Histone Methyl Transferases and Demethylases; Can They Link Metabolism and Transcription?

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Introduction
Increased life expectancy in industrialized countries goes hand in hand with a steady progression of multifactorial diseases, such as type 2 diabetes mellitus, obesity, and cancer, which result from the combined action of many genes and environmental factors, such as diet or exercise. Metabolic homeostasis is maintained by an intricate regulatory circuitry, controlled to a large extent by transcriptional mechanisms (Chawla et al., 2001; Francis et al., 2003; Feige and Auwerx, 2007). Metabolism therefore represents a sensitive indicator of the efficiency of these transcriptional mechanisms. Transcriptional control is achieved through a complex molecular circuitry that involves individual transcription factors, the basal transcriptional machinery, and multiprotein coregulatory complexes. These coregulators fine tune transcription and are proposed to act as metabolic sensors, which translate changes in metabolism into alterations in gene expression by affecting the activity of transcription factors, as well as changing the structure of the genome (reviewed by Smith and O’Malley, 2004; Spiegelman and Heinrich, 2004; Rosenfeld et al., 2006; Feige and Auwerx, 2007; Haberland et al., 2009).

Within the eukaryotic cell nucleus, genetic information in DNA is organized in a highly conserved structural polymer, termed chromatin, which supports and controls the functions of the genome. The fundamental repeating unit of chromatin is the nucleosome, which consists of 146 bp of DNA wrapped around an octamer of core histone proteins (an H3-H4 tetramer and two H2A-H2b dimers) (reviewed by Laskowski and Thornton, 2008). Linker histones of the H1 class associate with DNA between single nucleosomes establishing a higher level of organization, the so-called “solenoid” helical fibers (Laskowski and Thornton, 2008). Core histones are evolutionarily conserved and consist of a globular domain and a flexible charged N-terminal tail, which is covalently modified by different enzymes mainly at specific lysine and/or arginine residues. Those modifications of the histone tail include acetylation, phosphorylation, methylation (Figure 1), and ubiquitination. Together these histone modifications compose the “histone code,” which influences chromatin condensation and gene transcription (reviewed by Jenuwein and Allis, 2001; Berger, 2007; Kouzarides, 2007; Ruthenburg et al., 2007). Depending on nucleosome condensation, chromatin is more or less accessible to different enzymes and proteins, most of which regulate gene expression. Euochromatin is generally referred to transcriptionally active relaxed chromatin, while the silent inaccessible chromatin is called heterochromatin. Although chromatin modifications have been divided into those that correlate with activation and those that correlate with repression of transcription, the truth is likely to be that any given modification can activate or repress genes depending on the context (Vakoc et al., 2005; Berger, 2007; Bernstein et al., 2007; Kouzarides, 2007; Ruthenburg et al., 2007).

Since the well-established role of DNA methylation (reviewed by Suzuki and Bird, 2008 and Ling and Groop, 2009) and the emerging function of histone acetyltransferases and deacetylases (reviewed by Smith and O’Malley, 2004; Spiegelman and Heinrich, 2004; Rosenfeld et al., 2006; Feige and Auwerx, 2007; Haberland et al., 2009) in the control of metabolism has been recently reviewed, we focus here on the existing evidence for a potential role of histone methylation in metabolic adaptation.

Histone Methylation and Demethylation in the Control of Gene Expression
Histone methylation increases the basicity and hydrophobicity of histone tails and the affinity of certain proteins, such as transcription factors, toward DNA. There are three classes of histone methyltransferase (HMTs) enzymes: SET domain lysine methyltransferases, non-SET domain lysine methyltransferases, and arginine methyltransferases. All three classes use S-adenosylmethionine (SAM) enzymes and demethylases could link metabolic signals to chromatin and alter transcription, further research is indispensable to consolidate these enticing observations.
as a coenzyme to transfer methyl groups (Smith and Denu, 2009). Lysine methyltransferases have striking target specificity, and they usually modify only one single lysine on a single histone and their output can be either activation or repression of transcription.

The SET domain-containing class of methyltransferases is best characterized and has been associated with metabolic diseases (Lee et al., 2008b; El-Osta et al., 2008, and Brasacchio et al., 2009). The SET domain is an evolutionary conserved domain, initially identified in Drosophila PEV (positive effect variegation) suppressor SU(VAR)39 (Tschiersch et al., 1994), the polycomb group protein Enhancer of zeste (Jones and Gelbart, 1993), and the trithorax group protein Trithorax (Stassen et al., 1995). Although the major role of these methyltransferases is the modulation of gene activity via histone methylation and alteration of chromatin structure (Rea et al., 2000), they also target several nonhistone proteins. In particular, the tumor suppressors p53 (Chuikov et al., 2004; Huang et al., 2010) and pRb (Munro et al., 2010), and the estrogen receptor α (Subramanian et al., 2008) are substrates of SET-domain-containing methyltransferases, while the peroxisome proliferator-activated receptor γ coactivator 1α is a substrate of the arginine methyltransferase CARM1 (Teyssier et al., 2005).

For a number of years following the discovery of HMTs, the existence of histone demethylases (HDMs) was contentious. The discovery of the first HDM, lysine-specific demethylase 1 (LSD1) (Shi et al., 2004), led to the identification of additional demethylases. These HDMs are distinguished by the nature of their demethylase domains, with distinct reactions being catalyzed by the LSD1 domain and the JmJC (Junmonji C) domain demethylases. LSD1 is a highly conserved protein, homologous to other FAD-dependent oxidases (like the monoamine oxidases involved in serotonin metabolism), composed of two subdomains: a FAD-binding and a substrate-binding domain (Anand and Marmorstein, 2007). LSD-1 is recruited to its target genes by different multiprotein complexes, which drive LSD-1 activity toward mono- and dimethylated H3K9 or K4, leading to context-dependent transcriptional activation or repression (Shi et al., 2005 and Metzger et al., 2005).

The other class of HDMs got their name from the jumonji gene, which was identified by a mouse gene trap approach as essential for the development of multiple tissues (Takeuchi, 1997). Over 100 proteins from bacteria to mammals contain a JmJC domain, a predicted metalloenzymatic catalytic motif. More recently JmJC-domain-containing proteins were identified as transcriptional coregulators involved in the demethylation of H3K9 (Tsukada et al., 2006). Five JmJC domain subfamilies maintain histones demethylated, i.e., JMJD1, JMJD2, JMJD3, and JARID1 (Tsukada et al., 2006). Recently identified JmJC proteins are JMJD6, an HDM with specificity to H3R2me2 and H4R3me2, demonstrating that also arginine methylation marks are reversible (Chang et al., 2007), and the PHD finger and JmJC-domain-containing PHF8 protein, with specificity toward mono- and dimethylated H3K9 and H4K20 (Feng et al., 2010; Liu et al., 2010). JmJC enzymes convert substrates in a manner different from LSD1 and also act on trimethylated lysines (Smith and Denu, 2009). They feature a α-ketoglutarate (α-KG) / Fe2+-dependent dioxygenase activity. α-KG stabilizes the enzyme/substrate complex (Tsukada et al., 2006) and binds the catalytic iron center undergoing oxidative decarboxylation to give succinate as part of the reaction (Smith and Denu 2009). While the demethylation of H3 starts to be understood, demethylases of other proteins, including other histones, are with a few exceptions (Chang et al., 2007; Feng et al., 2010; Liu et al., 2010) still largely unknown (Figure 1).
methionine by 5-methyltetrahydrofolate-homocysteine methyl transferase (MTR) through transfer of a methyl group from 5-methyltetrahydrofolate (5-MTHF) (Finkelstein and Martin, 2000), or fully metabolized to cysteine and pyruvate by two rounds of transsulfuration reactions catalyzed by cystathionine-β-synthase (CBS) and cystathionine-γ-lyase (CTH) (Finkelstein and Martin, 2000). High intracellular energy levels increase SAM concentration and DNA methyl transfer (DNMT) activity with impact on global DNA methylation (Chiang et al., 2009); whether HMT activity and histone methylation are also affected is still unclear. Furthermore, it is not established whether physiological changes in energy levels, such as those induced by diet and exercise, change SAM levels. The fact that anaplerotic diets increase intracellular energy and SAM levels and thereby improve adult polyglucosan body disease, characterized by dysfunctional glycosidic metabolism and diminished methionine levels (Roe et al., 2010), argues that this is, however, the case.

Methylation-independent SAM metabolism can also influence transcription. SAM can be decarboxylated by SAM decarboxylase (SAM-DC) to decarboxy-SAM (DC-SAM) and enter the polyamine cycle (Figure 3). Polyamines are abundant multifunctional organocations, largely bound to RNA and DNA, that regulate many cellular functions, including transcription. Interestingly, mice transgenic for the rate-limiting enzyme in the polyamine pathway, spermine/spermidine N1 acetyltransferase (SSAT), have an improved metabolic profile, as the accelerated polyamine flux enhances ATP consumption (Pirinen et al., 2007).

To catalyze demethylation reactions, the LSD1 or the Jumonji domain classes of HDMs require different metabolic cofactors, flavin adenine dinucleotide (FAD) (Anand and Marmorstein, 2007) and α-KG (Tsukada et al., 2006), respectively. FAD is a redox coenzyme existing in two different redox states, involved in several important reactions in metabolism. FAD consists of a riboflavin moiety (vitamin B2) bound to the phosphate group of ADP (Figure 2), thus clearly requiring ATP to be synthesized. Starting from riboflavin, flavin mononucleotide (FMN) is first generated by riboflavin kinase (RFK)-dependent phosphorylation. FMN is then converted to FAD by FAD synthase (FLAD), which transfers an AMP moiety from an ATP molecule to the FMN (Figure 3). The reduced form of FAD, FADH₂, is an energy carrier. When oxidized back to FAD, FADH₂ sends its two high-energy electrons through the electron transport chain (ETC) to produce ATP by oxidative phosphorylation. Any oxidoreductase enzyme that, like LSD1, uses FAD as an electron carrier is called a flavoprotein. Flavoproteins are essential in many metabolic reactions, as illustrated by the enzyme complex succinate dehydrogenase (complex II) that oxidizes succinate to fumarate in the citric acid cycle, thereby reducing FAD to FADH₂. Other well-studied flavoproteins include acyl CoA dehydrogenase, α-ketoglutarate dehydrogenase (α-KGDH), and a component of the pyruvate dehydrogenase complex, illustrating the potential link between LSD1 and intermediary metabolism (Figure 3).

α-KG (Figure 2), the coenzyme of the JmJc class of HDMs (Tsukada et al., 2006), is a key intermediate in the Krebs cycle, coming after isocitrate and before succinyl-CoA. Anaplerotic reactions replenish the cycle at this juncture by synthesizing α-KG through the action of glutamate dehydrogenase on glutamate. α-KG is the substrate of the α-KGDH reaction by which it is converted in succinyl-CoA in the presence of FAD and NAD (Figure 3), a critical and highly regulated step of the Krebs cycle mainly controlled by feedback regulation. Succinyl-CoA, FADH₂, and NADH have all been shown to inhibit the reaction (Smith et al., 1974). Interestingly, α-KGDH is also inhibited by high intracellular ATP levels (Smith et al., 1974) (Figure 3).

Based on the dependence of HDMs and metabolon coenzymes, whose availability is governed by the intracellular energy content, these effectors of reversible methylation could in theory reprogram gene expression in function of the metabolic milieu. But, however tempting this hypothesis is, much further work is required to unequivocally establish whether these coenzymes are rate-limiting under physiological conditions.

Methylation/Demethylation in the Pathogenesis of Metabolic Disorders

The first SET-domain HMT to be studied for its role in metabolism was MLL3,
a member of the mixed-lineage leukemia (MLL) family (Ansari and Mandal, 2010). MLL3 is a component of the ASC-2/NCOA6 (ASCOM) complex, which possesses HMT activity toward H3K4 and is involved in transcriptional coactivation (Lee et al., 2008a). MLL3−/− mice have less white adipose tissue, associated with a favorable metabolic profile. The MLL3−/− mice are also resistant to high-fat diet-induced hepatosteatosis because of the role of ASCOM in coactivating the liver X receptor (Lee et al., 2008b). In line with these observations for MLL3, SETDB1 has also been shown to inhibit adipogenesis via Wnt-dependent inactivation of PPARα transcriptional activity (Takada et al., 2007).

The SET7 HMT has been recently suggested to be involved in the pathogenesis of vascular complications of diabetes (El-Osta et al., 2008; Brasacchio et al., 2009). Transient hyperglycemia causes both in vitro and in vivo a sustained expression and activation of the transcription factor NFkB-p65. This is associated on the one hand with SET7 recruitment and H3K4 monomethylation (El-Osta et al., 2008) and on the other hand with persistent H3K9 demethylation and recruitment of LSD1 to the NFkB-p65 promoter (Brasacchio et al., 2009). Another study also links LSD1 and the vascular impact of diabetes (Reddy et al., 2008). In mouse vascular smooth muscle cells (mVSMC) from db/db mice, a mouse model of diabesity, both the basal and Tumor necrosis factor-α-induced expression of the inflammatory genes monocyte chemoattractant protein 1 (MCP-1) and interleukin 6 (IL-6) are increased. This is associated with an increase in the activating H3K4 dimethylation mark and reduced LSD1 recruitment to the promoters. Interestingly, high-glucose treatment recapitulates this in vivo observation in cultured human VSMC, suggesting that glucose by itself increases the expression of inflammatory genes, like MCP-1 and IL-6, through alterations in histone methylation. Although it is tempting to speculate that metabolic alterations in diabetes, through changing the levels/activity of metabolic coenzymes, could impact on HMT/HDM activity, firm evidence in support of this hypothesis is still missing.

One of the best characterized JmjC-domain-containing HDMs is JHDM2a/KDM3A, which has been recently demonstrated to play a role in metabolism, as two coinciding studies show that JHDM2a−/− mice develop to become obese (Tateishi et al., 2009; Inagaki et al., 2009). The mechanisms proposed in these two studies are different. Tateishi et al. attribute the obesity to impaired brown adipose tissue function, likely due to a reduced expression of genes involved in energy metabolism, as UCP1 and PPARα, and an impaired β-adrenergic signaling (Tateishi et al., 2009). Interestingly, the JHDM2a−/− mice developed by Inagaki also show a full-blown metabolic syndrome, but brown adipose tissue was not affected (Inagaki et al., 2009). In contrast, a set of genes was specifically reduced in white adipose tissue, including the anti-adipogenic transcription factor COUP-TFI (Xu et al., 2008), an inhibitor of fat storage like ApoC1 (Jong et al., 2001), and ADAMTS9, a gene associated to type 2 diabetes by genome-wide association studies (Zeggini et al., 2008). As both

Figure 3. Schematic Representation of How Cofactor Biosynthesis Is Dependent on Intracellular Energy Status

The ATP produced by the intracellular metabolism of nutrients is the key molecule for the biosynthesis of coenzymes. MAT converts methionine to SAM by using ATP. RFK and FLAD use also ATP to synthesize FAD from riboflavin. High intracellular ATP levels in contrast inhibit α-KGDH, a key enzyme in the TCA (tri-carboxylic-acid cycle) that converts α-ketoglutarate to succinyl-CoA (Smith et al., 1974). Words depicted in blue indicate precursors for coenzymes biosynthesis; words in green indicate coenzymes directly involved in methylation (SAM) and/or demethylation (FAD and α-KG) reactions. Abbreviations are found in the text.
mechanisms proposed are not mutually exclusive, further studies are required to clarify the molecular pathways involved in the phenotypic changes in JHDM2a−/− mice.

Although all these studies suggest metabolic functions of HMTs/HDMs, they are far from establishing how these enzymes are integrated in metabolic control. In fact, no hard biochemical evidence is provided that the levels of the metabolic cofactors (SAM, FAD and α-KG) were affected by the metabolic changes, a condition sine qua non to establish the link between methylation/demethylation and metabolism. Furthermore, it is unclear how HMTs/HDMs are selectively targeted to certain promoters (e.g., NFKB-p65 gene) to achieve highly specific effects on certain genes but not others. From the apparent contradictory results, showing on the one hand LSD1 recruitment and persistent demethylation of H3K9 (Brasacchio et al., 2009) and on the other hand LSD1 depletion and hypermethylation of H3K4 (Reddy et al., 2008), it also emerges that the role of high glucose in the control of LSD1 activity and methylation is complex and contentious. Another key question is how transient changes in glycerol induce long-lasting alterations in chromatin state. Although this so-called “metabolic memory” concept has been invoked to explain the sustained legacy effect of blood glucose control observed in the Diabetes Control and Complications Trial and the United Kingdom Prospective Diabetes Study (Nathan et al., 2005; Holman et al., 2008), further mechanistic work is required to establish a role for HMTs/HDMs in this phenomenon.

Conclusions and Future Perspectives

The epigenome has been hypothesized to provide an important part of the interface between the environment and the regulation of gene expression (reviewed by Ladurner, 2006 and Feinberg, 2007). Clearly, one of the most important environmental factors is the availability of energy. In the body, energy-rich substrates, such as carbohydrates and fats, are converted into ATP, with concomitant increases in the levels of metabolites such as acetyl-CoA, NAD+/NADH, SAM, α-KG, and FAD by processes like glycolysis, fatty acid oxidation, and oxidative phosphorylation. These metabolites, in turn, are the high-energy substrates used as coenzymes by chromatin and DNA-modifying enzymes to drive epigenetic modifications and alter gene expression (Ladurner, 2006; Tsukada et al., 2006; Anand and Marmorstein, 2007; Feige and Auwerx, 2007; Wellen et al., 2009) (Figure 4). Sensing these intermediary factors is hence a prime way to inform cells about the energy availability.

For the lysine acetyl transferases (KATs) and deacetylases (KDACs), solid evidence is emerging that they act as real energy sensors that are respectively activated by high and low intracellular energy levels, and that they translate these metabolic signals into alterations in chromatin structure and transcription (reviewed by Feige and Auwerx, 2007; Dominy et al., 2010; Jeninga et al., 2010; Zhao et al., 2010). Furthermore, the characterization of these KATs and KDACs has demonstrated a role for some of them in the control metabolic flexibility and linked them to the pathogenesis of common complex disorders of the metabolic, cardiovascular, and nervous systems. In analogy to the KATs and KDACs, it is tempting to speculate that the HMTs and HDMs could fulfill similar functions. The phenotypic characterization of various genetically engineered mouse models for the HMTs and HDMs, which show some metabolic abnormalities, argues that they could in fact control metabolic processes. Further support for such a role is provided by studies in C. elegans and D. melanogaster, that assign a role for HMTs/HDMs in longevity (Chen et al., 2009; Greer et al., 2010; Siebold et al., 2010), which traditionally has been linked to metabolism (reviewed by Bordone and Guarente 2005; Houtkooper et al., 2010).

Despite the implication of HMTs and HDMs in metabolic control, at present evidence to support that they are bona fide energy sensors that directly synchronize metabolic information with transcription is still circumstantial. To turn this speculation into hard facts, further research is required. First, the dependence of HMTs/HDMs on their metabolic coenzymes was often determined in highly artificial test-tube cell-free conditions and therefore needs also to be established in cellular systems and in the intact organism. Evidence how the rapidly changing physiological and developmental homeostatic context affects the dynamic levels of metabolic coenzymes, and how this then is integrated by the HMTs/HDMs to alter transcriptional homeostasis in a highly specific manner, is also lacking. Within this context, particular attention should be given to solve the apparent contradiction that the cellular energy levels positively regulate the synthesis of both coenzymes for HMTs and HDMs. Furthermore, clarification is required about how these nuclear localized HMTs and HDMs capture these
metabolic coenzymes, which are often produced in different cellular compartments (mitochondria and cytoplasm). Finally, the temporal nature of an eventual coupling between metabolic changes, coenzyme levels, HMT/HDM activity, histone methylation, and changes in gene expression needs to be solved. In other words, how can extremely dynamic metabolic processes adapt transcription over extended periods? In the absence of unequivocal evidence for the role of HMTs and HDMs as energy sensors, the simple alternative that energy sensing involves other factors that ultimately transmit this information to transcription factors in the nucleus, which then recruit HMTs and HDMs to specific genomic loci to alter gene expression and maintain metabolic homeostasis, remains a safe explanation.

Despite these reservations, we think that over the next a number of studies will emerge that will help us to unequivocally define whether and how HMTs/HDMs contribute to metabolic control. We can only hope that, in analogy to the KATS and KDACs, these HMTs and HDMs may also provide us with new targets for prevention and treatment of metabolic diseases.

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REFERENCES


Perspective