more detailed discussions of DNA damage signalling involving chromatin ⁹, the detailed mechanisms of epigenetic transcriptional control ^{9–11} and the effects of non-coding RNAs on epigenetic regulation and transmission ¹².

Signal integration on histones

Cells must coordinate responses to many stimuli, including signalling cues from other cells, the extracellular matrix, hormones and active compounds. As they are subject to feedback

control, these response pathways are usually ‘on’ or ‘off’ in nature. By contrast, transcriptional changes can be ‘dialed’ up or down and provide a composite response to multiple inputs over time. In line with this, signal transmission to chromatin can be compared to a digital-to-analogue converter (DAC) that takes ‘on’ and ‘off’ signals and converts them into a continuous output that can vary in amplitude over time. We speculate that histone tails may function in this way, acting as a site that stores signals and integrates them to regulate the transcriptional programme (FIG. 1A). Indeed, the pure density and variety of post-translational modifications on histones suggests that they are uniquely placed to act as signal integration devices. Histone modifications work in combination, which adds to the diversity of outputs that they might facilitate. Crosstalk between histone modifications occur in *cis* and *trans*, by: influencing the binding of reader molecules to adjacent residues¹³; precluding or recruiting enzymes that modify additional sites^{14, 15}; or through combined effects on complex docking (termed ‘multiple site display’)^{16–20} (FIG. 1). Although the underlying mechanisms are not fully understood, the modification landscape can be fully or partially maintained throughout replication, allowing past signalling events to influence the current state of transcription²¹. As a result, the transcriptional output of a single gene over a time course, or even over the life of a cell, follows a continuous wave pattern that can be dialed up or down, but that differs from the inputs received at any specific time point. Circadian gene expression is one example of this, whereby chromatin surrounding the CLOCK gene promoter is methylated, acetylated, then deacetylated to control fluctuations in the amplitude of CLOCK gene expression over a 24-hour period²². Thus, histone tail posttranslational modification has all the features of a ‘storage and output’ device for epigenetic information. Nevertheless, it is ill defined how long a signal can be stored on chromatin. In addition, it is still largely unknown how the chromatin landscape is preserved during DNA replication and cellular division. Thus, determining the variables that define the longevity of marks on histones is critical for understanding the storage capacity for these marks.

Mechanisms of signalling to chromatin

Chromatin landscape alteration is emerging as a major consequence of signalling events. A signal can be relayed directly to chromatin-modifying enzymes to add or remove chemical modifications on histones and DNA. In addition, activated signalling cascades can communicate with **nucleosome remodellers** and **histone chaperones** to alter the chromatin structure. In this section, we will review such mechanisms and discuss the future work needed to clarify and build on our knowledge of how signalling to chromatin is mediated.

Direct targeting of nucleosomes by kinase cascades

At the chromatin level, phosphorylation creates docking sites for phospho-binding proteins and acts to temporarily oppose existing or potential modifications on histone tails, such as methylation. Histone methylation has a longer half-life compared with acetylation²³ and phosphorylation²⁴, implying that signal-induced changes in the methylation landscape on chromatin may facilitate longer-lived storage or ‘memory’ of cellular experience. Conversely, histone phosphorylation and acetylation seem to be more transient and short-lived signals that trigger an immediate switch in chromatin behaviour.

Kinase cascades can directly alter nucleosomes through phosphorylation of histones (for a review, see references^{25, 26}). For example, Aurora B (AurB) phosphorylation at Ser10 of histone H3 (H3S10) at the onset of mitosis is necessary for proper chromosome condensation and segregation²⁷. This phosphorylation perturbs the methylation and binding of many chromodomain-containing proteins to the adjacent lysine 9 residue (H3K9me2/3). As most H3K9me-binding proteins repress transcription and/or condense the chromatin, Ser10 phosphorylation during interphase activates transcription. The increased phosphorylation of S10 that is induced by mitogens is transient, gradually decreasing within an hour of the stimulus^{28, 29}; it is unknown whether this rapid decline requires active dephosphorylation or histone turnover. This path from stimulus to transcription has been elegantly tracked using the progesterone-responsive MMTV promoter as a model system; within 5 minutes of treatment with the synthetic steroid progestin, H3S10 became phosphorylated in the MMTV promoter region in an Msk1-dependent fashion. Furthermore, H3S10 phosphorylation and associated H3 acetylation blocked binding of chromo-domain protein HP1 γ and promoted ATP-dependent chromatin remodelling to fully activate target gene transcription³⁰. H3S10 phosphorylation also initiates a complex cascade of chromatin factor recruitment that ultimately culminates in transcriptional elongation. Specifically, by creating a docking site for 14-3-3 ϵ/ζ , H3S10 phosphorylation triggers histone acetylation, followed by BRD4/p-TEFb recruitment to modify the CTD of RNA Pol II and promote transcriptional elongation³¹. Similarly to H3S10 phosphorylation, H3Y41 phosphorylation by JAK2 perturbs HP1 α binding. As constitutive activation of JAK2 commonly occurs in haematological malignancies, increased H3Y41 phosphorylation in this cancer type has been proposed to contribute to genomic instability and aberrant gene activation through loss of HP1 α function at chromatin³².

Although many sites of histone phosphorylation have been identified, how these marks influence the deposition or activity of each other in *cis* or *trans* is much less characterized. For example, residues proximal to S10 on the H3 tail are also phosphorylated in response to pathway activation, including but not limited to, the phosphorylation of T6 and T11 by PKC α and Chk1, respectively. High resolution NMR spectroscopy has been used to evaluate the hierarchy of phosphorylation events at these three proximal sites on a single H3 molecule. This showed that PKC α and Chk1 can also phosphorylate S10 and that this initial event would prevent subsequent modification of T6 or T11 on the same molecule. By contrast, T6 or T11 phosphorylation has no effect on S10 phosphorylation by Chk1, PKC α or AurB. Thus, S10 phosphorylation sits atop the hierarchical ladder, preventing nearby phosphorylation events in *cis*³³.

Phosphorylation can also actively preclude demethylating enzymes from removing methyl-marks in *cis*³⁴, or block the recruitment of Lys methyltransferases and deacetylases to a particular residue to allow acetylation at that site³⁵. For example, phosphorylation of H3T11 by the oncogenic kinase PKM2 precludes HDAC3 retention on chromatin, resulting in H3 acetylation and transcription of the potent oncogenes MYC and CCND1³⁶. In summary, signal-induced phosphorylation of histones mediates the rapid conversion of one chromatin read-out to another. To completely understand this process, however, more research is

needed to define the relative permanence of the various signal-induced histone modifications, and to better understand the crosstalk between these marks.

Feedback and signalling out from chromatin

A series of positive and negative feedback loops must exist to achieve homeostasis. This is true both at the organismal level (for example, during endocrine signalling) and at the cellular level. During growth factor-stimulated signal transduction, for example, receptor activation usually involves phosphorylation of the receptor intracellular domain and factors in the downstream kinase cascade. Often, this same cascade activates a phosphatase that then removes the activating phosphorylation marks, thereby shutting down the very signal that activated it. In this way, receptor activation persists for a defined amount of time, to ensure a controlled response by the cell. Disruption of these negative feedback loops can lead to cancer³⁷. Feedback also occurs in transcriptional gene transcription programmes; early, middle and late rounds of gene transcription cross-regulate each other. Chromatin regulators affect this process; one round of gene activation can lead to the expression of enzymes that then alter the chromatin landscape to promote or reduce the expression of other genes in the same pathway. For example, the transcription factor FOXO1 targets the SIRT1 gene to increase SIRT1 expression. SIRT1 protein then binds to and deacetylates FOXO1 to further activate the transcription factor, thus forming a positive feedback loop³⁸. Aside from the traditional role of chromatin regulators in transcription, feedback control can also occur via signalling emanating from chromatin (that is, inside-out signalling). Taking the DNA damage response as an example, the specific event of double-strand break induction on chromatin triggers a response that repairs and monitors the damage while also controlling the cell cycle. Thus, the cascade is moving away from, instead of towards, the chromatin and is independent of transcription. This idea of direct (indirect being through transcriptional regulation) inside-out signalling from chromatin in contexts other than DNA damage is relatively new. It is established that histone modifications can influence other histone modifications, but whether histone modifications can signal to non-histone proteins is less clear. Such crosstalk with non-histone proteins has been shown for histone H2B ubiquitylation (H2BK123), which is required for methylation of the non-histone protein DAM1 at kinetochores³⁹. Additional studies to explore crosstalk --- between posttranslational modifications on histone and non-histone proteins, between histone proteins and DNA methylation and between DNA methylation and non-histone proteins --- will be important to fully understand the complexity of signalling around and away from chromatin, and how this process ensures cellular homeostasis.

Direct regulation of chromatin modifiers

The same mechanisms that regulate transcription factors can also regulate the epigenetic machinery, although key questions remain about how. When an external signal induces a major or minor overhaul of the transcriptional programme, how are chromatin factors regulated to act at the right time and place? Making a logical extension from the principles of transcription factor control, one can envision multiple ways to induce changes in the chromatin landscape in response to extracellular cues (FIG. 2). However, so far there is only direct experimental evidence for a few of these possibilities.

Metabolic control of chromatin factors

Metabolic pathways can communicate with chromatin, providing information about nutrient availability⁴⁰ (FIG. 2a). Indeed, nutrient availability is one major informational resource our bodies use to interpret current environmental conditions. It is therefore imperative that mechanisms be in place to relay this information to chromatin to impart changes in gene transcription, thus allowing organisms to adapt and survive. For example, in hypoxic conditions, HIF1 α and HIF1 β bind to and recruit the histone acetyltransferases p300 (and its homologue CREB-binding protein CBP) to chromatin, resulting in increased local nucleosomal acetylation and transcriptional activation of hypoxia-responsive genes. Oxygen levels may also directly regulate the expression and catalytic activity of the JmjC domain-containing lysine demethylases^{41,42}, as these enzymes are 2-oxoglutarate-dependent dioxygenases. Indeed, metabolites are used as cofactors by many of the chromatin-modifying enzymes, so it is possible that the local availability of these could affect enzymatic performance. Alternatively, metabolites pooling around chromatin territories can also be natural enzymatic inhibitors. Supporting this notion, the oncometabolite 2-hydroxyglutarate precludes cellular differentiation by inhibiting lysine demethylases^{43, 44}. Furthermore, during times of starvation or limited carbohydrate availability, levels of the ketone body β -hydroxybutyrate increase and inhibit HDAC activity⁴⁵. Glucose-sensitive O-GlcNAcylation of histones, histone-modifying enzymes and multiple transcription factors including Pol II has been reported^{46, 47}. O-linked β -d-N-acetylglucosamine (O-GlcNAc) transferase (OGT) uses uridine diphosphate N-acetylglucosamine (UDP-GlcNAc) as a donor towards the addition of GlcNAc at Ser or Thr residues, often in lieu of phosphorylation at that site. Multiple nutrient sensing pathways regulate the levels of UDP-GlcNAc and could therefore have an impact on the extent of O-GlcNAcylation occurring on chromatin. The consequence and longevity of chromatin O-GlcNAcylation are still largely unknown and warrant further research to better understand how nutrient availability may affect gene expression acutely and possibly epigenetically (for a more detailed discussion of crosstalk between metabolic pathways and chromatin see references^{48,49} and⁵⁰).

Kinase cascade regulation of chromatin-modifying enzymes

Aside from the metabolic pathways mentioned above, activated kinase cascades can actively signal to chromatin-modifying factors to alter the chromatin landscape (FIG. 2b). In particular, phosphorylation of these factors is the best-described mechanism of communication, and will thus be the focus here. However, it is reasonable to assume that signalling cascades also relay information to chromatin-modifying factors through other means, such as other types of PTM and sequestration. Here, we discuss illustrative examples of mechanisms of signalling to chromatin through chromatin-modifying factors, focusing on chromatin methylation, remodelling and deposition of histone variants. A more comprehensive list of signal-induced post-translational modification of chromatin-modifying enzymes is shown in TABLE 1; the better characterized examples of lysine acetyltransferases (HATs) and deacetyltransferases (HDACs) control by signalling are excluded owing to space consideration (for a review, see reference⁵¹).

The PI3K-AKT pathway targets chromatin-modifying enzymes

The PI3K/AKT signalling pathway is central to eliciting a pro-growth and pro-survival response to various cellular signals, including growth factors, integrin binding, cytokines, and hormones. AKT can phosphorylate chromatin-modifying enzymes, including the H3K27 methyltransferase EZH2⁵², the histone ubiquitylating enzyme BMI1⁵³, the histone acetyltransferase p300⁵⁴, and the DNA methyltransferase DNMT1, resulting in significant changes to the function of these enzymes (FIG. 3A). Other chromatin-modifying enzymes including KDM4B, hSET1A and DNMT3B contain phosphorylation sites (identified in large proteomic studies) within AKT consensus motifs, and are thus likely to be additional targets (FIG. 3B). In this section, we will review the reported consequences of AKT phosphorylation on two chromatin-modifying enzymes, DNMT1 and EZH2.

During replication, the pattern or ‘signature’ of methylated DNA is preserved by the DNA methyltransferase 1 (DNMT1). These patches of methylated DNA are positioned to maintain silencing of developmental genes, repetitive elements and endogenous retroviral sequences⁵⁵. It is essential that DNMT1 activity is regulated, as its misregulation could lead to loss of this DNA methylation pattern and gene expression changes after multiple rounds of cell division; this could in turn promote tumorigenesis if an oncogene were to be reactivated, or a tumour suppressor gene were to be silenced. DNMT1 can be regulated via phosphorylation by AKT, which is stimulated by growth factors, cytokines, hormones and pro-survival signals. AKT-induced phosphorylation of DNMT1 at Ser143 blocks monomethylation of the adjacent Lys142 residue by the methyltransferase SET7/9 (also known as SETD7). As methylation at Lys142 promotes DNMT1 degradation by the proteasome, AKT phosphorylation increases the stability of DNMT1⁵⁶. Phosphorylation of the same Ser143 residue of DNMT1 also prevents association with UHRF1 and PCNA⁵⁷, critical partners for the maintenance of DNA methylation during replication^{58–60}. Together, these findings suggest that S143 phosphorylation increases the protein levels of DNMT1, but prevents its association with the replication fork. It has been proposed that this mechanism could explain the global DNA hypomethylation observed in cancer cells. Indeed, the PI3K-AKT pathway is often overactive in cancer⁶¹, but it remains to be determined whether DNMT1 activity is promiscuous in such tumours, and if this alters the DNA methylation signature globally or at select gene promoters.

EZH2 is the catalytic subunit of the PRC2 repressor complex, and trimethylates Lys27 of histone H3 (H3K27me3) to silence gene expression. Intriguingly, H3K27me3 and opposing H3K4me3 demarcate **bivalent domains** that represses gene expression but is poised to respond to cellular cues⁶². In the bivalent state, H3K27me3 actively represses transcription; therefore removal of this modification alone is sufficient to activate gene expression. For this reason, EZH2 provides a target through which signalling cascades can activate poised genes. Indeed, EZH2 is a known target of AKT, and AKT phosphorylation at Ser21 reduces the ability of EZH2 to methylate H3K27⁶³. In addition to gene promoters, distal regulatory enhancer elements are also marked by defined H3K27 modification states; poised but silent enhancers display the H3K27me3 mark⁶⁴, whereas activated enhancers display H3K27 acetylation⁶⁵. The histone acetyltransferase p300 (or CBP) acetylates H3K27, and concordantly, p300 is found bound to active enhancers⁶⁶. Furthermore, the co-activator

activity of p300 is increased by AKT phosphorylation⁵⁴. It will therefore be interesting to determine whether PI3K-AKT signalling could activate gene enhancer elements by inhibiting EZH2 and activating p300 to switch H3K27 from a tri-methylated state to an acetylated state (FIG. 3c). We speculate that AKT may be a major regulator of enhancer status.

p38 phosphorylation of a chromatin remodelling subunit

The SWI/SNF family of chromatin remodelling enzymes can promote 'sliding' of nucleosomes to reveal or hide DNA sequences and thereby control the access of transcription factors to particular genes⁶⁷. For example, the MyoD transcription factor, a master regulator of muscle differentiation, sits poised at target genes in myoblasts so that it can trigger the differentiation programme in response to the appropriate cue. As the chromatin is condensed around muscle genes in myoblasts, MyoD retention depends on binding of the SWI/SNF subunit BAF60c here. Upon differentiation, BAF60c is phosphorylated by p38 α/β , creating a nucleating site for the remaining SWI/SNF components. This allows chromatin remodelling and exposes MyoD target sequences, thus allowing muscle-specific gene expression and muscle differentiation⁶⁸. Similar modes of regulation have been demonstrated in other contexts and, in each case, one subunit of a large protein machine sits at target regions, poised and possibly acting as a 'placeholder'. Upon stimulation, the remaining complex nucleated by this factor is formed for rapid and directed activation of **primary response genes (PRGs)**⁶⁹.

Inhibition of PRMT5 by JAK2 phosphorylation

Protein Arg *N*-methyltransferase 5 (PRMT5) sym-metrically dimethylates Arg residues in non-histone and histone substrates, resulting in a broad range of biological outcomes⁷⁰. The transcriptional influence of PRMT5 histone methylation can be positive or negative, depending on the histone site modified, the cell type and the particular gene. In one instance, PRMT5 methylation of arginine 3 in H4 results in gene repression through recruitment of DNA methyltransferase DNMT3B⁷¹. PRMT5 has an unexpected role in myeloproliferative neoplasms, in which the oncogenic forms of the JAK2 kinase phosphorylate PRMT5, inhibiting PRMT5 activity and preventing histone methylation. This loss of PRMT5 activity promotes hematopoietic progenitor cell proliferation and expansion. Thus, one oncogenic mechanism of mutant JAK proteins is to inhibit PRMT5 activity to alter the gene transcription profile and encourage progenitor cell expansion in myeloproliferative neoplasms⁷². This study elegantly demonstrated direct regulation of a histone-modifying enzyme by an activated kinase cascade. Unlike histone acetylation and methylation at Lys residues, it is still unclear how dynamic histone arginine methylation is, that is, whether arginine methylation is turned over by demethylating enzymes, and whether such turnover would be common or only occur selectively at particular times during development. Indeed, if histone arginine methylation is a long-lived epigenetic event similar to that of DNA methylation, regulating the PRMT family of enzymes by signal transduction would be a powerful way to leave a lasting impression on the gene expression signature.

PKC- ζ phosphorylation of NF- κ B controls histone methylation

The nuclear factor- κ B (NF- κ B) signalling pathway is central to cellular immune responses, and is activated by pro-inflammatory cytokines. Consequently, this pathway must be regulated to ensure an effective but controlled response. Indeed, its over reactivity has been implicated in autoimmune disease. Moreover, in cancer, NF- κ B pathway activation by the tumour microenvironment results in the activation of pro-survival genes and an enhanced local inflammatory response, creating a dangerous positive feedback loop. Therefore, mechanisms to properly regulate NF- κ B functions are clinically important. One intricate NF- κ B regulatory mechanism involves crosstalk between histones, histone-modifying enzymes and Lys methylation. Before pathway activation, a fraction of RelA (a component of the NF- κ B transcriptional unit) is located in the nucleus and is monomethylated by SETD6. This methylation event creates a docking site for the histone methyl-transferase GLP, and GLP subsequently methylates Lys9 of H3 to repress NF- κ B target gene expression. Upon pathway stimulation by tumour necrosis factor (TNF), RelA is phosphorylated by PKC- ζ on a residue that lies adjacent to the methylation site. This event abrogates GLP binding, allowing demethylation of H3K9 and NF- κ B target gene activation⁷³. Thus, pathway stimulation by an inflammatory response can change the methylation state of chromatin, and therefore transcriptional responsiveness, by regulating histone methyltransferase recruitment to chromatin via crosstalk between post-translational modifications on a classic transcription factor.

Calcium signalling control of histone variant deposition

How are the experiences of an organism converted and transformed into changes in neuronal morphology, neuronal responsiveness and neural wiring? This question has been intensely investigated for over a century. Chromatin-modifying enzymes are emerging as critical factors during brain formation *in utero* and perinatally, as many mutations causing neurodevelopmental disorders reside in genes encoding histone modifying enzymes (BOX 1). In post-mitotic neurons, long-term memory formation requires transcription and new protein synthesis after neuron stimulation. One emerging theory is that the chromatin of neurons is slightly restructured after each stimulation event (or organismal experience), providing a mechanism for tuning that neuron to a precise function within the brain circuitry and retaining heightened sensitivity to a particular stimulus. Indeed, histone acetylation is required in the brain for training-induced memory formation⁷⁴ (for a review of chromatin modification in the central nervous system and during memory formation, see reference⁷⁵). Upon neuronal activation, a rapid wave of gene expression is induced⁷⁶. This activation of immediate-early genes (IEGs) and subsequent protein translation are required for many types of memory formation *in vivo*⁷⁷. Chromatin regulation during this process is partially characterized, and includes calcium signalling-induced phosphorylation of key transcription factors such as cAMP-responsive element-binding protein (CREB) as well as activation of their associated cofactors.

Histone variant deposition provides another level of regulation in neuronal signalling. Histone variant H3.3 is deposited in a replication-independent manner, as opposed to H3.1⁷⁸, and therefore is predicted to be the primary histone that is newly deposited in post-mitotic neurons. After neuronal stimulation, calcium-dependent signalling leads to the

dephosphorylation of DAXX, an H3.3 chaperone. This increases DAXX binding to H3.3 and DAXX-dependent deposition of H3.3 after transcriptional initiation at select IEG regulatory regions, ultimately allowing full activation of these genes⁷⁹. Although all IEGs tested have increased deposition of H3.3 after activation, some events are independent of DAXX, implying that other H3.3 chaperones also regulate this pathway. Another outstanding question is how DAXX, and presumably the other H3.3 chaperones, are targeted to IEGs after neuronal stimulation. Importantly, the deposition of H3.3 is critical for cellular archival of active gene regions in the absence of transcription⁸⁰. This may prove essential for neurological memory, as long periods of time may pass between retrieval of a particular memory. It will be important to determine how neuronal signalling events coordinate histone variant deposition, and what the contribution and significance of variant deposition is for neuronal function, learning and memory.

Regulation through histone mimics

Signalling to chromatin requires that environmental information is transmitted not only to nucleosomes, but also to chromatin-associated factors, as discussed above. It is now becoming clear that some chromatin-associated factors are modified and 'read' by the same protein complexes that work on nucleosomes. Of particular relevance, simultaneous binding of reader proteins to similarly modified motifs provides an opportunity for signalling-mediated control. Combinatorial domain binding to chromatin is frequent, and may be more common in vivo than individual domain binding to a single chromatin modification. Indeed, many chromatin-associated proteins have multiple reader domains, including the chromatin-modifying enzymes themselves (FIG. 1). In addition to multiple chromatin-binding domains, many chromatin regulators are found as homodimers in their associated protein complexes^{81, 82}. This common feature suggests that protein complexes can recognize multiple histone molecules and/or non-histone proteins simultaneously, especially if the same motif within multiple proteins is modified in a similar way. This idea of modification cassettes was posited in 2003, and is now gaining support⁸³. For example, the function of a protein complex may be partly inhibited until the appropriate modifications have been added to multiple proteins or histones, allowing cellular events to be coupled with changes in chromatin. Basic local alignment search tools predict that a number of nuclear proteins have plausible histone-mimic motifs (FIG. 4A). It will be interesting to determine whether some of these proteins are co-modified with histones to synchronize cellular events. Alternatively, histone mimic modification may also create 'dummy' motifs that compete with 'reading' of the histone tails. Indeed, compounds that are structurally similar to modified histone tail motifs can selectively block reader domains from binding their targets on chromatin⁸⁴, and an influenza virus protein (NS1) that harbours an H3-like motif can 'siphon' away the human transcription machinery from chromatin and suppress the antiviral response⁸⁵.

G9A and GLP contain H3-like motifs

The methyltransferase G9A auto-methylates K165 within the same ARKS/T motif as its H3 target residue, Lys9^{86, 87} (FIG. 4b). Consistent with this, automethylation creates a docking site for chromo-domain-containing HP1 γ and this interaction has similar binding kinetics to that between HP1 γ and H3K9me3. In addition to Lys165 methylation, Thr166 is

phosphorylated *in vitro*, suggesting that this sequence within G9A is a hotspot for modifications. The function of G9A auto-methylation is unclear; mutation of this residue to alanine does not alter target gene transcription. Perhaps the G9A H3-like motif supports complex assembly. Indeed, GLP, a mammalian homologue and heterodimerization partner of G9A⁸⁸, automethylates at Lys205 to create a docking site for another chromo-domain-containing protein, MPP8. A MPP8 dimer then simultaneously binds methylated GLP and methylated DNMT3A (a *de novo* DNA methyltransferase) to tether two transcriptionally repressive events, namely H3K9 methylation and DNA methylation, to the same complex for tight co-regulation⁸². So, loss of the methyl-motif within G9A or GLP might result in gene expression changes linked to *de novo* DNA methylation (FIG. 4c).

P53 contains an H4-like motif

P53 is the most extensively studied tumour suppressor, and with good reason. This transcription factor is mutated or lost in up to 80% of cancers. P53 is crucial for diverse cell biological processes, including cell cycle control, DNA repair, metabolism, senescence, apoptosis, autophagy and necrosis. For p53 to function effectively at the right time and place, it must be tightly regulated. One mechanism by which p53 is controlled is through post-translational modification of its C-terminal regulatory domain (CTD). Although the precise function of the C-terminus of p53 has been debated, it is clearly required for p53-mediated transcription of naked DNA and chromatin templates⁸⁹. Interestingly, a stretch within p53 CTD looks similar to a motif within the tail of H4 (FIG. 4b). Indeed, this histone mimic sequence in p53, its multiple modifications by histone-modifying enzymes, and subsequent association of reader domains at this site is one of the best characterized models of a histone mimic^{90–92} (for a review, see reference⁹³). Interestingly, the crystal structure of PHF20 (PHD finger protein 20), a reader molecule that associates with methylated p53, has been solved. Supporting the idea that protein dimerization bridges two histone or histone-mimic motifs together, PHF20 forms a homo-dimer that has a greatly enhanced capacity to bind p53 modified on two different lysine residues, simultaneously, as opposed to a single modification⁹⁴. PHF20 works as a co-activator with the histone acetyltransferase MOF⁹⁵, and together they enhance the transcription factor function of p53⁹⁶, suggesting that double modification at the p53 CTD to allow PHF20 binding could greatly enhance p53 activity. One possible explanation for disparate reports of the p53 CTD functions and its importance may be because it is indeed an extensively modified region. Similarly to modifications of histone tails, each modification pattern on p53 might have a different downstream consequence.

The RNA Polymerase II C-terminal domain as a PTM hub

Although it is not an apparent histone mimic by sequence, the C-terminal regulatory domain of RNA polymerase II is a critical regulation module for controlling transcription initiation and elongation that, similarly to the histone tails, undergoes extensive post-translational modification⁹⁷. This may have possible implications for how transcription is regulated through coordinated control of both chromatin and Pol II. The established model is that the Pol II CTD is phosphorylated on different serine residues within a 52 heptad repeat sequence. Phosphorylation at Ser5 is observed on a polymerase that has progressed past the promoter region but remains paused, whereas phosphorylation at Ser2 and Ser5 marks the

elongating form of Pol II⁹⁸. The Pol II CTD is also tyrosine phosphorylated and O-GlcNAcylated, and the downstream consequences of these events are now being elucidated. Tyr1 phosphorylation, for example, blocks the tethering of termination factors to Pol II while in gene bodies, and so ensures that termination occurs only after transcription is complete^{99, 100}. O-GlcNAcylation of promoter-bound Pol II may temporarily oppose the phosphorylation events required for elongation¹⁰¹, an event that could be responsive to metabolic glucose levels.

There are some exceptional residues within the CTD repeat that differ from its canonical YSPTSPS sequence. Interestingly, this variation usually involves a lysine or arginine substitution in the Ser7 spot, providing the option for lysine ubiquitylation and lysine or arginine methylation. Indeed, arginine in the CTD is methylated by CARM1 and this recruits a methyl-arginine reader molecule TDRD3¹⁰². TDRD3 also binds methyl-arginines on histone tails (H3R17me2 and H4R3me2), and in so doing acts as a strong transcriptional coactivator¹⁰³. It remains to be determined whether more than one TDRD3 molecule exists within a single complex that could bridge methylated histone to methylated Pol II. This example may typify a common mechanism, whereby enzymes co-modify chromatin and Pol II in response to a cellular signal to synchronously coordinate a transcriptional event between chromatin and transcription factors. Supporting this theory, it has been shown that in response to serum stimulation, the CTD of RNA Pol II is phosphorylated by p-TEFb and this requires that H3S10 first be phosphorylated by PIM1 kinase³¹. Together, these findings paint an image of a histone-tail like region on Pol II, where a dense platform of PTMs precisely controls polymerase function.

Conclusion and perspectives

There have been many descriptions of how the chromatin landscape is ‘stamped, erased and transmitted’, but when this occurs physiologically is unclear. As we have discussed, one emerging mechanism is direct communication between signalling molecules and the chromatin-modifying machinery. Activated kinases, for example, can directly modify histone tail residues to switch the functional read-out and thereby influence transcription. Activated cascades can also modify methylating enzymes, remodelers and histone chaperones to change their activity on chromatin. However, the evidence to support this has been largely obtained using cell-based model systems. *In vivo* demonstrations of signal-induced chromatin alterations is still lacking, and is key for tying our current mechanistic knowledge together with that of organismal physiology. In the future, it may prove fruitful to further scrutinize well-characterized *in vivo* models of particular signalling pathways (such as the insulin pathway) for temporal changes in chromatin landscape, structure, occupancy and activity in response to stimulus or pharmacological inhibition. In particular, a further understanding into how different metabolic states influence the activities and modifications on chromatin *in vivo* would be especially timely considering the increasing rates of metabolic disease and type II diabetes in the western world.

Signalling events occurring throughout the lifetime of a cell can be integrated and stored on chromatin to influence the amplitude of gene expression. A key task for the future is to define which chromatin-altering signalling events are transient and which are truly

epigenetic (that is, allowing a heritable change in gene expression). The developmental stage at which a signal is initiated most certainly influences the extent of the epigenetic engagement it triggers. For example, during different stages, the expression level or cellular localization of epigenetic regulators may change their responsiveness to signal-lig pathways. Increased communication between the environment and our epigenomes is observed clinically as developmental windows of plasticity or susceptibility, occurring during embryogenesis, childhood and ado-lescence, when environmental factors or situations are more likely to have an impact on the health or develop-ment of the person. The effects of environmental expo-sure during these critical periods are also more likely to persist into adulthood. If the molecular mechanisms that define windows of epigenetic engagement can be better characterized, this knowledge may in turn increase the possibility of artificially recreating such windows for more effective treatment of developmental disorders.

Signal transduction resulting in epigenetic changes fixes a cellular experience into the epigenome. As is true for many pathological situations, the exploitation of these same epigenetic mechanisms can lead to disease later in life. For example, exposure to dietary restric-tions early in life results in increased susceptibility for various adult diseases ^{104, 105}¹⁰⁶, and traumatic events result in post-traumatic stress disorder (PTSD) ^{107, 108}. At the cellular level, cancer cells responding to growth factors capitalize on the pathways that feed into epigenetic regulation to convert themselves into a more stem cell-like state with greater proliferation potential and also adjust their programmed metabolic needs to tolerate limited oxygen and nutrient supply. Therefore, the elucidation of how epigenetic mechanisms respond to and feed back to the environment is crucial for fully understanding development, trans-generational effects of short-lived environmental occurrences and disease pathology.

Glossary

Writer	Enzyme with the ability to add chemical modifications to DNA or histones.
Eraser	Enzyme with the ability to remove chemical modifications from DNA or histones.
Reader	A protein that binds histones or DNA only when certain chemical modifications are present (or absent). They often contain similar protein domains for recognizing similar modifications; for example, many chromodomain containing proteins bind to di- or trimethylated Lys 9 on histone H3.
Histone variants	Multiple genes encode for each histone family (H1, H3, H4, H2A and H2B), resulting in family members with slight sequence variation (for example, H3.1 and H3.3). The subtle differences between family members can lead to different functional outcomes once incorporated into nucleosomes, and therefore histone variant utilization offers another level of control for transcription, replication and DNA repair.

Nucleosome remodellers	ATP-dependent enzymes that catalyse a shift in the nucleosome position relative to the DNA sequence. The various families of remodellers use slightly different mechanisms to facilitate nucleosome repositioning.
Histone chaperones	Proteins that associate with histones to facilitate a number of functions, including histone transfer, deposition into or eviction from chromatin, histone variant utilization and storage.
Bivalent Domains	Chromatin regions that harbour 'active' and 'repressive' histone modifications. Bivalent domains are thought to mark genes that are expressed at low levels only, but that are poised for activation upon a differentiation cue or other signalling events.
Primary response genes	(PRGs). A gene class that is rapidly induced upon cellular stimulation. Transcription of this gene class occurs independently of new protein production, via proteins that are already present in the cell nucleus.

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Biographies

Yang Shi received his PhD degree from NYU and postdoctoral training at Princeton. His lab at Harvard focuses on histone methylation and recognition and their roles in human diseases such as cancer and neurological disorders.

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Online summary

- The histone tails contain a plethora of posttranslational modification (PTM) sites, and are modified in a variety of ways. One explanation for this exceptionally dense region of PTMs within chromatin is to provide a platform for signal integration and storage.
- Histone phosphorylation appears to be a mechanism for rapid alteration of the epigenetic state. Phosphorylation can promote the binding of proteins to chromatin as well as antagonize the readout, deposition or removal of longer-lived histone tail PTMs.
- Metabolic states may directly regulate the activity of many chromatin-modifying enzymes.
- How activated signalling cascades control chromatin regulators is poorly understood. The best-characterized examples so far include direct regulation of the chromatin machinery through phosphorylation/dephosphorylation of enzymes and histone chaperones to tailor their activity.
- Signal storage and output in and from chromatin may work like a biological digital-to-analog converter, taking on/off signals received over a time course and integrating them to control the amplitude of gene transcription.
- During critical developmental periods, an organism is more sensitive to environmental inputs. This is partially due to increased communication between activated signalling pathways and the epigenetic regulators, as well as an increased ability to lay down long-lived epigenetic marks.

Box 1 | Depositing and maintaining the proper methyl-balance during neurodevelopment

Many different genetic mutations have been linked to neurodevelopmental disorders, such as autism and intellectual disability. Interestingly, a large number of causative genetic mutations have been found in genes encoding enzymes that catalyse the addition or removal of the trimethylation mark of Lys4 on H3 (H3K4me3) and H3K9me2^{109, 110, 111–115} (see the table). Histones at the promoter regions of transcriptionally active genes are marked with H3K4me3. Conversely, as developmental genes become silenced during differentiation, they are marked with H3K9me2. These histone markings do not just correlate with the transcriptional status but also functionally oppose each other by providing docking platforms for effector proteins and thereby contribute to gene expression control. The disease associated mutations highlighted here strongly suggest that the balance between two functionally opposing histone methylation marks, and perhaps uncharted methylation events on non-histone proteins, is important during neurodevelopment. One possibility is that epigenetic events such as this are integral in the precise repression kinetics of developmental and cell cycle genes as neurons exit the cell cycle and pattern the brain. Furthermore, in mature neurons, direct signalling to the enzymes that control these marks after synaptic transmission and membrane depolarization may be a crucial step in the establishment of individualized chromatin landscapes, influenced by the particular fate and experience of each neuron.

Gene	Protein function	Disease caused by mutation
<i>PHF8</i>	H3K9me1, H3K9me2, H3K27me1 and H4K20me1 demethylase	Intellectual, disability
<i>KDM4C</i>	H3K9me2 and H3K9me3 demethylase	Autism
<i>MLL3</i>	H3K4 methyltransferase	Autism, intellectual disability
<i>EHMT1</i>	H3K9me2 methyltransferase	Autism, intellectual disability, Kleefta syndrome
<i>KDM5C</i>	H3K4me2 and H3K4me3 demethylase	Autism, intellectual disability

EHMT1, euchromatic histone methyltransferase 1; KDM, Lys-specific demethylase; MLL3, mixed-lineage leukaemia 3; PHF8, PHD finger 8.

Figure 1. The histone tails act as a signal storage and converter device

Signals from multiple inputs occurring over time, which are ‘on’ or ‘off’ in nature, can be stored on chromatin through post-translational modifications (PTMs) of histone tails. These inputs may affect the chromatin directly or be transmitted through epigenetic enzymes, including factors that ‘write’ or ‘erase’ particular marks on histones and chromatin-remodelling factors. The chromatin landscape is constantly interpreted by ‘reader’ proteins, which are comprised of various domains. This ‘signal interpretation’ involves a collage of mechanisms whereby the marks present on histones, either directly or through crosstalk, recruit reader proteins and additional enzymes. The crosstalk between different histone modifications can have several effects including: changing the ability of a reader protein to recognize an adjacent mark; recruiting or precluding enzymes that modify additional sites; or creating a combinatorial display for recognition in multivalent binding events. Together, these outcomes can influence the amplitude of a continuous wave of transcriptional output. Ac, acetylation; Me, methylation; P, phosphorylation.

Figure 2. Possible modes of signalling to chromatin

Activated signalling cascades can alter the epigenetic landscape directly or indirectly by communicating with chromatin regulators. **a**, Metabolites and nutrient availability influence the activity of many enzymes regulating the epigenome, as these small molecules are used as cofactors. Foreexample, oxygen is required for the activity of the JumonjiC (JMJC) family of Lys demethylases. The extent to which a hypoxic environment inhibits the various JMJC demethylases is not known. Hypoxia can lead to histone acetylation around hypoxia-inducible factor 1 α (HIF1 α) and HIF1 β target genes via the recruitment of p300. Dietary Met is a precursor of the methyl donor *S*-adenosylmethionine (SAM), which is required for DNA, Lys or Arg methylation (Me) events, and glucose metabolism through the tricarboxylic acid (TCA) cycle produces α -ketoglutarate, which is also required for JMJC demethylase activity. Glucose metabolism through the hexosamine biosynthetic pathway produces uridine diphosphate *N*-acetylglucosamine (UDP-GlcNAc), which is used by *O*-linked β -D-*N*-acetylglucosamine transferase (OGT) to trigger *O*-GlcNAcylation of enzymes

that regulate chromatin modification, histones and transcription factors (TFs). Acetyl-CoA that is produced from citrate exiting the TCA cycle also provides the donor group for protein acetylation (Ac). **b**, A simplified and generic kinase cascade that collides with chromatin modifying factors in the nucleus. Signal-induced post-translational modifications (PTMs) of enzymes (such as phosphorylation) may alter enzyme activity in modifying histone or DNA residues. Enzyme modification of ATP-dependent chromatin remodellers can also alter their effects on nucleosome repositioning, changing the modification landscape or DNA accessibility around particular gene promoters or enhancers. Controlling enhancer activity or responsiveness to signals through nearby histone tail modifications and transcription factor binding site accessibility may be a crucial process in cell fate decisions, as enhancer utilization drives tissue-specific gene expression¹¹⁶. Phosphorylation–dephosphorylation events on histone chaperones can alter their ability to deposit histone variants into functionally important genomic areas, such as around the transcriptional start sites of genes. Reader proteins may also be direct targets of signalling cascades; changing the ability of these proteins to recognize the PTMs on chromatin and therefore the recruitment of additional regulators can completely switch the biological consequence of a chromatin modification. Another mechanism at work is complex nucleation initiated by a signalling event. In this case, one or a few proteins are maintained on or nearby their target genes, poised but not active, and acting as a ‘placeholder’ for rapid induction. Upon a signalling event, the placeholders nucleate the remaining protein complex and a function is executed. Due to the rapid nature of this response, complex nucleation may be most prevalent during **primary response gene** (PRG) induction.

Figure 3. AKT signalling to chromatin-modifying enzymes

Chromatin-modifying enzymes are emerging as a new class of AKT phosphorylation targets. **A**, The AKT signalling cascade is induced by ligand binding and subsequent activation of receptor Tyr kinases, cytokine receptors and integrin receptors, to name a few. This results in activation of PI3K at the intracellular domain of the receptor, followed by PI3K-induced production of phosphatidylinositol-3,4,5-triphosphates (PtdIns(3,4,5)P₃). PtdIns(3,4,5)P₃ production creates docking sites for phosphoinositide-dependent kinase 1 (PDK1) and AKT, thus allowing phosphorylation and activation of AKT by PDK1. Many cellular pathways are regulated by AKT signalling. Depicted are epigenetic enzymes that are targeted by AKT, including the histone methyltransferase EZH2, the histone ubiquitylating enzyme BMI1, the histone acetyltransferase p300 and the DNA methyltransferase DNMT1. AKT-mediated phosphorylation of these enzymes may have several effects, for example altering catalytic activity in the case of EZH2 and p300, altering protein stability or retention at the replication fork in the case of DNMT1, or increasing the ubiquitin ligase activity of BMI1 towards H2A after DNA damage. **B**, Other chromatin-modifying enzymes contain AKT target motifs (that is; R-X-R-X-X-S/T; Arg residues are boxed in blue and Ser/Thr

residues in orange). Listed are potential phosphorylation sites for KDM4B (a demethylase for histone H3 Lys 9 dimethylation (H3K9me2) or H3K36me3), SET1A (a H3K4 methyltransferase) and DNMT3B (a *denovo* DNA methyltransferase). Although more enzymes contain putative AKT sites, each of these highlighted sites has been identified multiple times as phosphorylated peptides in large proteomic studies aimed at identifying new AKT targets ([PhosphoSitePlus](#)). **c**, The modification state of H3K27 is one major chromatin feature that is highly predictive of enhancer activity. Methylation at this residue by EZH2 marks an inactive, and possibly poised, enhancer, whereas acetylation of H3K27 by p300 marks an active enhancer that is engaged in transcription with promoter regions. AKT phosphorylation of EZH2 limits its ability to methylate H3K27, whereas AKT phosphorylation of p300 promotes transactivation.

Figure 4. Histone-mimic sequences may be an integral part of signal-induced chromatin-mediated processes

A, The amino-terminal tails of histones H3 and H4 are depicted. Basic local alignment search tools predict that several nuclear proteins contain plausible histone-mimic motifs, and notable examples are shown here. Residues that are common with H3 or H4 are depicted in pink. These highly conserved histone tail-like sequences illustrate that it is likely that multiple non-histone proteins modulating chromatin are subject to modification by the same enzymes that modify the histone tails. **B**, Histone-mimic sequences have been well-characterized within the methyltransferase G9A and the tumour suppressor p53. Examples of respective homologous protein motifs are shown and the common residues highlighted in yellow. **C**, The reader protein MPP8 (M phase phosphoprotein 8) can recognize methylated histone-mimic motifs within the histone methyltransferase GLP and the *de novo* DNA methyltransferase DNMT3A, linking dimethylation of Lys9 on histone H3 (H3K9me2) with *denovo* DNA methylation by DNMT3A. One possibility is that a single signalling event might trigger simultaneous automethylation of GLP and methylation of DNMT3A to coordinate histone and DNA methylation events in close vicinity. ATF7IP, activating transcription factor 7-interacting protein 1; BRWD3, bromodomain and WD repeat-containing protein 3; CREBZF, CREB/ATF bZIP transcription factor; DHX34, DEAH box protein 34; FMR2, fragile X mental retardation 2; FOXC2, forkhead box C2; MLL, mixed-lineage leukaemia; TAF11, transcription initiation factor TFIID subunit 11; ZNF, zinc-finger.

Table 1

Direct signal-induced PTMs on chromatin-modifying enzymes

Chromatin-modifying Enzyme	Histone or DNA Substrate	Sites of PTM and Enzyme Responsible (if known)	Functional Consequence of modification event
Su(Var)3-9 (<i>D. melanogaster</i>)	H3K9	Ser191 phosphorylation by JIL1 ¹¹⁷	Unknown
EHMT2/G9A/KMT1C	H3K9	Lys171me2 and Lys242me2 automethylation ¹¹⁸	Promotes binding of G9A to HP1
EHMT1/GLP	H3K9	Lys205me ⁸² automethylation	Regulates complex assembly with MPP8
ESET/SETDB1/KMT1E	H3K9	Ser1066 phosphorylation by CDK2 ¹¹⁹	Unknown
MII1/KMT2A	H3K4	Ser516 phosphorylation by ATR ¹²⁰	MII1 stabilization and promotion of S-phase checkpoint
MII2/KMT2B	H3K4	Thr440 O-GlcNAcylation by ¹¹⁹	Unknown
MII5/KMT2E	H3K4	Thr440 O-GlcNAcylation by OGT ⁴⁶	Increased catalytic activity of MII5
Pr-Set7/8/KMT5A	H4K20	Ser100 phosphorylation by CDK1; Ser29 phosphorylation by CDK1/cyclinB ¹²¹ ; Lys151 and 172 SUMOylation ¹²²	Cell cycle regulation (Pr-Set7/8phosphorylation); important for repressive function (sumoylation)
EZH2/KMT6	H3K27	Ser21 phosphorylation by AKT ¹²³ ; • Thr350 phosphorylation by CDK1 and CDK2 ¹²⁴ ; Thr487 phosphorylation by CDK1 ¹²⁵ ; Thr372 phosphorylation by p38 ¹²⁶	Phosphorylation of EZH2 suppresses enzymatic activity important for repressive activities; blocks EZH2 interaction with SUZ12; promotes complex assembly
Lysine Demethylases			
PHF2	H3K9me2	Ser655 phosphorylation by PKA ¹²⁷	Promotes PHF2 binding to ARID5B and H3K9me2 demethylation
PHF8	H4K20me1, H3K9me1/2 H3K27me1/2	Ser69 and Ser120 phosphorylation by CDK1 ¹²⁸	Cell cycle regulation
Arginine Methyltransferases			
PRMT4	H3R17	S217p ¹²⁹ ; S228p ¹³⁰	Reduced PRMT4 activity, possibly during mitosis; Reduced PRMT4 activity and homo-dimerization
PRMT5	H4R3	Tyr297, Tyr304 and Tyr307 phosphorylation by JAK2 ^{V617F} ⁷²	Reduced PRMT5 activity on histones
DNA Methyltransferases			
DNMT1	Cytosine, hemimethylated DNA	Lys142me1 by Set7; Ser143 phosphorylation by AKT ⁵⁶ ; Lys148, Lys194, Lys739, Lys957, Lys1135, Lys1609 SUMOylation; Ser146 phosphorylation (mouse) by Ck1d/e; Ser154 phosphorylation	DNMT1 stability and abundance
DNMT3A	Cytosine, <i>de novo</i> methylation	Lys44me2 (mouse) and Lys47me2 (human) ⁸²	Promotes DNMT3 binding to MPP8 and bridging to G9A/GLP

Chromatin-modifying Enzyme	Histone or DNA Substrate	Sites of PTM and Enzyme Responsible (if known)	Functional Consequence of modification event
ATP-dependent Chromatin Remodelers			
BRM	N/A	Lys1540, Lys1542, Lys1549 and Lys1551 acetylation ¹³¹	Dampens BRM-mediated transcriptional activation
CHD4/Mi2a	N/A	Thr529 phosphorylation ¹¹⁹ by CDK2; Ser1349 phosphorylation ⁹² by ATM	DNA damage response
CHD7	N/A	Lys119 SUMOylation ¹³²	Unknown
CHD8	N/A	Lys592 SUMOylation ¹³³	Unknown

Key: p -- phosphorylation, s -- sumoylation, me -- methylation, a -- acetylation, GlcNAc -- N-Acetylglucosamine