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Metabolic Mechanisms of Epigenetic Regulation

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Abstract

Chromatin modifications have been well-established to play a critical role in the regulation of genome function. Many of these modifications are introduced and removed by enzymes that utilize cofactors derived from primary metabolism. Recently, it has been shown that endogenous cofactors and metabolites can regulate the activity of chromatin-modifying enzymes, providing a direct link between the metabolic state of the cell and epigenetics. Here we review metabolic mechanisms of epigenetic regulation with an emphasis on their role in cancer. Focusing on three core mechanisms, we detail and draw parallels between metabolic and chemical strategies to modulate epigenetic signaling, and highlight opportunities for chemical biologists to help shape our knowledge of this emerging phenomenon. Continuing to integrate our understanding of metabolic and genomic regulatory mechanisms may help elucidate the role of nutrition in diseases such as cancer, while also providing a basis for new approaches to modulate epigenetic signaling for therapeutic benefit.

Keywords

epigenetics; metabolism; histones; posttranslational modification; IDH1; 2-hydroxyglutarate; genomics; chromatin

I. Introduction

> "The one-dimensional script of the human genome, shared by essentially all cells in all tissues, contains sufficient information to provide for differentiation of hundreds of different cell types, and the ability to respond to a vast array of internal and external influences. Much of this plasticity results from the carefully orchestrated symphony of transcriptional regulation."
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> -- Lander et al., Nature 2001

The above quote, from a report of the draft sequence of the human genome in 2001, remains a paean to the importance of transcriptional regulation in human biological processes.[1] Transcriptional regulation underlies differentiation, the process responsible for cellular identity,[2] while aberrant transcriptional regulation is a hallmark of many pathological states, including cancer.[3, 4] One way in which transcription and other genomic processes are regulated is by controlling access to DNA through packaging in chromatin. Chromatin consists of DNA wrapped in 147-bp segments around histone octamers to form nucleosomes, and serves to compact the 3×10^9-bp human genome into a nucleus of a few hundred μm^3. Chemical modifications of chromatin, including histone acetylation, histone methylation, and DNA methylation, can alter the dynamics of transcription by regulating the accessibility of the genome and facilitating the binding of trans-acting factors (Figure 1a).[5–7] These chemical modifications of chromatin, the enzymes that implement them, and the phenotypic effects they elicit have all been termed "epigenetic".

Although the precise definition of epigenetic has been the subject of much debate,[8, 9] here we use the term simply to denote molecular events regulating transcription and other genomic functions that do not directly depend on the primary nucleotide sequence of DNA. Whether causal, heritable, or neither,[10, 11] one indisputable fact is that chemical modifications of histones and DNA are biologically relevant. For example, Kouzarides and Dawson recently annotated 40 genes involved in the establishment or recognition of histone modifications that show recurrent mutation in cancer.[12] These genetic errors can lead to the mistargeting or overactivity of chromatin modifiers due to translocation and gain-of-function mutations, and have been found to stimulate cellular transformation and proliferation in diverse cell lineages. [3, 12] Non-mutated chromatin modifiers also play a significant role in cancer, as oncogenic gene expression can demonstrate an absolute requirement for chromatin activities that are dispensable in phenotypically normal cells.[13]

"Big science" initiatives such as ENCODE,[14] the Cancer Genome Atlas,[15] and the Roadmap Epigenomics Project[16] have provided an invaluable service discovering and cataloguing epigenetic drivers of disease. However, manipulating epigenetic processes requires moving from the macro-level of genome function to the micro-level of enzyme activity. At the molecular level, one characteristic that many KAT, HDAC, KMT, KDM, DNMT, and TET enzymes share in common is their use of cofactors derived from primary metabolism (Figure 1b). The binding sites for these cofactors have been used for the design of synthetic inhibitors and cofactor analogues targeting chromatin-modifying enzymes.[17] More recent research has shown that endogenous cofactors and metabolites are also capable of directly influencing chromatin modifications. These studies were in part stimulated by the discovery that mutations in the metabolic enzymes SDH, FH, and IDH can cause cancer by producing metabolites that inhibit the activity of chromatin-modifying enzymes.[18, 19] This has led to the hypothesis that the activity of chromatin-modifying enzymes may serve as central signal integrators, modulating genome function in response to rate-limiting levels of cofactors derived from metabolism. Several recent reviews have persuasively outlined the evidence supporting this hypothesis, and are highly recommended.[20–23] To avoid redundancy, here we approach this topic from a different direction, focusing on the mechanistic basis for metabolic regulation of epigenetics. Limiting our scope to chromatin modifiers that utilize acetyl-CoA, NAD+, SAM, and α-KG as cofactors, we identify three mechanisms by which cellular metabolism can impact the activity of chromatin-modifying enzymes: i) competitive inhibition, ii) cofactor depletion, and iii) subcellular localization of cofactor biosynthesis. We detail case studies for each mechanism with an emphasis on their role in cancer, and then draw parallels between natural mechanisms and strategies devised by chemical biologists to modulate epigenetic states. Finally, we discuss future prospects for chemical biology to advance studies of genomic regulation, as well as to draw inspiration from metabolic strategies for new approaches to the targeted perturbation of genomic processes.

Note: much of this review assumes a working knowledge of chromatin modifications as well as their proposed role in transcription and associated genomic processes. For convenience, a glossary is provided at the end of the text summarizing some of this information. Chromatin-modifying enzyme activities are also defined in Figure 1. Further background and discussion can be found in recent reviews.[24, 25]

II. Regulation of Epigenetic Signaling by Cofactor Competitive Metabolites

The most straightforward mechanism by which metabolism can affect the activity of a chromatin modifier is through the production of a competitive inhibitor. Many chromatin-modifying enzymes are sensitive to feedback inhibition by the cofactor-derived product of their enzymatic reaction. For instance, the KAT enzyme Gcn5 binds acetyl-CoA and CoA with similar dissociation constants (Table 1). Cofactor competitive molecules can be

produced as byproducts of enzymatic reactions that modify macromolecules, or directly as products of rewired metabolic pathways.[26]

A spectacular example of the competitive inhibition mechanism is exhibited in cancers driven by mutations in the metabolic enzymes succinate dehydrogenase (SDH), fumarate hydratase (FH), and isocitrate dehydrogenase (IDH). Each of these mutations results in the cellular accumulation of a metabolite that antagonizes the activity of Fe(II)/α-KG-dependent dioxygenases. SDH and FH-driven cancers are associated with inactivating mutations.[19] In both cases heterozygous germline mutations predispose an individual to cancer, but are not triggered until loss of the second allele occurs. This interrupts TCA cycle turnover and leads to accumulation of succinate and fumarate (Figure 2). SDH and FH-deficient tumor samples contain ~10–20 fold higher levels of succinate and fumarate than wild type tumors. [27] Notably, both metabolites contain a dicarboxylate moiety similar to that of the α-KG cofactor required for prolyl hydroxylase (PHD), KDM, and TET activity.

Early studies centered on the ability of succinate and fumarate to activate the transcription factor HIF-1, a well-known oncogene in hereditary cancers driven by mutations to the Von-Hippel Lindau (*VHL*) tumor suppressor gene.[28] Mechanistically this could proceed by succinate/fumarate inhibition of PHD2, a Fe(II)/α-KG-dependent prolyl hydroxylase whose activity promotes HIF-1 degradation. Indeed, cell-based models of SDH and FH inhibition demonstrated that accumulation of either TCA cycle intermediate could antagonize PHD2 activity, resulting in HIF-1 stabilization.[29, 30] *In vitro* biochemical measurements indicate fumarate and succinate are mid- to high micromolar inhibitors of PHD2 activity, with fumarate possessing greater inhibitory activity than succinate (Table 1).[31]

Despite these compelling findings, more recent evidence suggests succinate and fumarate may exert their oncogenic effects through mechanisms unrelated to PHD2/HIF1. For example, when HIF1 is genetically inactivated in mice engineered to lack *FH*, tumorigenesis is actually accelerated.[32] Similarly, paragangliomas associated with SDH and VHL mutants form two distinct gene expression subgroups, signifying they may function through different mechanisms.[33] Furthermore, yeast deficient for SDH show increased levels H3K36me2, a histone mark normally removed by KDM activity.[34] This same study found that succinate could inhibit human KDM4D *in vitro*, evoking a role for KDM enzymes in SDH-driven cancers. A recent survey of clinical tumor samples found that *SDH*-mutated tumors possess a distinct hypermethylated DNA profile that is conserved across developmentally distinct tumors harboring *SDH* mutations.[35] This makes sense conceptually, as increased histone methylation – a consequence of KDM inhibition – can provide binding surfaces for plant homeodomain-containing proteins that can ultimately stimulate DNMT activity and lead to aberrant DNA methylation. Alternatively, increased DNA methylation could result from antagonizing active DNA demethylation processes mediated by TET enzymes.[36] Supporting this, molecular pathology of SDH-deficient gastrointestinal stromal tumors has found these cancer exhibit decreased levels of 5-hydroxymethylcytosine.[37] Succinate and fumarate accumulation may also impact the cell through mechanisms completely orthogonal to chromatin, such as chemical modification of proteins[32] or interaction with membrane receptors.[38] Interestingly, although individuals with inborn mutations to *SDH* and *FH* harbor these errors in every cell of their body, only a subset of tissues such as brain and kidney are predisposed to cancer. This suggests epigenetic reprogramming mediated by *SDH* and *FH* inactivation collaborates with tissue-specific genomic factors to generate the tumorigenic phenotype. However, the identity of these epigenetic collaborators, as well as exactly which Fe(II)/α-KG-dependent enzymes mediate the transformed phenotype in cancers driven by SDH and FH mutations, remain to be determined.

As with SDH and FH, mutations in IDH enzymes can also result in the production of a cofactor competitive metabolite. However, IDH mutants differ significantly in terms of occurrence, clinical impact, and mechanism. Cancers associated with mutations of cytosolic *IDH1* and mitochondrial *IDH2* span a wide variety of cell types and are relatively common compared to *SDH*/*FH*-driven cancers, occurring with particularly high incidence in cases of glioblastoma and acute myeloid leukemia (AML).[39–42] For *IDH1*, highly specific somatic mutations affect a single amino acid in the active site, R132, and result in various amino acid substitutions, with R132H being the most common.[43] Rather than inactivate IDH1, this mutation results in the mutant IDH1 gaining the abililty to convert α-KG to the R-enantiomer of 2-hydroxyglutarate (R-2HG; Figure 2).[44] R-2HG shares the connectivity and dicarboxylate chemotype of α-KG. Similar to SDH and FH, early reports focused on the ability of R-2HG to inhibit PHD2 and stabilize HIF-1.[45, 46] However, biochemical and cellular studies have now shown conclusively that R-2HG activates PHD2, while serving as an inhibitor of KDM and TET dioxygenases.[47, 48] Although the inhibition constants of R-2HG for KDM and TET enzymes are weak (Table 1), R-2HG levels in tumor cells are increased ~1000-fold over wild type tumors (Table 2) making inhibition of dioxygenase activity biochemically feasible.[41, 44] Genetic studies of AML patient blood samples found that *IDH1* and *TET2* mutations occur in a mutually exclusive manner, supporting the hypothesis that R-2HG production by mutant IDH phenocopies TET2 inactivation.[49] Moreover, this study found that *IDH1/2* and *TET2* mutated tumors share similar genome-wide DNA hypermethylation profiles. This mirrors observations initially made in *IDH1*-mutated glioma, which also exhibits a hypermethylator phenotype.[50, 51] In cell-based models, R-2HG itself is sufficient to block differentiation and promote growth factor independence, two hallmarks of leukemic transformation.[52] These effects are recapitulated by TET2 knockdown, but are also dependent on the ability of R-2HG to activate PHD2 and induce HIF-1 degradation. Together these data implicate TET2 as a physiologically relevant epigenetic target that is inhibited by cofactor competition in IDH-mutated AML. Therapeutics that preferentially inhibit mutant IDH1 over the wild type enzyme induce changes in histone and DNA methylation, and have also shown promise in pre-clinical studies of *IDH1*-mutated AML.[53, 54]

While seemingly a well-plowed field, recent data indicates the epigenetic consequences of IDH inhibition may still not be completely understood. For instance, at low doses inhibitors targeting mutant IDH1 do not affect histone or DNA methylation, yet still have anti-tumor activity in a mouse model of glioma. This suggests mutant IDH1 and/or IDH1 inhibitors are capable of acting independently of chromatin methylation.[53] Studies of *IDH1* mutations in other cell types have also implicated non-TET2 dioxygenases as physiologically relevant targets of R-2HG. Thompson and coworkers found that stable transfection of the IDH1-R132H mutant in immortalized astrocytes resulted in an accumulation of histone methylation on H3K9 and H3K27.[55] R-2HG blocked differentiation in a cell-based model, an effect that was phenocopied by knockdown of the H3K9 demethylase KDM4C. This indicates R-2HG can utilize cell lineage-specific mechanisms to promote disease-relevant epigenetic change. Interestingly, although altered DNA methylation was observed in an IDH1 R132H conditional knock-in mouse, large changes in H3K9me3 levels were not observed.[56]

Cancers driven by mutants such as IDH1 R132H provide a fascinating example of how competitive metabolites can drive phenotypic change through epigenetic mechanisms. Further study is necessary to assess whether more transient changes in metabolism are also capable of exerting epigenetic effects. However, several lines of evidence suggest this may be the case. For example, in both yeast and human cells, changes in glucose availability can regulate transcription of growth-related genes through direct effects on the acetylation state of histones.[57, 58] These histone acetylation events are mediated by Gcn5, a KAT enzyme. As

mentioned above, Gcn5 is distinct from many KAT enzymes in that it possesses similar binding constants for Acetyl-CoA and CoA. Mutant studies of yeast Gcn5 suggest acetyl-CoA levels are sufficient to saturate Gcn5 KAT activity *in vivo* under normal growth conditions.[59] However, animal models have shown that in many tissues Acetyl-CoA/CoA ratios fluctuate from >1 in fed to <1 in fasting states, even while overall CoA pools stay relatively constant.[60] Together, these data support the premise that feedback inhibition by intracellular CoA may help regulate the ability of Gcn5 and other CoA-sensitive KATs to activate transcription of genes required for cell growth.[61] Conclusive testing of this hypothesis will require integrated measurements of acetyl-CoA/CoA ratio, chromatin modifications, and gene expression under carefully defined cell growth conditions.[57] Notably, several chromatin-modifying enzymes have K_i values for competitive metabolites that are estimated to be near their intracellular levels, suggesting competitive mechanisms may be operable *in vivo* (Table 1). Alternatively, chromatin modifications may be sensitive to transient changes in the combined level of competitive metabolites sharing a common chemotype (such as the dicarboxylates succinate/fumarate/2-HG) rather than any single competitive inhibitor alone.[18] Finally, different chromatin modifications are introduced and removed with very different kinetics.[62] Histone acetylations have a half-life on the order of minutes, while methylation persists on a time scale of hours. Thus, it is tempting to speculate that a wide-spectrum of metabolic sensitivity exists, in which some chromatin modifiers (such as KMT/KDM/TETs) may require only a short metabolic stimulus to elicit a sustained epigenetic response, while others (such as KAT/HDACs) may react more dynamically to changes in metabolism or nutritional status.

III. Regulation of Epigenetic Signaling by Cofactor Depletion

A second metabolic mechanism of epigenetic regulation is cofactor depletion. Cofactor depletion inhibits enzyme-catalyzed chromatin modifications by decreasing the concentration of enzyme cofactors to rate-limiting levels. Cofactor depletion can function independently of or collaborate with metabolic competition mechanisms, and disproportionately impacts chromatin-modifying enzymes with a high K_m for their obligate cofactor. Cellular cofactors may be depleted by i) nutrient limitation of essential vitamin precursors, ii) inhibition of cofactor biosynthesis, or iii) overconsumption of the cofactor by a competing enzyme. An illustrative example of the latter is the inhibition of KMTs by nicotinamide-*N*-methyltransferase (NNMT).[63]

NNMT is a metabolic methyltransferase that is overexpressed in a variety of cancers and is phenotypically associated with increased tumor cell migration and invasion.[64, 65] To understand how NNMT impacts cancer pathogenesis, Ulanovskaya et al. utilized an integrated profiling approach to study the effects of NNMT overexpression on metabolism, chromatin modification, and gene expression. Unbiased metabolomic profiling of NNMT overexpressing cells identified only two molecules as consistently increased: 1-methylnicotinamide (1MNA) and the cofactor byproduct SAH (Figure 3). Treatment of cells with 1MNA itself did not enhance tumor cell invasion or produce new metabolites, suggesting the primary effects of NNMT may derive from its ability to consume SAM and produce SAH. By coordinating NNMT overexpression with depletion of the SAM precursor methionine (Figure 3), the authors were able to distinguish between biological effects sensitive to SAM cofactor depletion (observed only with low methionine media) and cofactor competition caused by reduced SAH:SAM ratios (observed in low and high methionine media) (Table 2). Notably, increased SAH levels by themselves were not sufficient to reduce histone methylation, indicating that in this model KMTs are more sensitive to overall cellular concentrations of the SAM cofactor than the SAM/SAH ratio. The reduced levels of histone methylation correlated with changes in gene expression and increased migratory ability of tumor cells. At a molecular level NNMT overexpression leads

to a ~40–50% decrease in H3K4me3, H3K9me2, and H3K27me3 levels, while having less effect on arginine and DNA methylation. This is consistent with biochemical data showing that some KMTs possess higher K_m values for SAM than protein arginine methyltransferases (PRMTs), and thus might be expected to be disproportionately sensitive to cofactor depletion (Table 1). The finding that bulk DNA methylation was unchanged by NNMT overexpression is actually surprising based on the published K_m and IC_{50} values of human DNMT1 for SAM and SAH;[66] this may reflect the vagaries of cell culture conditions, or suggest changes in the DNA methylation machinery during NNMT overexpression.

The integrated measurements of metabolism, chromatin modifications, and gene expression found in the NNMT study provide a textbook approach to dissecting metabolic mechanisms of epigenetic regulation. Although such efforts are still rare in the literature, evidence suggests other metabolic methyltransferases may also be capable of regulating chromatin methylation through cofactor depletion. Glycine-*N*-methyltransferase (GNMT) consumes SAM to methylate glycine, yielding sarcosine.[67] As with NNMT, tumor cells often overexpress GNMT, creating an environment that inhibits histone and DNA methylation.[67, 68] Hormone-induced expression of GNMT results in a global loss of DNA methylation.[69] Catechol-*O*-methyltransferase (COMT) can reduce DNMT activity *in vitro* through similar mechanisms.[70] Methylthioadenosine phosphorylase (MTAP) deletions are found in many tumors, and can inhibit protein arginine methylation by a combination of cofactor depletion (reducing methionine salvage) and competitive inhibition mechanisms (increased S-methylthioadenosine).[71, 72]

Contrasting with these cases, in some cancers cellular transformation and proliferation is stimulated by methylation-associated gene silencing.[73] In these cancers, reducing methylation potential by cofactor depletion may provide a new avenue for therapy. For example, the SAH hydrolase inhibitor 3-deazaneplanocin A (DZNep) decreases protein and DNA methylation through disruption of SAM recycling (Figure 3).[74] Inhibition of SAH hydrolase by DZNep globally inhibits protein and DNA methylation, indicating a severe disruption of cellular SAM and SAH levels.[75] DZNep has shown promising activity as a single agent and combination therapy against hematological malignancies and solid tumors in preclinical models.[76, 77]

Cofactor depletion can also occur by limiting essential building blocks required for SAM biosynthesis. This manifests at the phenotypic level in yellow agouti mice, who acquire a brown coat when exposed to diets rich in folate, a SAM precursor, during development (Figure 3).[78] This brown coat is the direct result of increased DNA methylation of the agouti gene, whose silencing allows mouse skin cells to produce a black pigment.[79] The resulting brown coat phenotype is maintained for the entire life of the mouse and can even be inherited by the next generation. In addition to folate, changes in amino acid metabolism can also affect cofactor levels. For instance, glycine and serine provide methyl groups to SAM via the activities of serine hydroxymethyltransferase (SHMT) and the glycine cleavage system (GCS; Figure 3). Inhibition of threonine dehydrogenase (TDH) reduces glycine production in mouse embryonic stem cells, and has the downstream effect of depleting cells of the SAM cofactor.[80] Cofactor depletion selectively reduced histone methylation at H3K4 (but not H3K9 or H3K27) and caused cells to differentiate due to an inability to induce the expression of genes necessary for self-renewal.[80] While humans do not utilize TDH for glycine production, glycine and serine metabolism have been found to play critical roles in a number of cancers.[81, 82] An important goal of future research will be to determine how the uptake and biosynthesis of amino acids, including non-essential amino acids such as proline,[83] impact epigenetic states in disease.

Finally, while we have focused on SAM and chromatin methylation, cofactor depletion may also regulate other chromatin modifiers. Early studies of IDH1 mutation showed ectopic overexpression of IDH1 R132H could decrease cellular α-KG levels in cell lines, although the *in vivo* relevance of this effect is unclear.[45] NAD+ levels are depleted by PARP enzymes in response to DNA damage, which may affect the activity of NAD+ utilizing HDACs such as SIRT1 (Table 1).[84] NADH levels can also affect the activity of carboxyl-terminal binding protein (CtBP) a transcriptional corepressor active in breast cancer.[85] In yeast and humans, histone acetylation is sensitive to reduced acetyl-CoA biosynthesis.[58, 86, 87] Histone acetylation is also sensitive to inhibition of pantothenate kinase, the rate-limiting enzyme in CoA biosynthesis.[88] In most of these cases, both how cofactor depletion collaborates with other metabolic mechanisms, and whether metabolic perturbations manifest global or targeted epigenetic effects, remain to be established.

IV. Regulation of Epigenetic Signaling by Localization of Cofactor Biosynthesis

All of the preceding examples involve regulation of epigenetic signaling by global changes in metabolite concentrations. Recent discoveries indicate chromatin-modifying enzymes may also be capable of responding to local changes in cofactor concentration. This emerging mechanism has been most well-studied in the context of MafK, a protein belonging to the Maf family of transcription factors whose overexpression is associated with poor prognosis in multiple myeloma.[89] Spurred by proteomic studies that identified the SAM synthetase MATIIα as a MafK interaction partner in mouse cells, Katoh et al. analyzed how the genomic localization of MATIIα affected transcriptional repression by MafK.[90] Chromatin immunoprecipitation showed that induction of the stress-inducible gene *HO-1* led to displacement of MATIIα but not MafK from the *HO-1* locus, suggesting MATIIα genomic occupancy is required for transcriptional repression. Transcriptional repression of *HO-1* by MATIIα was dependent on an active catalytic domain, supporting a direct role for cofactor biosynthesis. Knockdown of MATIIα reduced levels of H3K4me2 and H3K9me2 at the *HO-1* locus, but did not affect H3K36 or DNA methylation. Together, these observations support the hypothesis that KMTs may be capable of using increased SAM production at specific genomic loci as a signal to mediate discrete transcriptional outcomes (Figure 4). Genomic analyses of MATIIα occupancy and correlation with histone methylation levels will be required to understand whether this mechanism operates genome-wide or exclusively in the context of MafK transcription. However, several candidate KMTs were identified as interaction partners of MafK that may mediate this effect, including G9a, EHMT, and MLL1.[90]

Genomic localization of cofactor biosynthesis is a relatively recent discovery and therefore has not been extensively studied. However, several observations support a potentially broad role for this mechanism. The KAT cofactor acetyl-CoA is produced by two main biosynthetic enzymes, ATP citrate-lyase (ACL) and acetyl-CoA synthetase (ACS). Both of these enzymes can be found in the nucleus in human cell lines.[58] Furthermore, electron microscopy has found >95% of yeast ACS localizes inside the nucleus when cultured in rich media.[86] The NAD+ synthase nicotinamide mononucleotide adenylyltransferase-1 (NMNAT-1) has been shown to co-localize in the nucleus with genes occupied by the HDAC SIRT1 and increase deacetylase activity.[91] In addition to these examples, it has also been shown that primary metabolic enzymes directly regulate genome function without a cofactor intermediary. Pyruvate kinase M2 (PKM2), which catalyzes the final step in glycolysis, can directly phosphorylate histones at H3T11 using phosphoenolpyruvate as the phosphate group donor.[92] This histone modification stimulates transcriptional activation at

the *MYC* locus, and correlates with increased malignancy and progression in glioblastoma clinical isolates.

V. Mimicking Metabolic Mechanisms of Epigenetic Regulation with Synthetic Cofactors and Small Molecules

Understanding the metabolic basis of epigenetic regulation is not only an intellectual pursuit, but also one ultimately undertaken to provide a basis for therapeutic intervention in disease. Regulatory insights resulting from mechanistic study can be used to validate new targets, as in the case of mutant IDH1, or provide fodder for the development of new approaches to alter epigenetic signaling. Thus, it is useful to briefly compare metabolic strategies with those devised in the laboratory to study and perturb genome function.

The design and application of cofactor competitive inhibitors predates our knowledge that competitive metabolites may also regulate chromatin-modifying enzymes in cells, and is a topic worthy of its own discussion, provided by several superb review articles.[17, 93, 94] However, consideration of a few examples is valuable. Synthetic cofactor analogues have been extremely useful for inhibition of KAT and KMT enzymes. Lys-CoA was one of the first designed KAT inhibitors, and consists of a high affinity bisubstrate formed by covalent linkage of the CoA cofactor to the ε-amine of lysine through a non-hydrolyzable acetyl bridge (Figure 5a).[95] Lys-CoA inhibits the p300/CBP KAT reaction with a K_i of 19 nM *in vitro*, while related histone peptide-CoA conjugates show enhanced affinity and specificity for KATs in the pCAF and MYST families.[95, 96] Rational design has also led to the development of potent cofactor-based inhibitors of the KMT DOT1L.[97] These SAM analogues, exemplified by EPZ-5646 (Figure 5), take advantage of a unique open conformation DOT1L adopts upon SAM analogue binding to inhibit H3K79 methylation with high affinity (K_i = 0.08 nM) and selectivity.[98] These SAM analogues reverse epigenetic patterning in cell and animal models, and show selective toxicity towards cancers harboring *MLL* gene fusions that are dependent on DOT1L activity.[13, 97] A number of different molecular scaffolds containing the Fe(II)-chelating and dicarboxylate moieties of α-KG have been explored for inhibition of KDM and PHD enzymes, and show varying degrees of affinity and selectivity.[99, 100] In contrast, aside from structural studies,[101] inhibition of sirtuin-type HDACs by synthetic NAD+ analogues has been relatively unpursued.

In general, synthetic cofactor analogues show much higher affinity for KATs, KMTs, and KDMs than endogenous metabolites. However, due to their hydrophilic nature, one limitation synthetic cofactor analogues face is their low cell permeability. For example, Lys-CoA and related bisubstrate-CoA analogues must be delivered to cells using cell penetrating peptides or transfection agents.[102] Cellular biosynthesis of Lys-CoA from a metabolic precursor has been explored, but appears to be incompatible with the substrate requirements of the human CoA biosynthetic pathway.[103] SAM-based DOT1L inhibitors require prolonged administration to manifest biological effects,[97] and cellular data for a number of highly active SAM-based KMT inhibitors have not been reported,[104] both of which may reflect their poor cell penetrance. KDM inhibitors based on α-KG also suffer from poor cell permeability of dicarboxylates, a limitation that can be circumvented through their administration as esterase-labile prodrugs.[93, 99] These challenges highlight one advantage endogenous metabolites have over synthetic cofactors as competitive inhibitors, namely that because their production occurs in the cell, high gradients of inhibitor can accumulate. This may be mimicked experimentally by using prodrug or metabolic precursor strategies to produce active species intracellularly. Another attractive approach is the discovery of chemotypes that directly compete with cofactors but are not structurally cofactor-based, such as the pyrimidone class of KMT inhibitors.[105, 106]

Cofactor depletion also provides fertile ground for epigenetic reprogramming by small molecules. Cofactors can be depleted pharmacologically by inhibition of salvage and recycling pathways, as with the aforementioned inhibition of SAH hydrolase by DZNep, which reduces histone and DNA methylation (Figure 3).[75] Similar results can be elicited by treatment of cells with adenosine dialdehyde (Adox), an amine-reactive inhibitor of SAH hydrolase.[75] Sirtuin HDAC activity is sensitive to cofactor depletion caused by small molecule inhibition of nicotinamide phosphoribosyltransferase (NAMPT), the rate-limiting enzyme of the NAD+ salvage pathway (Figure 5b).[107] Treatment of cells with the NAMPT inhibitor FK866 can mimic effects observed during circadian oscillation of NAD+ levels, and increases acetylation of the regulatory protein BMAL to similar levels as observed in SIRT1 knockout mice.[108] ATP-citrate lyase (ACL) inhibition reduces histone acetylation in cultured cells, although its effects on overall acetyl-CoA/CoA levels were not studied in detail.[58] SAH, NAMPT, and ACL inhibitors all show potent antitumor activity.[77, 109, 110] Further study will be required to understand what role inhibition of specific chromatin modifications plays in the effects of these pleiotropic agents.

In contrast to the previous two examples, no synthetic approach for controlling the genomic localization of cofactor biosynthesis has yet been developed. However, of relevance is a recent study in which approaches with a foundation in chemical biology have been applied to localize and study the effects of chromatin-modifying enzymes themselves.[111] Hathway et al. recently developed an experimental system that applied chemical inducers of dimerization[112] to control the dynamics of association of HP-1α and a transcriptional activator domain (TAD) at a designed *Oct*4 locus in mouse embryonic stem cells (Figure 5c).[111] HP-1α is a KMT-recruiting protein involved in the establishment of transcriptionally silenced heterochromatin, while TAD domains stimulate transcription. Controlling the genomic localization of these two antagonistic protein domains with orthogonal small molecules (Figure 5c) allowed the experimental examination of how histone methylation propagates and is transmitted over multiple generations. Small molecule-induced recruitment of HP-1α silenced transcription from the *Oct4* locus, and caused H3K9 trimethylation to spread ~10 kB from the site of targeting, similar to the span of natural heterochromatin territories. In mouse embryonic fibroblasts, induced histone methylation was inherited and maintained through multiple cell divisions even after removal of the chemical inducer of dimerization. These effects could be overcome by recruitment of the TAD domain, indicating that while HP-1α-induced histone methylation is self-propagating and persistent, they are also malleable. As methods for targeted genomic localization continue to develop,[113] an interesting future question will be whether local epigenetic effects can also be stimulated by loci-specific recruitment of cofactor biosynthetic enzymes.

VI. Conclusion and Future Directions

It is becoming increasingly clear that metabolic control of epigenetic signaling is a real phenomenon that can contribute to phenotypic change and disease. While we have artificially limited our discussion here to a handful of well-studied chromatin-modifying enzymes that utilize acetyl-CoA, SAM, NAD+, and α-KG as cofactors, the constellation of protein and nucleic acid modifications is vast. Evidence supports a role for metabolism in regulating the activity of other cofactor-utilizing chromatin modifiers not discussed here, including non-sirtuin HDACs (flavin mononucleotide), PRMTs (SAM), PARPs (NAD+), histone threonine kinases (ATP), sirtuin lysine deacylases (succinyl-CoA, myristoyl-CoA), and O-GlcNAc-transferases (UDP-GlcNAc). [20–23, 114] Similarly, because acetylation and methylation of lysine residues block their modification by ubiquitin and ubiquitin-like modifiers, these modifications can also have a metabolic component.[115] RNA methylation/hydroxymethylation is another cofactor-dependent (SAM/α-KG) epigenetic process that has been directly linked to obesity; however, the sensitivity of these enzymes to changes in cell

metabolism has not been studied.[116] Finally, chromatin modifiers underwent a nomenclature change in 2007 due to the realization that many of these enzymes modify and regulate the function of non-histone proteins.[117] Understanding how metabolism co-regulates the posttranslational modification of histones,[25] transcription factors,[118] multiprotein complexes,[119] and metabolic enzymes[120] will be an important step to defining the feedback and feed-forward loops that connect metabolism and gene expression *in vivo*.

Looking forward, we see many valuable ways in which chemical biology can help explain how the metabolic state of the cell impacts epigenetic mechanisms. First and foremost, chemical biologists can accelerate our understanding of metabolic regulation of epigenetics by developing new tools to study biochemistry in living systems. The development and widespread application of genetically-encoded fluorescent metabolite sensors for imaging metabolite concentrations and gradients in living cells would provide a real-time view into how metabolism is rewired in response to nutrient uptake and/or metabolic perturbation.[121, 122] Sensor-based approaches would complement traditional methods for measuring absolute metabolite concentration[123] by differentiating free vs. bound cofactors and allowing metabolic compartmentalization to be directly visualized. A second crucial barrier is to correlate observed changes in metabolite concentration with enzyme activity. Activity-based protein profiling (ABPP) has proven a powerful approach to determine the activity of entire enzyme classes in living cells and cell lysates.[124]

Studies of kinases have demonstrated the feasibility of using active-site probes to profile the cofactor sensitivity of native cellular enzymes.[125, 126] Notably, several active site-directed affinity probes for chromatin modifiers have been reported.[127–130] As with metabolite profiling, the continued development of geneticallyencoded[131, 132] and peptide-based[133] reporters of chromatin-modifying enzyme activities will be an important complementary approach, providing insight into the dynamics and subcellular localization of specific posttranslational modification events. Real-time approaches may be especially valuable in determining how the kinetics and equilibrium of different chromatin marks relate to the metabolic sensitivity of their chromatin modifiers. Data from each of these methods will also provide valuable inputs for computational approaches to model the pleiotropic effects of metabolic perturbation.

Together with these approaches, understanding the functional consequences of metabolic flux and chromatin-modifying enzyme activity will also require new methods to profile the targets of cofactors and enzymes in living systems. Engineered “bump-hole” cofactor-enzyme pairs have been applied artfully to identify potential protein[134, 135] and genomic[136] targets of specific KMTs and PRMTs. Although this method does not currently report on endogenous KMT activities, this limitation is likely to be circumvented in the future through the use of genome editing tools to incorporate mutant KMTs into endogenous loci.[113] From the perspective of metabolic regulation, it will be critical to find out whether KMT mutants differ from endogenous enzymes in their sensitivity to metabolites such as SAH and methylthioadenosine. Recent studies suggest chemical genetic approaches may also be useful for the identification of KAT substrates.[137] This method faces the additional challenge of correcting for background due to the promiscuity of the KAT p300 for engineered cofactors[138] as well as the high chemical reactivity of acyl-CoA species, which are known to non-enzymatically modify proteins.[138, 139] Linked to this latter observation, recent studies that have shown that non-enzymatic modification of proteins by chemically reactive metabolites can directly modulate metabolic and transcriptional programs.[32, 140] Therefore, another vital objective will be to understand how metabolism mediates protein modifications that result solely from chemical reactivity, and what, if any, downstream effects these modifications have on genome function.

A final way in which chemical biology can contribute to the studies of both metabolism and epigenetics is by continuing to develop new agents for the targeted perturbation of these processes. Organizations such as the Structural Genomics Consortium have made great strides in the development and dissemination of specific, high affinity probes for a variety of chromatin-modifying and chromatin-interacting proteins,[141–143] and the identification of mutant IDH1 as an oncogenic driver has renewed interest in the development of specific small molecule inhibitors of metabolism.[144] Cell permeable inhibitors and light-activated probes remain the gold standard for studying temporally defined effects of enzyme activation or inhibition.[145] Combining carefully designed chemical agents with ensemble[146] and single-cell methods[147] for monitoring gene expression will aid the systematic identification of transcriptional programs sensitive to metabolic flux. Thus, as these techniques continue to develop and mature, it is our view that chemical biology may demonstrate a fine ear for “new melodies” in the carefully orchestrated symphony of transcriptional regulation.

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Glossary

Genome function	The functions of the genome are to serve as a template for replication and transcription, as well as to maintain fidelity of the template itself (repair) - this term refers to those three processes
Epigenetic regulation	The regulation of transcription and other genomic functions by molecular events that are not directly dependent on the primary nucleotide sequence of DNA. Most commonly associated with histone/DNA modifications. Additional epigenetic modifiers beyond the scope of this review include ATP-dependent chromatin remodeling enzymes, non-coding RNAs and histone variants
Chromatin (Heterochromatin, Euchromatin)	147-bp segments of DNA wrapped around histone octamers containing two copies each of the core histones H2A, H2B, H3, and H4. Open chromatin is referred to as euchromatin and is associated with transcriptionally active genes. Compacted chromatin is referred to as heterochromatin and is associated with transcriptionally inactive genes
Histone acetylation	Posttranslational modification of histone lysine residues. Added by KATs, removed by HDACs. Commonly referred to by the name of the histone, residue modified, and modification. Example: H3K27Ac, monoacetylation of histone H3 on lysine 27. Lysine acetylation is associated with transcriptionally active euchromatin. Acetyl-lysine residues are bound by bromodomain-containing proteins
Histone methylation	Posttranslational modification of histone lysine and arginine residues. Added by KMTs and PRMTs, removed by KDMs. Commonly referred to by the name of the histone, residue

	modified, number of modifications, and symmetry of modification (arginine only). Example: H3K9me3, trimethylation of histone H3 on lysine 4. H3R17me2a, dimethylation (assymetric) of histone H3 on lysine 4. Lysine methylation can promote DNA methylation through recruitment of repressive complexes; however, lysine methylation may be associated with either euchromatin or heterochromatin depending on the context. H3K9me3 residues interact with a variety of protein domains, including plant homeodomains
DNA methylation	Methylation of the cytosine base at the C5 position. Added by DNMTs, oxidized by TETs to promote removal. Often occurs at genomic regions rich in 5′-CG-3′ sequences known as CpG islands. Associated with transcriptionally silenced heterochromatin. DNMTs can be recruited by plant homeodomain-containing proteins, thus linking them to histone lysine methylation
Chromatin marks associated with transcriptionally active chromatin	Histone acetylation, H3K4me2, H3K4me3, H3K27Ac, H3K36me3, H3K79me2
Chromatin marks associated with transcriptionally repressed chromatin	DNA methylation, H3K9me2, H3K9me3, H3K27me3
Chromatin marks associated with bivalent states, poised for rapid induction of either transcription or repression	H3K4me3, H3K27me3

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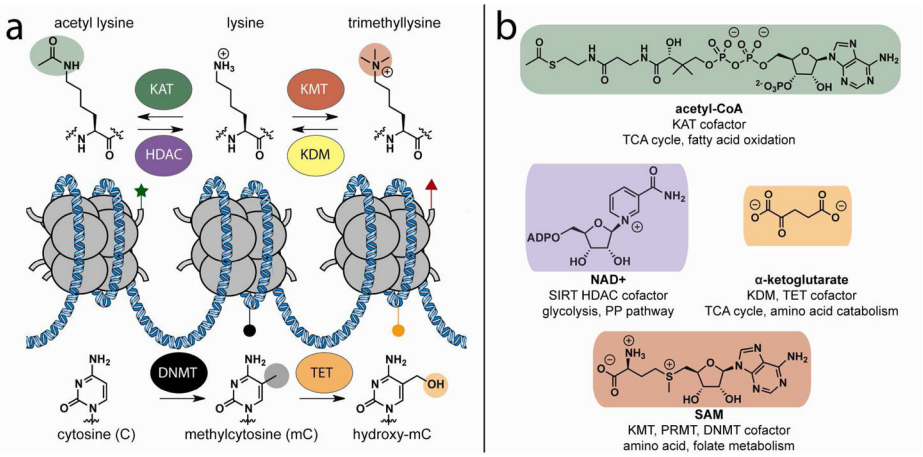

Figure 1.

Regulation of genome function by cofactor-dependent enzymes. (a) Chromatin-modifying enzymes modulate the posttranslational modifications of histone amino acids and cytosine methylation state. These modifications can affect the physical accessibility of genomic loci, provide specific binding surfaces for effector proteins, or influence posttranslational modification of neighboring residues. (b) Enzyme cofactors, their associated chromatin modifiers, and examples of metabolic pathways that produce and consume each cofactor. Enzymes are abbreviated according to nomenclature established by Allis et al.[117] KAT, lysine acetyltransferase; HDAC, sirtuin histone deacetylase; KMT, lysine methyltransferase; KDM, lysine demethylase; DNMT, DNA methyltransferase; TET, cytosine hydroxylase.

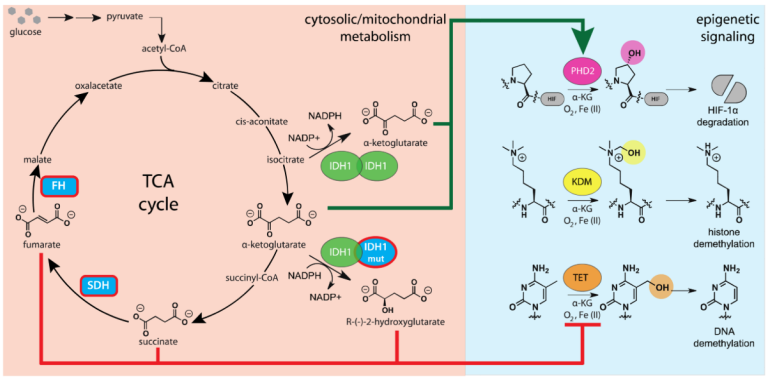

Figure 2.

Regulation of epigenetic signaling by cofactor competition. Diverse mechanisms of genomic regulation including transcription factor stability, and histone and cytosine methylation status are regulated by α-ketoglutarate-dependent dioxygenases (green arrow). Inactivating mutations in the TCA cycle enzymes FH and SDH or missense mutations in cytosolic IDH1 result in the production of high concentrations of fumarate, succinate, and (*R*)-2-hydroxyglutarate, which compete with α- ketoglutarate for enzyme active sites (red arrow). Metabolic enzymes are color-coded light blue, with those proteins mutated or overexpressed in cancer outlined in bold red. HIF, hypoxia-inducible factor; SDH, succinate dehydrogenase; FH, fumarate hydratase; IDH, isocitrate dehydrogenase.

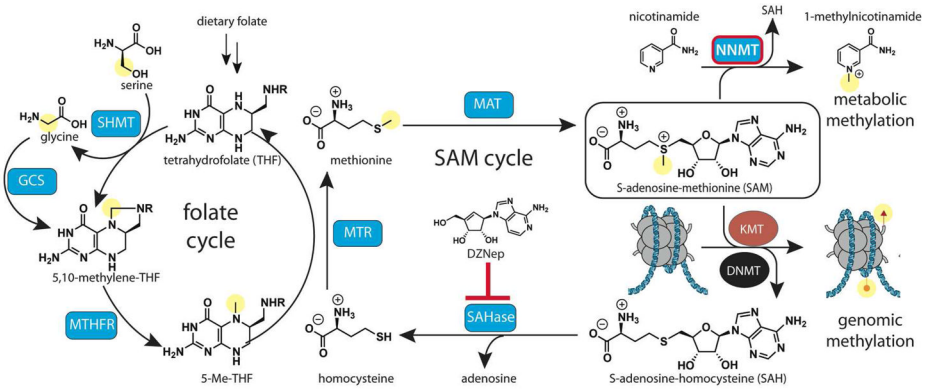

Figure 3.

Regulation of epigenetic signaling by cofactor depletion. SAM functions as a universal methyl donor for methylation of macromolecules and metabolites. Overactivity of metabolic methyltransferases such as NNMT can result in depletion of SAM and decreased SAM/SAH ratios, thereby reducing KMT/DNMT activity and genomic methylation. Replenishment of SAM requires the activity of the folate and SAM cycles, which utilize methyl groups derived from folate or choline (not shown) to replenish intracellular methionine for SAM biosynthesis. Disruption of the SAM cycle by inhibitors has been shown to decrease the activity of KMT enzymes in cancer. Cofactors and byproducts of most enzymatic reactions have been omitted for simplicity. NNMT, nicotinamide N-methyltransferase; SAHase, S-adenosylhomocysteine hydrolase; MTR, 5-methyltetrahydrofolate-homocysteine methyltransferase; MTHFR, methylenetetrahydrofolate reductase; SHMT, serine hydroxymethyltransferase; MAT, methionine adenosyltransferase; DZNep, 3-deazaneplanocin A.

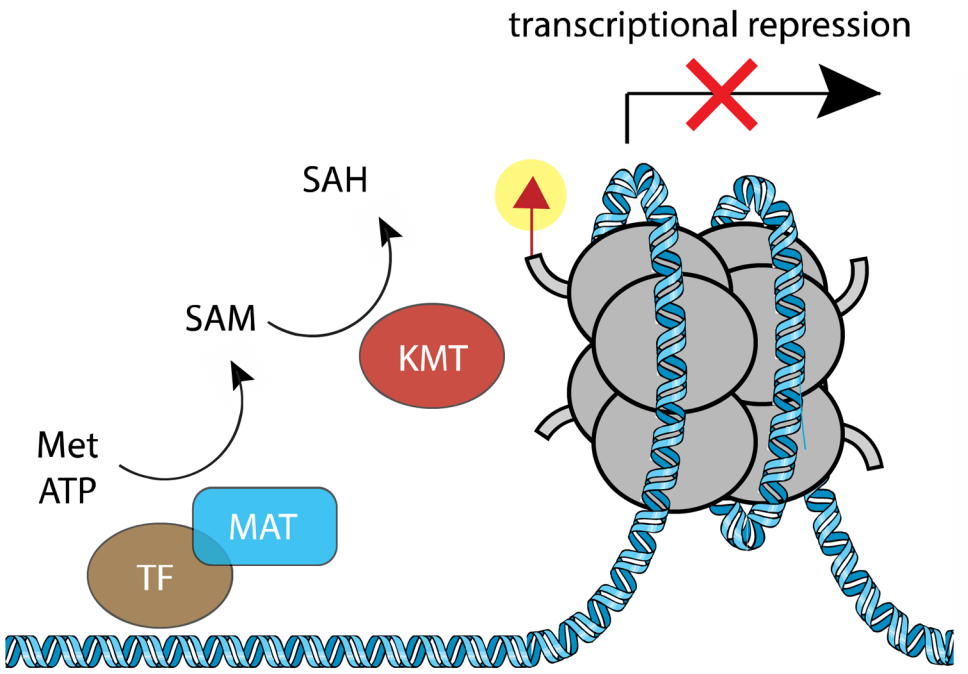

Figure 4.
Regulation of epigenetic signaling by subcellular localization of cofactor biosynthesis. The SAM biosynthetic enzyme MAT associates with transcription factors at specific genomic loci, producing SAM that is used by KMT enzymes to methylate histones and establish a repressive heterochromatin state. Notably, addition of exogenous SAM does not stimulate the same effect, suggesting genomic-localized biosynthesis of SAM is required for transcriptional repression.

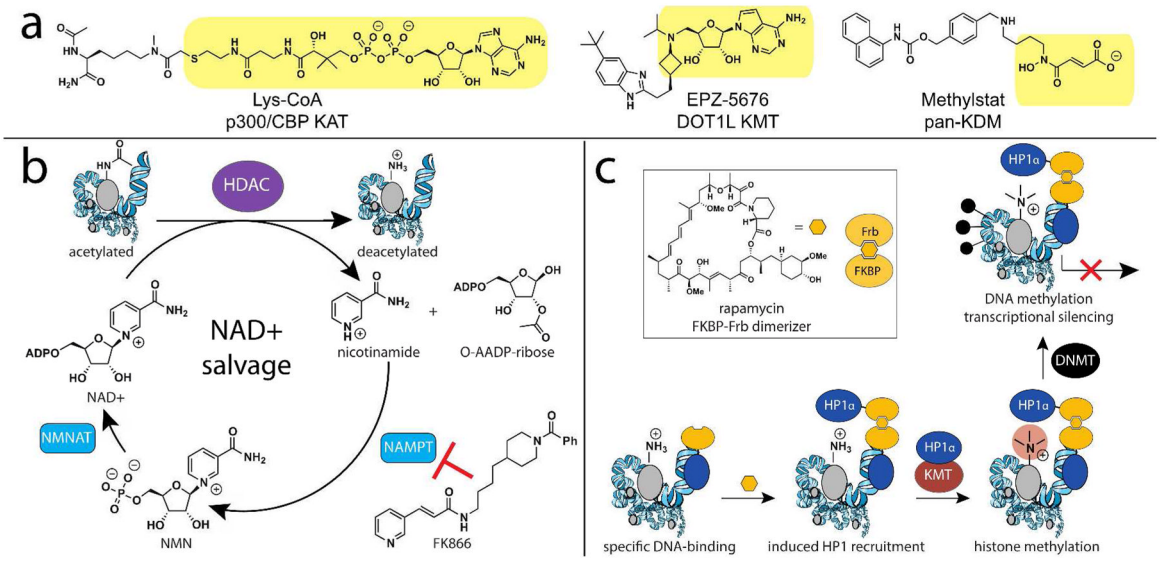

Figure 5.

Mimicking metabolic mechanisms of epigenetic regulation with small molecules. (a) Synthetic cofactors applied for inhibition of chromatin-modifying enzymes. Cofactor-derived portions of each molecule are highlighted in gold. (b) NAD+ salvage pathway and NAMPT inhibitors. Blockade of NAD+ salvage has been shown to deplete cellular NAD+ levels and inhibit SIRT HDAC activity. (c) Chemical inducer of dimerization approach used to localize genomic function of HP1, which stimulates local KMT and DNMT activity. NAMPT, nicotinamide phosphoribosyltransferase; NMNAT, nicotinamide mononucleotide adenyltransferase; HP-1, heterochromatin binding protein.

Table 1

Biochemical measurements of interactions between epigenetic modifiers and regulatory metabolites. Metabolic sensitivity indicates evidence for regulatory metabolites promoting or impeding chromatin modification in cells (y = yes, n = no, nd = not determined). References for data: Gcn5,[148] p300,[149, 150] pCAF,[151] KDM4A/KDM4C,[46] PHD2,[31, 46, 48] EZH1/EZH2,[152] CARM1,[152, 153] SIRT1,[154] CtBp.[155] Gcn5 values from yeast homologue, Kd for CoA obtained with etheno-CoA; p300 Ac-CoA and CoA Kd and Ki obtained with acetonyl-CoA and desulfo-CoA respectively; SIRT1 Ki value from yeast homologue (Sir2). All values are given without standard deviations and have been rounded to the nearest decimal for simplicity.

Epigenetic modifier	Regulatory metabolite	Km (μM)	Kd (μM)	IC50 or Ki (μM)	Chromatin mark	Metabolic sensitivity
Gcn5	Ac-CoA	2.5	8.5	-	H3K14Ac	y
	CoA	-	5.1	6.7		
p300	Ac-CoA	1.2	0.7	-	H3/H4Ac	nd
	CoA	-	7.3	8.1		
Pcaf	Ac-CoA	1	0.6	-	H3K9Ac	nd
	CoA	-	-	0.4		
KDM4A	a-KG	6	-	-	H3K9me3 (removal)	y
	R-2-HG	-	-	24		
KDM4C	a-KG	4	-	-	H3K9me3 (removal)	y
	R-2-HG	-	-	79		
PHD2	a-KG	1	-	-	HIF-1 genomic occupancy reduced	y
	R-2-HG	210	-	7300		
	succinate	-	-	350		
	fumarate	-	-	80		
EZH1	SAM	2.5	-	-	H3K27me	y
	SAH	-	-	8.3	(me2/me3)	
EZH2	SAM	1.2	-	-	H3K27me	y
	SAH	-	-	7.5	(me2/me3)	
CARM1	SAM	0.2	0.8	-	H3R17me2a	n
	SAH	-	6.9	0.1		
SIRT1	NAD+	154	-	-	H3K9Ac (removal)	y
	NADH	-	-	28000		
CtBP	NAD+	-	11	-	co-repressor	y

Epigenetic modifier	Regulatory metabolite	Km (μM)	Kd (μM)	IC50 or Ki (μM)	Chromatin mark	Metabolic sensitivity
	NADH	-	0.07	-		

Table 2

Effect of metabolic perturbations on ratios and overall levels of cofactors used by epigenetic modifiers. Sources for perturbation data: Ac-CoA/CoA, liver levels in fed/fasted rats;[60] α-KG/R-2HG, tumor levels in IDH1 R132 wild type/mutant tumors;[44] SAM/SAH, cell lines in control/NNMT overexpressing cell lines.[63] α-KG/R-2HG levels were calculated as the average of wild type/mutant samples from reference. All data are provided as unit-less ratios and fold-change to account for differences in units of measurement from each study, and have been rounded to the nearest decimal for simplicity.

Epigenetic modifier	Regulatory metabolite	Perturbation	Native ratio	Perturbed ratio	Fold change - metabolite
KAT	Ac-CoA	fasting	1.2	0.7	1.1
	CoA				1.9
KDM	a-KG	IDH1 mutation	0.2	0.002	0.9
	R-2-HG				64.9
PMT	SAM	NNMT overexpression	1.2	0.2	1.9
	SAH				0.4

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