HHS Public Access

Author manuscript

Curr Opin Chem Biol. Author manuscript; available in PMC 2018 August 01.

Published in final edited form as:

Curr Opin Chem Biol. 2017 August ; 39: 109–115. doi:10.1016/j.cbpa.2017.05.015.

Allosteric Regulation of Epigenetic Modifying Enzymes

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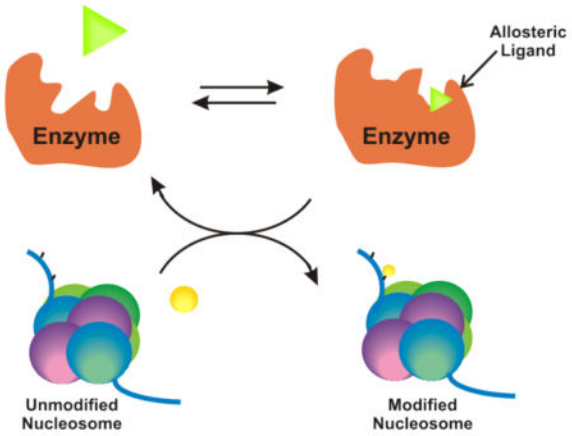
Abstract

Epigenetic enzymes including histone modifying enzymes are key regulators of gene expression in normal and disease processes. Many drug development strategies to target histone modifying enzymes have focused on ligands that bind to enzyme active sites, but allosteric pockets offer potentially attractive opportunities for therapeutic development. Recent biochemical studies have revealed roles for small molecule and peptide ligands binding outside of the active sites in modulating the catalytic activities of histone modifying enzymes. Here we highlight several examples of allosteric regulation of epigenetic enzymes and discuss the biological significance of these findings.

Graphical Abstract



Funding

Introduction

Epigenetic enzymes are pivotal determinants of cell fate by regulating chromatin modifications on both nucleosomal proteins and DNA. These modifications result in changes

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in the “timing” and “volume” of gene expression; and when occurring on histone residues, constitute the proposed histone code. This code of histone modifications is generated by “writers,” interpreted by “readers,” and removed by “erasers.” The most intensively studied writer enzymes include the Lys acetyltransferases and the Lys and Arg methyltransferases. The best understood family of acetyl-Lys readers is the bromodomain containing proteins. There are several classes of methyl-Lys readers including chromodomains, PHD fingers, tudor domains, and MBT proteins. Eraser enzymes for acetyl-Lys belong to two major families, the classical HDACs which are Zn hydrolases and the more chemically unusual NAD-dependent sirtuins. Two major families of Lys demethylases have been identified including the flavin-dependent demethylases and the non-heme iron monoxygenase Jumonji enzymes [1–5].

Within each of these writer, reader, and eraser families are multiple well-characterized examples making the epigenetic machinery intricate and complex [4,6,7]. Moreover, a wide array of acyl chain modifications have been identified recently including propionylation, butyrylation, 2-hydroxyisobutyrylation, succinylation, malonylation, glutarylation, crotonylation and β-hydroxybutyrylation [4,8,9]. Specific modifications on particular histone residues are generally associated with “open” or transcriptionally active gene states while others are associated with “closed” or transcriptionally silent chromatin [7,10,11]. Aberrant activity or mutation of histone modifying enzymes can alter the chromatin structure and gene expression profile contributing to cancer, developmental abnormalities, and other diseases [1,6,7,12].

Understanding how these enzymes are regulated in both normal physiology and disease is of great fundamental importance and may offer therapeutic opportunities. The broad significance of epigenetic writers and readers as factors in disease processes has stimulated researchers to identify and design small molecule modulators of these protein activities. Targeting the enzyme active sites of the writers and erasers has been the primary focus of drug discovery programs. However, given the conserved active sites of many epigenetic enzyme families, achieving specificity for particular enzyme family members can prove challenging [13–15]. In contrast, allosteric modulators of their activities pave the way to unique and specific pharmacologic agents. In addition, dissecting allosteric mechanisms within epigenetic enzymes can facilitate a fundamental understanding of the principles of their biological regulation. Accordingly, the past six years has seen the budding of allosteric regulation of epigenetic enzymes. Lessons from cell signaling enzymes such as protein kinases indicate how various domains and structural features can dramatically impact the activity of phosphoryl transfer. The protein tyrosine kinase Src has served as a paradigm in this regard. In Src, engagement of its SH2 and SH3 adaptor domains by phosphotyrosine and proline-rich ligands can relieve autoinhibition of its catalytic activity [16–18]. Related themes are beginning to emerge in epigenetic modifying enzymes. Below, we describe several examples of epigenetic enzyme allosteric mechanisms and their connection to opportunities in pharmacology.

Allosteric regulation of histone demethylase KDM5A

The retinoblastoma binding protein KDM5A (RBP2, JARID1A) is a histone demethylase that catalyzes the removal of methyl groups from histone H3K4me3 and H3K4me2 [11,19]. KDM5A has been shown to have a role in adipocyte development, osteogenesis, and immunoactivation [20–22]. It has been implicated in numerous cancers, including multiple myeloma, gastric, lung, and breast [23–28]. Like many histone demethylases, the protein KDM5A contains both reader and eraser domains within a single polypeptide. KDM5A contains both a Jumonji (Jmj) catalytic domain and three plant homeodomain (PHD) reader domains. The Jmj enzymes require iron(II) and α-ketoglutarate as cofactors [1,2,29]. In KDM5A as in other KDM5 enzymes, the JmjC domain is preceded indirectly by a JmjN domain which folds with the JmjC domain to form a stable, catalytic core [29–32]. Inserted between the JmjN and JmjC domains in this subclass of Jmj enzymes is the first PHD finger and ARID DNA binding domain (Figure 1). In general, PHD domains recruit methyltransferases and demethylases to chromatin in a sequence/modification specific paradigm. Seemingly promiscuous, PHD domains can bind acetylated, methylated and unmethylated lysines depending on the context [1,33,34]. PHD1 of KDM5A can bind unmodified H3K4 peptide with low micromolar affinity [35], and deletion of PHD1 leads to increased cellular H3K4me3 [36].

Studies with an unmodified H3(1-18) tail peptide demonstrate that the affinity of PHD1 of KDM5A is dependent upon the first four residues of the H3 tail. By studying intact KDM5A protein, it was revealed that the binding of unmodified H3(1-18) by PHD1 stimulates the demethylation of H3K4me3 by the JmjC catalytic domain of KDM5A (Figure 2 and Table 1). This enhancement of catalysis was even more dramatic on homogenously modified H3K4me3 nucleosomes, where the k_{obs} of demethylation increased over 20-fold in the presence of unmodified H3(1-18) peptide. In the current model, binding of the PHD1 of KDM5A to the unmodified tail of H3 on one nucleosome may perform the role of feedback activation through allosteric changes to dramatically enhance demethylation of adjacent nucleosomes by the JmjC domain and propagate spreading of nucleosome demethylation [37]. Such propagation may explain gene cluster silencing as KDM5A is known to silence *Hox* gene clusters which are marked by H3K4 methylation [38,39]. This catalytic link between a reader domain and nucleosome demethylation suggests that these domains structurally communicate although the molecular details remain to be elucidated. This mechanism also raises the possibility that small molecule ligands for PHD1 may provide a tool to inhibit gene expression for genes under the control of KDM5A.

Multi-domain regulation of p300/CBP

p300 (EP300), as well as its paralog CREB-binding protein (CBP), is a promiscuous and prolific lysine acetyltransferase (KAT). p300 and CBP have been shown to play key roles in regulating a myriad of biological processes and diseases [8]. Consistent with its name, p300 is a large protein (264 kDa) with multiple domains (Figure 1). Acetyl transfer from a tightly-bound acetyl-CoA cofactor to the substrate Lys-containing protein or peptide substrate is catalyzed by p300's histone acetyltransferase (HAT) domain via a hit and run catalytic mechanism [8]. For p300, several mechanisms of allosteric regulation have been described.

The HAT domain is preceded by a bromodomain which “reads” PTMs by binding acetylated lysine side chains. Between the bromodomain and the HAT domains are the RING and PHD domains, also known together as the CH2 domain [40]. These have been shown to have inhibitory effects on p300 activity by an unidentified mechanism [40,41]. Also the HAT domain contains an autoacetylation loop (AL) that binds to the substrate binding pocket of the HAT domain, blocking substrate binding. However, upon acetylation of the AL by the HAT active site, the loop is released and

p300 acetylation of target proteins increases [42]. p300 has over 400 known protein-protein interactions and over 100 known protein substrates [8]. One of these is the transcriptional coregulator Mastermind like-1 (MAML1) [43]. Not only does p300 acetylate MAML1, but also the presence of MAML1 increases p300 autoacetylation. This effect is independent of the p300 AL but is dependent upon the subsequent CH3 domain with which MAML1 interacts. The MAML1-p300 interaction also increases the acetylation of the histone H4 tail peptide and the colocalization of p300 and H4-ac in nuclear bodies. Full-length MAML1 dramatically increases the expression of p300 target genes (Figure 2 and Table 1). The potential exists that a portion of the CH3 domain may interact with the HAT domain to inhibit its activity, and MAML1-CH3 binding abrogates this inhibition [44]. This would pose the CH3 domain as a potential drug target in an attempt to make specific activators of p300 acetylation.

The bromodomain of p300/CBP has also been investigated as a regulator of p300/CBP function. For example, after being acetylated by p300/CBP, the tumor suppressor p53-K382ac peptide can bind to the CBP bromodomain and this can enhance p300/CBP recruitment to transcriptional loci [45]. Blocking this interaction with a cyclic p53-K382 peptide, blocks p53 induction of p21 expression [46]. Relatively recently, several high affinity small molecule ligands for the p300/CBP bromodomains have been developed [47–52]. Each of these was shown to induce some cellular phenotype, and several were crystallized bound to the p300/CBP [50–52]. It was further shown that one of the p300/CBP bromodomain ligands, I-CBP112, specifically activates acetylation of H3K18ac in the context of intact nucleosomes (Figure 2 and Table 1). This activation of acetylation is seen both *in vitro* and in cell culture. The requirement of p300/CBP domains beyond the HAT domain and the intact nucleosome substrate points toward a multifaceted interaction shift propagated by I-CBP112, potentially unique to this bromodomain ligand among other small molecules [53]. The importance of the bromodomain to activate p300 acetylation was consistent with an earlier study in which deletion of the bromodomain abolished p300 acetylation of an entire library of nucleosome substrates [54]. Even weakening the bromodomain-Ac-Lys interaction by bromodomain point mutations [55] diminished p300’s propensity to acetylate “primed” nucleosomes (with preexisting H4ac) consistent with a “cooperative acetylation model” [54]. These and other data point to allosteric binding ligands regulating the acetylation activity of the HAT domain. The precise mechanisms of these actions are largely unknown but are areas of active investigation.

Catalytic regulation of Gcn5 by its bromodomain

Gcn5 is a histone acetyltransferase with much more narrow substrate selectivity compared with p300/CBP. Gcn5 has been shown to be especially important for hepatic metabolism and may also be an anti-cancer target [12,56,57]. Like p300/CBP, Gcn5 also possesses a bromodomain (Figure 1), and the role of its bromodomain has been explored in the context of histone site selectivity. As a part of the catalytic ADA complex (Gcn5 + Ada2 + Ada3), Gcn5 acetylates H3 tail lysines with the following specificity: H3K14 > H3K23 > H3K9 ≈ H3K18 > H3K27 > H3K36. However, if the Gcn5 bromodomain is mutated to make it incompetent to bind Ac-Lys, H3K18ac was most severely diminished. Accordingly, the same result was found with H3K14R mutants and wild type Gcn5. Therefore, a model is proposed in which Gcn5 acetylates H3K14 and then this Ac-Lys is bound by the bromodomain of Gcn5. H3K14Ac-Gcn5-bromodomain association then enhances H3K18ac by the Gcn5 HAT domain (Figure 2 and Table 1) [58]. Reminiscent of p300/CBP regulation by I-CBP112 or of KDM5A by unmethylated peptide, the acetylation of H3K18 seems to be susceptible to allosteric regulation. The short distance between H3K14 and H3K18 makes it unlikely that the Gcn5-K14ac association results from orienting the K18 of the same histone tail into the HAT active site [59]. More likely the activation occurs in *trans*. This *trans* acetylation activation model is similar to a proposal for the activation of H3 acetylation when p300 is incubated with nucleosomes already containing penta-acetylated H4 tails or at least one H4 tail acetylation site [54]. The recent reports of potent Gcn5 bromodomain selective ligands [60,61] offer promise to more deeply probe this allostery.

Allosteric inhibitors of PRMT3 arginine methyltransferase

PRMT3 is an example of a protein methyltransferase than methylates the Arg guanidinium sidechain. In general, protein methyltransferases catalyze the transfer of 1–3 methyl groups to either arginine or lysine side chains. Methylation of the terminal (ε) nitrogen of lysine side chains can be mono-, di-, or tri-methylated. Methylation of the guanidinium arginine group can occur as mono-, symmetrical di- or asymmetrical di-methylation [1,13,15]. Beyond the multiple sites on histone tails, over one-thousand cellular proteins have been identified as containing methylated residues (www.phosphosite.org) [62]. The transcriptionally repressed state of heterochromatin and polycomb genes is marked by histone H3 trimethylated at lysine 9 or 27 (H3K9me3 or H3K27me3); whereas H3K4me marks active chromatin regions including enhancers and promoters [2,3,63,64]. The mutation or dysregulation of many protein methyltransferases gives rise to disease, especially cancer and neurological disorders [6,13,15] and therefore poses an enticing pharmacological target.

All protein methyltransferases use *S*-adenosylmethionine (SAM) as the methyl donor. Thus, generating agents that specifically target the SAM binding site of a particular methyltransferase such as PRMT3 may prove challenging. PRMT3 can catalyze mono- or asymmetrical di-methylation. PRMT3 methylates the 40S ribosomal protein S2 (rpS2) which is mandatory for mature 80S formation [65]. Additionally, PRMT3 is associated with oculopharyngeal muscular dystrophy likely via PAPN1 methylation, with cardiomyocyte excitability via sodium voltage channel expression, and methylates the H4 tail *in vitro*

among many other substrates [66–69]. Therefore, inhibitors of PRMT3 have therapeutic potential in disorders involving these pathways.

Like other Type 1 PRMTs, PRMT3 is active as a homodimer (Figure 1) [70]. Using high throughput screening, moderately potent allosteric inhibitors of PRMT3 were discovered. Siaheyeva *et.al.* discovered a urethane compound from a library screen of over 16,000 compounds as a molecule that inhibited PRMT3 methylation of H4 tail peptide (1-24) [71]. After synthetic optimization, SGC707 (Figure 2 and Table 1) was developed as a highly potent and selective PRMT3 inhibitor probe. SGC707 was demonstrated to inhibit the methyltransferase activity of PRMT3. Consistent with earlier generation compounds, PRMT3 was bound over 15 A away from the active site and non-overlapping with the substrate peptide and SAM cofactor binding pockets. SGC707 binds in the large cavity of an inactive PRMT3 conformation between the β-barrel and the homodimerization arm near the dimer interface and likely forces the catalytically regulatory N-terminal α-helix into a conformationally inactive state. Enzymology confirmed the non-competitive inhibition mode. In contrast to parent compounds, SGC707 shows inhibition of H4R3me2a (asymmetric demethylation) in cells with an IC50 that is submicromolar [72]. This model of a small molecule binding to a conformationally dynamic and catalytically regulatory protein domain may be applicable to other epigenetic allosteric ligands.

Summary

KDM5A, p300, Gcn5, and PRMT3 are recent examples of histone modifying enzymes subject to allosteric regulation. In fact, many epigenetic enzymes possess non-catalytic domains whose biochemical functions are not well-characterized and may well participate in allosteric regulation. Emerging chemical tools to investigate allosteric mechanisms offer promise for clarifying biological function and opportunities for therapeutics development. As the catalytic functions and mechanisms of the wider array of histone modifying enzymes are increasingly defined, we believe that a rich tapestry of allosteric modulation will be identified and found actionable for pharmacologic application. Beyond small molecule probes, chemical techniques that allow for post-translational modifications to be introduced site-specifically into proteins [73,74] have the potential to greatly deepen our understanding of epigenetic regulation.

Acknowledgments

Funding: This work was supported by the National Institutes of Health [GM62437] and FAMRI Foundation and the Jane Coffin Childs Memorial Fund (B.E.Z.).

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Highlights

- Allosteric ligands of histone modifying enzymes pose a new drug targeting strategy.
- KDM5A, p300/CBP, Gcn5, and PRMT3 have allosteric ligands that modulate function.
- SGC707, an allosteric ligand of PRMT3, functions by inducing conformational changes.

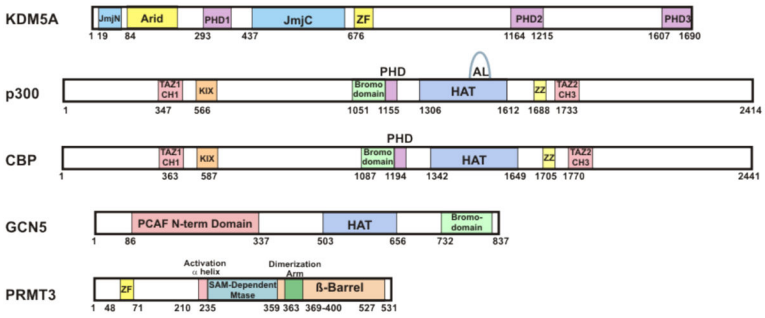

Figure 1.

Protein domains of each of the epigenetic enzymes discussed. Catalytic sites in shades of blue, allosteric ligand interacting domains in shades of green or purple, and DNA interacting regions in yellow. All other domains as labeled.

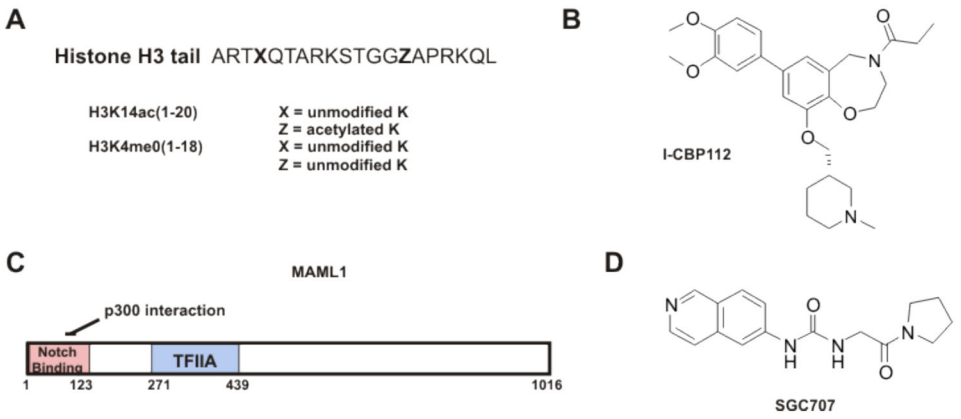

Figure 2.

Allosteric ligands discussed in this review. A) Histone peptides interacting with GCN5 and KDM5A, (un)modified as listed, respectively. B) I-CBP112 is a bromodomain ligand for p300 and CBP. C) MAML1 binds the CH3 domain of p300 to activate enzyme activity. D) SGC707 binds to the dimerization interface of PRMT3 to disengage the activation helix and inhibit activity.

Table 1

Epigenetic enzymes, their allosteric ligands, and functional effects.

Enzyme	Active Site	Ligand	Ligand Binding Site	Ligand Action
KDM5A	JmjC/N	H3(1-18) unmodified	PHD1	activate H3K4me3 demethylation
p300/CBP	HAT	MAML1 Proline-rich region	CH3	increase H4 acetylation and p300 target gene expression
p300/CBP	HAT	CBP112	Bromodomain	increase H3K18ac
GCN5	HAT	H3K14ac(1-18)	Bromodomain	increase H3K18ac
PRMT3	SAM-Dependent MTase	SGC707	Distal interface between SAM-Dependent MTase, α-Helix, and Dimerization Arm	decrease H4R3me2a