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Chromatin-modifying enzymes as therapeutic targets – Part 2

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Abstract

Background—Part 1 of this review described the importance of histone acetylases, deacetylases, methylases and demethylases in transcriptional control and their potential as therapeutic targets. However, precise gene regulation requires the involvement of more than just the addition or removal of acetyl and methyl groups on histones. Histone phosphorylation, ubiquitylation, SUMOylation and poly-ADP-ribosylation, as well as ATP-dependent nucleosome remodeling complexes, play equally pivotal roles in the maintenance of transcriptional fidelity. Accordingly, the enzymes responsible for these modifications are also misregulated in various disease states.

Objective—To review the complex roles of chromatin-modifying enzymes in gene regulation and to highlight their potential as therapeutic targets.

Methods—This review is based on recent published literature and online resources.

Results/conclusion—In this second and final part of the review, we discuss the importance of these other histone and nucleosome modifying enzymes in gene transcription as well as their therapeutic potential.

Keywords

chromatin; chromatin-modifying enzymes; epigenetics; histones; nucleosome remodelling; phosphorylation; ribosylation; SUMOylation; ubiquitylation

1. Introduction

Gene regulation is a highly active process involving multiple tiers of control. At the level of transcription, the degree of chromatin compaction dictates gene expression. Chromatin itself is a dynamic structure consisting of the cell's DNA tightly wrapped around core histone proteins to form nucleosomes (see part 1 and [1]). Depending on the cellular stimuli, histones can be modified to either loosen chromatin and allow for transcriptional initiation or condense chromatin and deny promoter access. The enzymes which carry out these modifications include histone acetyl transferases (HAT), histone deacetylases (HDAC), histone methyl transferases (HMT), histone demethylases (HDM), kinases, E3 ubiquitin ligases, small ubiquitin-related modifier (SUMO)-cojugating enzymes and ADP-ribosyl transferases (ADPRT). ATP-dependent nucleosome remodeling complexes do not covalently alter histones but they do play a role in regulating DNA accessibility. Together, these enzymes work in concert to compose distinctive signals which can trigger either the recruitment or denial of any number of additional regulatory transcriptional elements.

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Because this high level of precise control is required to maintain transcriptional fidelity, any alteration in regulation can have a profound effect as is seen in many disease states. Along the same lines, researchers have found that manipulating histone-modifying enzymes with agonists or antagonists is a promising therapeutic approach. In Part 1 of this review, we discussed the roles and therapeutic targeting potential of histone-modifying enzymes such as acetyl transferases, deacetylases, methylases and demethylases. Here we will focus on the less well studied histone modifications, such as phosphorylation, ubiquitylation, SUMOylation and ADP-ribosylation, as well as ATP-dependent nucleosome remodeling, and how further elucidation of the mechanisms and outcomes of these modifications may yield superior therapeutic strategies.

2. Phosphorylation

Many cellular processes are controlled, at least in part, by protein phosphorylation. It stands to reason therefore, that kinase-regulated chromatin remodeling also plays a critical role in transcriptional regulation. Phosphorylation adds a single negative charge to the histone, thereby altering chromatin structure and influencing transcription by facilitating interactions with transcription factors and other chromatin machinery. Histone phosphorylation has been linked with transcription, mitosis, DNA repair, apoptosis and chromosome condensation [1]. Prominent among the kinases responsible for this histone-phosphorylation-based regulation are those differentially initiated by mitogens, cytokines and stress, typically via the Ras-mitogen-activated protein kinase (MAPK) pathway. Depending on the stimuli, either single or multiple MAPK cascades may result in gene-specific transcriptional control.

Histones H1, H2A, H2B, H3 and H4 have all been identified as being susceptible to phosphorylation at either serine or threonine residues, although histone H3 N-terminal tail phosphorylation has been characterized the most (Table 1). The distinct phosphorylation pattern on histone H3 plays a role in signaling and targeting *trans*-acting factors to specific promoters, thereby eliciting either transcriptional repression or activation. During mitosis, and following mitogen stimulation, for example, H3 is highly phosphorylated at Ser10 and is associated with condensed chromosomes [2]. On the other hand, phosphorylation of H3-S10 is also highly associated with the induction of immediate-early genes, such as the proto-oncogenes c-fos, c-myc and c-jun [3]. This paradox can be explained by the fact that mitosis-specific H3 phosphorylation also occurs on Ser28 and Thr3, thus generating a different histone code than H3-S10 phosphorylation alone, which occurs during transcriptional activation. In addition, H3-S10 phosphorylation during transcriptional activation promotes H3-K14 acetylation and H3-K4 methylation, thereby allowing for chromatin relaxation [4].

Only a small number of the kinases responsible for the rapid yet transient phosphorylation of H3 have been identified. These include but are not limited to, protein kinase B (PKB)/Akt, ribosomal S6 kinase-2 (Rsk-2), mitogen- and stress-activated protein kinase 1 and 2 (Msk1/2), mixed lineage triple kinase-alpha (MLTK- α) and the aurora kinases. Rsk-2, an AGC kinase family member and a p90^{msk} kinase, is first activated itself via phosphorylation by extracellular-signal-regulated kinase (ERK)1/2 MAPKs. Once activated, its physiological role is debatable. Initial results on Rsk-2, typically involved in proliferation and differentiation, implicated the kinase in EGF-induced phosphorylation of H3-S10, particularly at the c-fos promoter, and revealed that the *rsk-2* gene was mutated in Coffin-Lowry syndrome (CLS), an affliction displaying the same clinical symptoms as Rubinstein-Taybi syndrome [5]. Patients afflicted by CLS, an X-linked disorder, typically display severe psychomotor retardation, facial and digital dysmorphisms and progressive skeletal abnormalities. EGF-induced H3 phosphorylation was found not to occur in fibroblasts from a CLS patient, although this modification could be restored upon the reintroduction of the wild type *Rsk-2* gene [6]. More recent data, however, indicates that Rsk-2 phosphorylates histone H2B and not H3 *in vitro*

[7], and that Rsk-2-deficient CLS cells display normal phosphorylation patterns in response to mitogens. Furthermore, Soloaga *et al.* demonstrated that Msk1/2, also members of the AGC family of kinases, and not Rsk-2, are the primary histone H3 kinases in response to mitogenic and stress stimuli in fibroblasts resulting in rapid immediate-early gene induction [8]. Msk1/2, also initially activated via the Ras-MAPK pathway, are phosphorylated by either ERK1/2 or p38 kinase. Msk1/2-knockout mice have displayed severely reduced levels of H3 phosphorylation and immediate-early gene expression, thus providing additional evidence supporting Msk1/2 as the principal H3 kinases [8]. Despite the fact that the downstream kinase responsible for H3 phosphorylation remains contentious, it is accepted that the cascade is initiated by ERK1/2 and p38 MAPKs, making them potential therapeutic targets.

Aurora kinases are a highly conserved family of serine/threonine phosphokinases, consisting of aurora-A, -B and -C, which physiologically serve to control spindle dynamics, cytokinesis and chromosomal segregation, condensation and orientation [9]. Aurora kinases are essential factors in mitotic programming and aurora-B is the primary kinase responsible for H3 phosphorylation of S10 and S28 during mitosis and meiosis. Aurora-A is actually a better H3 phosphokinase as compared with aurora-B [10], however, aurora-B is regarded as a superior H3-S10 kinase due to their different subcellular localization [11]. Aurora-B-mediated H3-S28 phosphorylation is associated with mitotic chromosome condensation [12], whereas aurora-B mediated H3-S10 phosphorylation is associated with chromosome condensation and segregation as well as initiating pericentromeric heterochromatin.

Because of the cellular importance of these physiological processes, the actions of these kinases are counter-balanced by phosphatases such as protein phosphatase type 1 (PP1) in order to ensure tightly controlled transcriptional regulation. For example, aurora-A interacts with PP1 during mitosis as a mechanism of feedback regulation [13] while aurora-B and PP1 work in concert to regulate chromosome segregation and condensation during cell division [14].

A number of aggressive human cancers display elevated levels of all three aurora kinases leading to defective chromosome segregation and aneuploidy [9,10]. The aurora-A gene is amplified in multiple forms of cancer and its protein is overexpressed in a number of solid tumors [15]. Elevated levels of aurora-B are intimately associated with H3-S10 hyper-phosphorylation and chromosome instability. In colorectal cancer, for example, aurora-B overexpression results in multinuclearity, increased ploidy and cytokinesis malfunction [16].

Additional H3 kinases possessing important roles in both normal physiological processes and in tumorigenesis are MLTK- α and Akt. MLTK- α plays a significant role in neoplastic cell transformation and cancer development by regulating oncogenes such as *c-jun* and *c-myc* [17]. MLTK- α overexpression activates the ERK1/2 and p38 pathways and leads to H3-S28 phosphorylation [18]. The oncogene-activated PI3 kinase/Akt pathway has also been shown to be misregulated in a number of cancers [19].

Environmental carcinogens have been found to act as MAPK-dependent inducers of H3 phosphorylation, resulting in immediate-early gene expression. Arsenite, for example, initially activates Akt1, which in turn phosphorylates H3-S10. Ultraviolet light, another tumor promoter, also induces MAPK pathways, leading to ERK2- or p38 kinase-mediated H3-S10 phosphorylation [20].

Aside from H3, phosphorylation of other histones also contributes to transcriptional regulation and cellular function. Oncoproteins can constitutively activate the Ras-MAPK pathway leading not only to increased steady-state levels of H3 phosphorylation but also to H1 phosphorylation, which plays a critical role in chromosome condensation [21]. Phosphorylation of histone H1 by cyclin-dependent kinase 2 (Cdk2) is associated with cell-cycle progression and typically occurs during transcription or replication when chromatin is relaxed enough to expose this

linker histone [22]. Once phosphorylated, H1 is unable to properly compact and condense chromatin, resulting in unabated transcription, as is the case in ras-transformed cells [21]. In opposition, mammalian Sterile20-like 1 (Mst1)-mediated H2B-S14 phosphorylation is associated with chromatin compaction during apoptosis [23]. In *Drosophila*, Mst1 functions as a tumor suppressor by promoting apoptosis and preventing cell proliferation [24].

Although histone phosphorylation alone may modify nucleosome architecture, H3 phosphorylation is synergistically coupled to histone acetylation, thereby adding additional levels of complexity and control to transcriptional regulation [4]. Combinations of phosphorylated and acetylated histones will display different recruitment signals for transcription factors and regulatory complexes than a singly modified histone. The precise mechanism and physiological roles of this multifaceted cross-talk relationship is not fully understood but two models have been proposed: the synergistic model and the parallel-independent model (Figure 1) [25]. A number of HATs have been found to preferentially acetylate H3-S10 phosphorylated forms of their substrates as opposed to the unmodified forms, thus giving rise to the synergistic model. For example, Msk1/2-mediated H3-S10 phosphorylation enhances the binding affinity of the HAT general control of nuclear-5 (GCN5) to H3, which in turn leads to H3-K14 acetylation and transcriptional activation (Figure 1A) [26]. In the parallel-independent model, H3-S10 phosphorylation does not necessarily inevitably lead to H3-K14 acetylation, and the absence of H3 phosphorylation does not necessarily decrease levels of H3 acetylation (Figure 1B). This model is supported by the observation that, at times under certain conditions, Msk1/2 mutations or inhibition resulting in decreased H3-S10 phosphorylation do not affect levels of histone acetylation.

Phosphorylation and methylation are also believed to be interlinked when it comes to regulating the recruitment of repressive complexes to chromatin, although the precise mechanisms have not been fully elucidated and are partially hypothetical. The binary switch hypothesis is a form of cross-talk based on the fact that most HMT lysine targets which lead to a repressive state are found adjacent to serine or threonine residues (Figure 2) [27]. Therefore, phosphorylation of these serines or threonines will block the binding of repressive complexes to the methylated lysine residues or rather block specific lysine methylation. On the other hand, lysine methylation can also prevent serine or threonine phosphorylation in a similar manner (Figure 2A). For example, the chromosomal destabilization found in colorectal cancer caused by aurora-B overexpression is believed to occur as a result of suppressor of variegation 3-9 homolog 1 (SUV39H1) inhibition [9,16]. Phosphorylation of serine 10 on H3 prohibits SUV39H1-mediated H3-K9 methylation (Figure 2B) and subsequent heterochromatin protein 1 (HP1) recruitment (Figure 2C) [28]. Because HP1 associates with chromosome passenger proteins at the centromere during mitosis, aurora-B-mediated mislocalization of HP1 results in inappropriate chromosomal segregation and improper cytokinesis [9]. Additional data in support of this relationship include the fact that genetic mutations encoding the H3-K9 HMT SUV39H1 increase the levels of H3-S10 phosphorylation in mouse cells [29], and the observation that aurora-B can phosphorylate unmodified or K9-acetylated H3 but not K9-methylated H3 [1]. Additionally, phosphorylated S10 on H3 could theoretically recruit as yet unidentified demethylases (Figure 2D) [25]. The absence of a repressive complex can then lead to the recruitment of HATs which can acetylate and activate transcription. Alternatively, dephosphorylation by protein phosphatases type 2A (PP2A) can reverse the kinase-mediated modification and revert the chromatin back to a repressed state. Phosphorylation can also affect methylation machinery directly as Akt phosphorylates enhancer of zeste homologue 2 (EZH2), thereby suppressing its HMT activity and reducing its affinity for H3, resulting in decreased H3-K27 methylation [30].

Phosphorylation, acetylation and methylation machinery also all function together in a cross-talk tertiary loop (Figure 3). For example, on histone H3, K9 methylation inhibits S10

phosphorylation to repress gene transcription as part of the binary switch (Figure 2). On the other hand, in the case of gene activation, S10 phosphorylation facilitates K4 methylation, inhibits K9 methylation and enhances K14 acetylation, which in turn helps to further inhibit K9 methylation (Figure 3) [4,29,31]. The absence of a repressive methyl group on K9 then allows for K9 acetylation and further chromatin decondensation. These modifications work in concert to compose distinct histone codes, which are recognized by specific chromatin-interacting proteins in order to precisely drive the necessary physiological processes throughout the cell.

Understanding how kinases and phosphatases work together is of the utmost importance in terms of therapy and disease prevention. These enzymes are intimately involved in regulating correct chromosome dynamics and normal cellular proliferation and differentiation, therefore linking their dysregulation to oncogenesis. In fact, H3-S10 phosphorylation is vital for neoplastic cell transformation, thus making H3 and/or the kinases that phosphorylate it prime therapeutic suspects.

A number of kinase inhibitors have been used as pharmacological agents to elucidate the physiological effects of disrupting various Ras–MAPK cascades. Issues involving kinase selectivity, however, have made this task difficult, as most inhibitors are promiscuous, or at best preferentially selective. However, although most of these compounds are not therapeutically relevant, some small molecules are being developed which show some promise. H3-S10 phosphorylation can be indirectly inhibited by SB 203580, a pyridinyl imidazole, which inhibits p38 MAPK with an IC_{50} of 50 nm [32]. Msk1, an H3-S10 kinase, is inhibited by Ro 318220, which was originally developed as a PKC inhibitor with an IC_{50} of 8 nm [33], and by H89, a PKA inhibitor marketed by Calbiochem with an IC_{50} of 120 nm [34]. However, the kinases which have attracted the most attention as candidates for therapeutic inhibition are the aurora kinases. A number of aurora kinase inhibitors have shown promise in preclinical studies [35] and there are presently several in clinical trials including MK0457 (also known as VX-680), MLN8054, AZD1152 and PHA-739358.

MK0457, a 4,6-diaminopyrimidine and one of the first small molecule inhibitors of aurora, has recently entered Phase II clinical trials [36]. MK0457 displays nanomolar efficacy toward all three aurora kinases by targeting the ATP-binding catalytic domain and has been shown to decrease H3-S10 phosphorylation, block cell-cycle progression, induce apoptosis and suppress the growth of leukemic, colon and pancreatic tumors. MK0457 also inhibits Fms-related tyrosine kinase-3, a protein which is overexpressed in acute myelogenous leukemia [37]. MLN8054 is selective for aurora-A and causes abnormal mitotic spindle formation and growth inhibition in a number of cancers and xenograft models [38]. MLN8054 is presently in Phase I clinical trials for the treatment of advanced solid tumors. AZD1152, a quinazoline derivative and selective aurora-B inhibitor in Phase I clinical trials, induced abnormal mitosis, polyploidy, cell death and reduced H3-S10 phosphorylation in cell line and xenograft studies [37,39]. PHA-739358, another non-selective aurora inhibitor, is in Phase II clinical trials for the treatment of chronic myelogenous leukemia [40,41]. The precise mechanisms of action of the majority of these aurora kinase inhibitors are not fully elucidated, however, they are believed to activate p53-dependent postmitotic checkpoints resulting in the disruption of mitosis and cell cycle arrest [42].

3. Ubiquitylation

In comparison to other histone modifications, ubiquitylation is one of the least well understood, however, recent advances have shed light on the increasingly complex role of ubiquitylation and deubiquitylation in transcriptional regulation. Cellular ubiquitylation is now known to not

only be a marker for degradation but can also serve as a signal in a variety of physiological processes [43].

In vivo, H1, H2A, H2B and H3 can all be ubiquitylated at lysine residues; however H2A and H2B ubiquitylation is most common. Typically, histone ubiquitylation is associated with active and 'open' chromatin, however, histone ubiquitylation has been attributed to both transcriptional activation and silencing [44]. Ubiquitylated histones, once believed to affect gene transcription by altering higher-order chromatin structure and exposing DNA due to their size, are now believed to behave as key signals for successive histone modifications by recruiting additional regulatory molecules [45].

In yeast, radiation-resistance protein 6 (Rad6), an E2 ubiquitin conjugating enzyme with roles in DNA damage repair, meiosis and protein turnover, is recruited by brefeldin-resistance gene 1 (Bre1) [46], an E3 ubiquitin ligase, to monoubiquitylate H2B at Lysine123. This modification requires the presence of and is regulated by the RNA polymerase II-associated factor (PAF) transcriptional elongation complex [47]. Subsequent SET domain-containing protein 1 (Set1)-, disruptor of telomeric silencing-1 (Dot1)- or complex of proteins associated with Set1 (COMPASS)-mediated H3-K4 and H3-K79 methylation and transcriptional activation is also dependent on this PAF-mediated H2B ubiquitylation (Figure 4) [48]. These observations suggest that there is significant cross-talk between histone ubiquitylation and histone methylation [49]. In fact, rad6 gene deletions, H2BK123R mutations or the presence of ubiquitin-specific protease 8 (Ubp8), a ubiquitin protease and member of the suppressor of Ty (Spt)-adaptor protein (Ada)-GCN5-acetyl transferase (SAGA) complex, will decrease the levels of H3K4 and H3K79 methylation (Figure 4) [50]. Interestingly, decreased H3-K4 methylation does not affect ubiquitylated H2B-K123 levels and histone ubiquitylation is not a requirement for H3-K36 methylation [45,50]. The precise mechanisms by which ubiquitylated histones signal for the methylation of H3 are not known but may involve the presence of Ub-interacting domains on HATs.

In opposition to H2B ubiquitylation, H2A ubiquitylation has been linked to X chromosome inactivation and polycomb silencing [51]. Human polycomb repressive complex 1-like (hPRC1L), an E3 ubiquitin ligase complex containing a number of polycomb-group proteins, monoubiquitylates H2A at Lys119. In addition, targeted depletion of hPRC1L complex members results in the loss of H2A ubiquitylation [52].

Histone deubiquitylation, carried out by ubiquitin proteases, is also a vital histone modification for tightly controlled transcriptional regulation. In fact, recent data demonstrates that sequential ubiquitylation and deubiquitylation are both involved in gene activation [53]. This observation seems counterintuitive as all other reversible histone modifications discussed thus far affect transcription in equal but opposite ways. However, following Rad6-mediated H2B K123 monoubiquitylation and the subsequent methylation of H3, Ubp8 is recruited to H2B to then remove the ubiquitin moiety, a necessary step prior to transcriptional initiation [53]. Furthermore, Ubp8 mutations result in increased levels of H2B ubiquitylation and decreased SAGA-regulated transcription [53].

Presently, little is known about the full potential of ubiquitylation and deubiquitylation to regulate gene transcription but research is ongoing and promising. Therefore, while histone ubiquitylation and deubiquitylation enzymes alone may not represent obvious therapeutic targets, their involvement and requirement in cross-talk with other histone modifications underlines their importance and potential therapeutic relevance.

4. SUMOylation

In a fashion analogous to ubiquitylation, lysine residues can also undergo a related modification upon the addition of small ubiquitin-related modifiers (SUMO). SUMO moieties share only 18% sequence identity to ubiquitin, however, they possess similar secondary and tertiary structural elements. E1 SUMO-activating enzymes [SAE1/activator of sumo1 (Aos1)/sumo activator 1 (Sua1), SAE2/ubiquitin-like modifier activating enzyme 2 (Uba2)], E2 SUMO-conjugating enzymes [ubiquitin conjugating enzyme 9 (Ubc9)] and E3 SUMO ligases [protein inhibitor of activated STAT (PIAS)/PIAS1/PIAS γ (PIASy), ras-related nuclear protein binding protein 2 (RanBP2)] work in cooperation to SUMOylate lysine residues on target proteins, such as histone H4, via a mechanism analogous to that for their ubiquitin counterparts [54]. SUMOylation, though, does not translate into a degradation signal as does ubiquitylation but rather serves solely as a regulatory signal. Furthermore, unlike ubiquitylation, which is typically associated with active transcription, SUMOylation is associated with decreased gene expression [55]. H4 SUMOylation, for example, has been found to increase concomitantly with histone acetylation for the purpose of attenuating gene expression that was first initiated by acetylation [56]. SUMO-modified H4 is believed to induce this repressed chromatin state by enhancing substrate interaction with corepressors such as HDAC and allowing for repressive modifications such as methylation and subsequent recruitment of HP1 [55].

The modification that antagonizes SUMOylation is carried out by isopeptidases. Isopeptidases, such as the human SUMO-1-specific protease (Susp1), act to remove SUMO off of target proteins and are also required for SUMO precursor maturation [57]. Therefore, therapeutically inhibiting isopeptidases may result in decreased SUMOylation.

The targeting of SUMOylation as a therapeutic approach is in its infancy, however, the increasing number of correlations between SUMO machinery and cancer calls for increased attention to this therapeutic target. Ubc9, the only identified E2 SUMO-conjugating enzyme, is the most studied and best characterized protein in the SUMO pathway and is associated with cell-cycle regulation, proliferation, mitosis, apoptosis and DNA repair. It stands to reason, therefore, that Ubc9 and SUMOylation are intimately involved in cell growth and cancer development. In fact, Ubc9 has been found to be upregulated in a number of human cancers including ovarian carcinoma, melanoma and lung adenocarcinoma [58]. Additionally, Ubc9 dominant-negative-expressing tumors are smaller than Ubc9 wild-type-expressing tumors [59]. However, histone H4 is not the only protein SUMOylated in the cell. Other proteins, especially transcription factors, are regulated by SUMOylation. Thus, the cancer-related role of histone H4 SUMOylation is not well understood, though progress is being made.

5. Poly-ADP-ribosylation

Histones may also be modified by the attachment of multiple anionic ADP-ribose monomers. The resultant poly(ADP-ribose) chains are generated by the catalytic action of poly(ADP-ribose) polymerases (PARPs), also known as poly-ADP-ribosyl transferases (poly-ADPRTs). Using NAD⁺ as a substrate, PARPs transfer successive ADP-ribose moieties to glutamic acid or aspartic acid residues of substrate proteins. This results in an increased negative charge that alters the biological properties of the histone and affects condensation of the chromatin fiber. The removal of poly(ADP-ribose) chains is catalyzed by poly-ADP-ribose glycohydrolases (PARGs).

The PARP superfamily of proteins contains 18 members, however, PARP-1 is the most abundant [60]. PARP-1 is involved in transcription, replication, recombination, genomic stability, cell death and DNA-damage signaling and repair. Typically, PARP-1-mediated poly-ADP-ribosylation of histone H1 weakens the H1/DNA interaction, initiates chromatin unfolding and facilitates interactions between promoters and other proteins such as

transcription factors [61]. However, PARP-1 is not always associated with euchromatin. In fact, the transcriptional effects of PARP-1 depend on the surrounding chromatin environment [62]. PARPs also have the ability to poly-ADP-ribosylate the other histones of the nucleosome octamer but less is known about these modifications [63].

Evidence exists suggesting that histone acetylation and poly-ADP-ribosylation can occur synergistically and have the ability to cross-talk [64]. The hypothesis accepted by most is that histone acetylation triggers the recruitment of poly-ADP-ribosylation, which provokes a combinatorial effect on transcription. Data also indicates that HDACs are coupled with mono-ADP-ribosyl transferases (MARTs). In fact, the histone deacetylase activity-possessing sirtuins (SIRT)1/2/6 also display intrinsic MART activity [65]. MARTs will not be discussed further here but have been reviewed recently [63].

PARPs have a significant role in the cellular cytotoxic mechanism and are therefore closely associated with carcinogenesis. Excessive cellular damage initiates a suicidal response, which implicates PARP in both apoptotic and necrotic mechanisms [66,67]. These mechanisms activate chromatin-bound PARP, which depletes the energy metabolite NAD^+ . As a result, the coupled cellular NAD^+ /ATP energy pools are exhausted leading to enhanced cytotoxicity.

A number of cancers including breast, colon, prostate and laryngeal cancer all exhibit dysfunctional PARP regulation. In addition, pharmacological tumor promoters such as phorbol-12-myristate-13-acetate (PMA), benzoyl peroxide (BP), mezerein and 6-keto-lithocholic acid (KA) increased PARP activity in a human epithelial carcinoma cell line [68]. PARP agonists could therefore prove to be effective adjuvants to radiation and/or chemotherapeutic drugs. Alternatively, PARP antagonists inhibit the DNA repair mechanism and thus sensitize the cell to cytotoxic stress [69].

6. ATP-dependent chromatin remodeling

ATPases contained within chromatin remodeling complexes use the energy derived from ATP hydrolysis to non-covalently alter chromatin structure by disrupting histone–DNA interactions, thereby allowing nucleosome sliding or repositioning [70]. Four related ATPase families exist in eukaryotes including mating type switch/sucrose non-fermenting (SWI/SNF), imitation SWI (ISWI), chromodomain-helicase-DNA-binding (CHD) and inositol/choline-responsive element dependent gene activation mutant 80 (INO80), a swi2/snf2 paralogue [71]. These remodeling complexes recruit transcriptional co-activators and co-repressors to DNA promoters in order to control and regulate gene expression.

SWI/SNF family members have been defined the most and are found to interact with a number of oncogenesis-related transcription factors including retinoblastoma (Rb), Myc, p53, myeloid/lymphoid or mixed-lineage leukemia (MLL) and breast cancer 1 (BRCA1) [72]. There are two human SWI/SNF homologs: human brahma/swi2-related gene 1 (hBrg-1) and human brahma (hBrm). Brg-1/Brm-containing complexes have functional roles in transcription, cell-cycle progression and DNA replication and possess the ability to activate transcription, as in the case for p53 [73] and several nuclear receptors [74], and repress transcription, mainly through their interaction with the tumor suppressor Rb [75].

SWI/SNF is deemed a tumor suppressor complex due to the fact that certain necessary subunits are mutated in a variety of cancers [76,77]. Brg-1 and Brm, for example, are downregulated in a number of different human adenocarcinoma cell lines [78]. Additionally, the observations that Brg-1 haploinsufficiency leads to epithelial malignancies and that Brg-1 mutations have been found in a number of cancer cell lines including breast, lung, pancreatic and prostate has led to the suggestion that Brg-1 be considered a tumor suppressor [72]. Mutations in additional SWI/SNF family members, such as proliferation-associated gene (PASG) [79] and alpha

thalassemia/mental retardation syndrome X-linked homolog (ATRX) [80], are also found in a number of leukemias and myelodysplasias, respectively. SNF5/BAF47, a non-catalytic but essential Brg-1 chromatin remodeling complex subunit, has been accepted as a tumor suppressor as mutations of the gene have been found in malignant rhabdoid tumors, chronic myeloid leukemia, pediatric choroid plexus carcinomas, meningiomas, medulloblastomata and primary neuroectodermal tumors [81,82].

Cross-talk between ATPase-mediated nucleosome remodeling and histone modification machinery is vital in transcriptional regulation. Different members of the CHD family of ATPases, for example, are components of the NRD deacetylating complex and SAGA acetyl transferase complex [83]. Additionally, the H3K4 methyltransferase MLL interacts with SNF5/BRG1-associated factors (BAF)47 [84] and H3K4 methylation has been found to recruit and mediate the association of ISWI with chromatin to initiate transcription [85]. Furthermore, acetylation of lysine 56 within the globular domain of histone H3 leads to the recruitment of the SWI/SNF chromatin remodeling complex in yeast [86].

Presently, little work is being done to target these ATPase complexes as a therapeutic approach due to the overwhelming abundance of additional cellular ATP-dependent enzymes and the concern over drug specificity. However, seemingly broad-spectrum kinase inhibitors have been found to exhibit surprisingly specific anticancer effects, some of which have been FDA-approved. Therefore, the diverse and unambiguous roles of ATPases in transcriptional regulation and carcinogenesis require our attention as they represent obvious candidates for future generations of chromatin-modifying enzyme-targeted drugs.

Alternatively, as opposed to targeting ATPase activity directly, one approach to target these ATPase complexes is to manipulate the cellular factors which regulate them. Nuclear hormone receptors (NHR) constitute a large family of transcription factors, which often regulate ATP-dependent chromatin remodeling complexes [87]. NHRs play crucial roles in gene regulation as they control many cellular processes such as reproduction, development, homeostasis, metabolism, growth and energy balance. NHR-dependent transcription is highly reliant on SWI/SNF complexes as first evidenced for glucocorticoid and estrogen receptors [74,88]. One of the best model systems developed to elucidate the NHR-remodeling complex relationship was that for the mouse mammary tumor virus (MMTV) promoter. This model described NR-mediated remodeling and transcriptional activation mechanisms in detail [89]. Upon binding by ligands, such as steroid hormones, vitamin D3, retinoids and other lipophilic molecules, activated NHRs are able to access their response elements in repressed chromatin, which results in the recruitment of ATPase chromatin remodeling complexes as well as histone-modifying-enzyme complexes, followed by transcriptional initiation machinery [90,91]. Each ligand–NHR interaction alters the receptor's conformation in a unique fashion and exposes a distinctive ligand-specific interface capable of interacting with different subunits of chromatin remodeling complexes as well as co-activators or corepressors [90,92]. A number of NHR co-activators possess intrinsic HAT activity, such as p300/cAMP responsive element binding protein (CREB) binding protein (CBP)-associated factor (PCAF)/GCN5 [93,94], p300/CBP [95], TATA binding protein-associated factor_{II}250 (TAF_{II}250) [96] and steroid receptor co-activator-1 (SRC-1) [97]. In addition, many NHR corepressors are complexed with HDACs, such as the nucleosome remodeling and deacetylating (NuRD) complex, the nuclear receptor corepressor (N-CoR) complex and the silencing mediator of retinoid and thyroid hormone receptor (SMRT) complex [83,98-100].

7. Expert opinion and conclusions

The multifaceted nature of transcriptional regulation is complex and not fully elucidated. The synergistic and parallel-independent models describing cross-talk between acetylation and

phosphorylation, the binary switch hypothesis linking methylation and phosphorylation and the tertiary loop relationship connecting all three of these modifications are all vital to the precise mechanisms of gene control. Complicating the issue even more are additional, less well defined cross-talk mechanisms such as those linking methylation with ubiquitylation, acetylation with poly-ADP-ribosylation and acetylation and methylation with ATPase-dependent remodeling complexes. Furthermore, some well-defined chromatin modifying enzymes such as HDACs are being shown to possess additional distinct intrinsic catalytic activity, as is the case with SIRT1/2/6 displaying mono-ADP-ribosyl transferase capabilities.

An additional layer of cross-talk not discussed here which is vital to transcriptional regulation exists between histone modifications and DNA methylation. Histone acetylation and methylation and DNA methylation mechanisms are tightly interwoven. In fact, covalently modified histone residues can recruit DNA methylating or demethylating enzymes and methylated DNA can recruit histone-modifying enzymes.

The potential for modulating chromatin-modifying enzymes as therapeutic targets continues to grow as our knowledge and understanding of their involvement in cellular function and control deepens. The design strategy for future novel inhibitors will greatly benefit from a deeper understanding of the various mechanisms involved. Unidentified correlations and relationships between the different chromatin modifications and their respective enzymes and the elucidation of their upstream signaling pathways will undoubtedly add to this complexity. Chromatin-modifying enzymes geared toward the removal of histone modifications, such as demethylases, phosphatases and ubiquitin proteases, have received relatively little attention and could prove to be therapeutically valuable. Targeting NHR signaling pathways in order to affect the chromatin- and histone-modifying complexes that they govern is another practicable therapeutic approach. Because of the functional roles of combinations of histone modifications, the use of chromatin-remodeling-enzyme agonists, as opposed to hormone antagonists, may also be therapeutically relevant. Combination therapies targeting multiple chromatin-modifying enzymes simultaneously or using these drugs as adjuvants to treatments such as radiation will probably prove to be the preferred therapeutic modality.

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Figure 1. Cross-talk between phosphorylation and acetylation

A. The synergistic model. H3K14 acetylation is enhanced by H3S10 phosphorylation. Enzyme actions are indicated by blue arrows. Induction is indicated by the red arrow. **B.** The parallel-independent model. Lysine acetylation occurs equally on both phosphorylated and unmodified histones.

GCN5: General control of nuclear-5; HDAC: histone deacetylase; Msk1/2: Mitogen- and stress-activated protein kinase 1 and 2; Pptase: Phosphatases.

Figure 2. The binary switch hypothesis

The close proximity of lysine and serine residues on histones gives rise to cross-talk between phosphorylation and methylation. **A.** H3K9 methylation can preclude H3S10 phosphorylation, and vice versa. H3S10 phosphorylation can **(B)** inhibit suppressor of variegation 3–9 homolog 1 (Suv39h1)-mediated methylation, **(C)** block recruitment of heterochromatin protein 1 (HP1) to methylated H3K9, or **(D)** hypothetically recruit demethylating enzymes. Proteins and their directed actions are indicated by blue arrows. Induction and inhibition are indicated in red.

Figure 3. Tertiary cross-talk between acetylation, methylation and phosphorylation

On histone H3, S10 phosphorylation facilitates gene activation by blocking K9 methylation and facilitating K4 methylation and K14 acetylation. K14 acetylation, in turn, serves to further block K9 methylation and allow for chromatin decondensation. Induction and inhibition are indicated in red.

Figure 4. Cross-talk between ubiquitylation and methylation

In yeast, the ubiquitylation of H2BK123 determines the methylation of H3. Ubiquitinated H2BK123 facilitates H3K4 and H3K79 methylation and inhibits H3K36 methylation. Deubiquitinated or unmodified H2BK123, in contrast, inhibits H3K4 and H3K79 methylation and facilitates H3K36 methylation.

Bre1: Brefeldin-resistance gene 1; Dot1: Disruptor of telomeric silencing-1; Rad6: Radiation-resistance protein 6; Set: SET domain-containing protein; Ubp8: Ubiquitin-specific protease 8.

Table 1**Histone kinase specificity**

Histone kinases	Histone substrate specificity
PKB/Akt	H3-S10
Rsk-2	H2B; H3-S10
Msk1/2	H3-S10, -S28
MLTK- α	H3-S28
Aurora-A	H3
Aurora-B	H3-S10, -S28
Cdk2	H1
Mst1	H2B-S14

This table highlights only those histone kinases discussed in the text and is not fully inclusive of all existing histone kinases.

Cdk2: Cyclin-dependent kinase 2; MLTK- α : Mixed lineage triple kinase-alpha; Msk1/2: Mitogen- and stress-activated protein kinase 1 and 2; Mst1: Mammalian Sterile20-like 1; PKB: Protein kinase B; Rsk-2: Ribosomal S6 kinase-2.