Connecting Threads: Epigenetics and Metabolism

Sayako Katada,[1] Axel Imhof,[1,2] and Paolo Sassone-Corsi[1,*]
[1]Center for Epigenetics and Metabolism, School of Medicine, University of California, Irvine, Irvine, CA 92697, USA
[2]Munich Center of Integrated Protein Science, Adolf-Butenandt Institute, Ludwig Maximilians University of Munich, 80336 Munich, Germany
*Correspondence: psc@uci.edu
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Chromatin-modifying enzymes have long been proposed to be the authors of an epigenetic language, but the origin and meaning of the messages they write in chromatin are still mysterious. Recent studies suggesting that the effects of diet can be passed on epigenetically to offspring add weight to the idea that histones act as metabolic sensors, converting changes in metabolism into stable patterns of gene expression. The challenge will now be to understand how localized fluctuations in levels of metabolites control chromatin modifiers in space and time, translating a dynamic metabolic state into a histone map.

The excitement about epigenetics and chromatin remodeling that has characterized biological research during the past two decades is in large part focused on a single question: how is gene expression stably reprogrammed in response to transient external stimuli? Plasticity is at the heart of all biological functions, and, in the case of epigenetic control, it involves a large variety of mechanisms that have evolved to ensure adaptability to a multitude of signals, conditions, and organisms (Turner, 2009).

Epigenetic mechanisms control chromatin structure through DNA methylation, RNA interference, histone variants, and posttranslational modifications. The interplay of these regulatory mechanisms suggests that the coordinate and progressive combination of these processes may "lock" the epigenome in specific states, thereby determining the fate and physiology of a given cell (Borrelli et al., 2008). The molecular machines implicated in interpreting changes in the environment and translating them into ad hoc modulations of the epigenome are constructed from various interacting components, including kinases, acetyltransferases, and methyltransferases (Lee and Workman, 2007). These enzymes use cellular metabolites as sources of phosphate, acetyl, or methyl groups, respectively. Considering the myriad of residues on either DNA or histone tails that can potentially undergo modifications at a given time in the genome, appropriate levels of phosphate, acetyl, and methyl groups need to be available to the enzymes eliciting the modifications. This raises an interesting possibility: do changes in the levels of cellular metabolites influence the epigenome? Also, to what extent do those alterations induce inheritable effects? Here we review some recent ideas and discoveries that illustrate how the link between metabolism and epigenetics extends to previously unappreciated levels.

Metabolic States Influence Chromatin Structure

Because histone-modifying enzymes consume key metabolites, it is conceivable that they interpret the metabolic state of a given cell by changing chromatin modification patterns (Figure 1). Consistent with this, a global reduction of nuclear acetyl-CoA levels decreases histone acetylation, whereas reduced levels of NAD^+ have the opposite effect, inhibiting histone deacetylation (Nakahata et al., 2009; Wellen et al., 2009). However, the global level of a given metabolite is unlikely to be the sole determinant of the enzymatic activity of a specific chromatin remodeler. Indeed, rising levels of ATP do not increase the phosphorylation of all substrates equally, clearly indicating that changes in metabolite levels alone are not the only impetus for epigenetic editing. Histone-modifying enzymes can be recruited to specific chromosomal domains via their interaction with DNA-binding factors, and this can stimulate enzymatic activity locally. Alternatively, the inhomogeneous distribution of metabolites within a cell could lead to a local depletion or an excess of cofactors for histone-modifying enzymes with a similar outcome. Such spatial concentration differences could be achieved by the subcellular localization of enzymes responsible for the synthesis of individual metabolites, and hence the distribution would be different for each metabolite.

Similarly, enzymes that use the same metabolite but modify different substrates, such as DNA or histone methyltransferases, may compete with each other leading to either one or the other methylation product. This raises the question of whether the affinities of enzymes for their cofactors differ from each other such that they would be able to gauge metabolite concentration. Unfortunately information on the kinetic parameters of histone-modifying enzymes is sparse, and studies of the dynamic changes in metabolite concentrations are limited. Despite this paucity of available data, it is clear that modifying enzymes vary significantly in their affinities for their cofactors and have K_M values that are similar to the corresponding

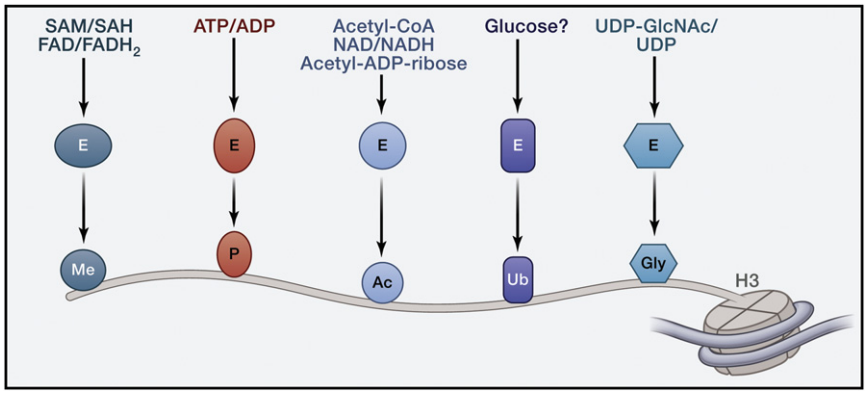

Figure 1. Chromatin-Remodeling Enzymes "Sense" Cellular Metabolism
Schematic representation of the histone H3 tail with residues that can be modified by various enzymes (E), leading to phosphorylation (P), acetylation (Ac), methylation (Me), ubiquitination (Ub), and glycosylation (Gly). These modifications have been associated with changes in chromatin organization, gene activation, silencing, and several other nuclear functions. Each enzyme utilizes cellular metabolites, whose availability would dictate the efficacy of the enzymatic reaction.

cofactor concentrations in vivo (Albaugh et al., 2011; Sauve et al., 2006). Global and local fluctuations of cofactor concentrations would therefore have an effect on the ability of these enzymes to fulfill their function. Such changes in concentrations may be caused by circadian rhythmicity, nutritional inputs, variation in carbon sources, or changes in oxygenation of the cell. Most of these external influences will globally change the cofactor concentration within the cell and will therefore have a global impact on histone modifications. However, as mentioned above, accumulating evidence indicates that histone-modifying enzymes could also make use of local changes in metabolite concentration to elicit domain-specific chromatin remodeling (Katoh et al., 2011; Wellen et al., 2009).

Subcellular Distribution of Metabolites: Chromatin "Niches" or Microdomains?

The cytosol and, even more strikingly, the nucleus of a eukaryotic cell contain a very high concentration of biological macromolecules such as proteins and DNA (200 mg/ml). This dense and very viscous medium severely restricts free diffusion of small molecules, thereby significantly slowing down biochemical reactions that are purely diffusion controlled. Intriguingly, several metabolic pathways are conveniently organized in multiprotein complexes to allow reaction channeling, which facilitates signaling. A classical example in this respect is fatty acid synthase (FAS), a large multiprotein complex that has been proposed to be constructed in a "molecular assembly line" to promote efficient channeling of substrates from one enzyme to the next (Leibundgut et al., 2008). Recent data suggest that close coupling of histone-modifying enzymes with enzymes critical for cofactor synthesis also exists in the nucleus (Katoh et al., 2011; Wellen et al., 2009). For example, Mat IIIa, an enzyme that catalyzes the formation of SAM from methionine and ATP, interacts with a sequence-specific transcription factor in order to maintain a high SAM concentration, which is ultimately used by an H3K9-specific histone methyltransferase to repress transcription (Katoh et al., 2011). Similarly, depending on the main carbon source, ATP-citrate lyase (glucose) or the acetyl-CoA synthetase ACS1 (acetate) localize to the nuclei of LN229 cells (Wellen et al., 2009) or *S. cerevisiae* (Takahashi et al., 2006) to provide a sufficient source of acetyl groups for histone acetyltransferases. Within those nuclear metabolic domains, eraser enzymes such as deacetylases might generate a local increase in acetate groups, which could be used by ACS1 to generate acetyl-CoA for the corresponding HAT enzyme. It is plausible that such a dynamic turnover of acetyl groups could be critical for transcriptional activation. The presence of "niches" or microdomains of chromatin modifications, where the substrates for histone modifiers are immediately replenished after the modification, could also help to insulate specific domains against spikes of metabolic changes (Figure 2). Importantly, the degree of insulation would not be homogenous along the chromatin. Indeed, it could very much depend on the level of molecular crowding, making constitutive heterochromatin much less sensitive to metabolic alterations than euchromatic regions (Bancaud et al., 2009).

Posttranslational Regulation of Histone Modifiers

Histones are not the only proteins that are modified by acetyltransferases, methyltransferases, or kinases. Fluctuations in cofactor concentrations could therefore lead to alterations not only in histone modifications but also in posttranslational modifications of the enzymes that then in turn modify histones. Indeed, many of these enzymes or accessory proteins are modified, with the modification affecting their enzymatic activity (Vaquero et al., 2007), their ability to bind chromatin (Wei et al., 2011), or the subunit composition of a large complex (Huang et al., 2007). Such modification networks result in complex feedback loops that translate physiological changes into changes within the epigenome. High glucose levels can, for example, stimulate the activity of the mammalian methyltransferase MLL5 through increased GlcNAcylation (Fujiki et al., 2009). This modification is catalyzed by the interacting O-GlcNac transferase (OGT), which is dependent on the presence of sufficient nuclear UDP-GlcNac. UDP-GlcNac is synthesized from extracellular glucose by the hexosamine biosynthesis pathway, thereby linking the activity of a histone-modifying enzyme directly to the extracellular concentration of glucose. This connection between glucose levels and chromatin structure seems to be evolutionarily conserved, as a mutation of the OGT gene in *Drosophila* leads to a *polycomb*-like phenotype (Sinclair et al., 2009).

The systematic analysis of reversible lysine acetylation (Kim et al., 2006; Choudhary et al., 2009) has revealed a role for acetylation in directly regulating energy metabolism as most of the

acetylated proteins in mitochondria are involved in various catabolic pathways. However, acetylation is not limited to the mitochondria as a large fraction of acetylated proteins are either exclusively nuclear or shuttle between cytoplasm and the nucleus (Choudhary et al., 2009). Interestingly, many components within large protein assemblies that either bind or modify chromatin are heavily acetylated. Despite this wealth of information on lysine acetylation, only in very few cases have the function or the regulation of the modification been explored. For example, HATs are acetylated on lysine residues, an event that increases their activity by protecting them from degradation (as in the case of p300), modulating their nuclear transport (as for P/CAF), or increasing the binding affinity for acetyl-CoA (as for Rtt109). In contrast to most acetyltransferases, the methyltransferase SUV39H1 is inhibited by reversible lysine acetylation of a single lysine within the catalytic SET domain (Vaquero et al., 2007). In light of the known importance of acetyl-CoA levels on HAT activity, it is very likely that all of these modifications are in fact coupled to metabolism and could translate physiological states into alterations of gene expression.

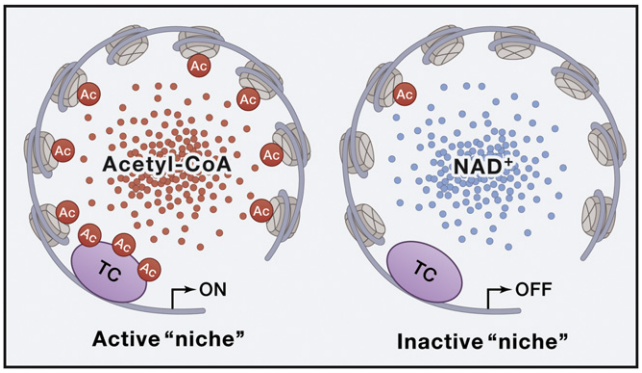

Figure 2. Hypothetical Organization of Chromatin "Niches" or Microdomains

The concentration of metabolites and their biosynthetic enzymes may vary within subdomains of chromatin, leading to localized transcriptional activation or inactivation. In this example, in a microdomain with high levels of acetyl-CoA, there will be higher availability of acetyl groups, facilitating acetylation of histone and nonhistone proteins in transcriptional complexes (TC), leading to activation of gene expression. On the other hand, high levels of NAD^+ within a "niche" would lead to the activation of HDACs of the sirtuin class, inducing deacetylation of substrates and transcriptional silencing. Such local differences could be achieved by local "trapping" of enzymes responsible for critical metabolic pathways within the domain.

NAD^+, a Master Metabolite?

A large variety of enzymes depend on the coenzyme NAD^+, including at least two groups of chromatin regulators, the class III HDACs (sirtuins) and the PARPs (poly-ADP ribose polymerases). Changing levels of a single metabolite may therefore impact independent groups of enzymes with different functions. Other enzymes such as HATs may also be indirectly affected by changing concentrations of NAD^+. Mutual interplays are also conceivable within the sirtuin family. There are seven sirtuins, which display distinct distributions in the cytoplasm, nucleus, and mitochondria. All seven sirtuins are thought to require NAD^+, although it is still unclear whether their affinities for the coenzyme differ (Sauve et al., 2006). In any case, it could be envisaged that the relative activity of the three sirtuins present in the nucleus (SIRT1, SIRT6, and SIRT7) may modulate the local concentration of NAD^+, thereby resulting in reciprocal regulation of the other sirtuins that may be localized in the same or nearby chromatin microdomains. In this respect, it is essential to evaluate how the concentration of specific metabolites may vary in various physiological conditions.

It has been shown that levels of NAD^+ are regulated in a circadian manner, establishing a direct link between cyclic rhythms and energy metabolism in the cell (Nakahata et al., 2009; Ramsey et al., 2009). Although expression levels of SIRT1 are noncyclic, HDAC activity is known to fluctuate in a circadian manner (Nakahata et al., 2008). Subsequent studies revealed that the cyclic availability of its own coenzyme, NAD^+, is responsible for these oscillations in SIRT1 HDAC activity (Nakahata et al., 2009; Ramsey et al., 2009). NAD^+ synthesis is directly regulated by the circadian clock machinery, which controls transcription of the *Nampt* gene. This gene encodes an enzyme (nicotinamide phosphoribosyltransferase; NAMPT) that elicits the rate-limiting step in the NAD^+-salvage pathway. Thus, changes in NAMPT activity directly dictate levels of intracellular NAD^+. These findings suggest that several SIRT1 targets are likely to display circadian oscillations in their acetylation. This is indeed the case for K9/K14 histone H3 sites at circadian gene promoters, as well as BMAL1, a nonhistone target of SIRT1 that operates as a transcriptional coactivator of the circadian regulator CLOCK (Nakahata et al., 2009).

These findings suggest that the circadian clock and energy metabolism are directly coupled. This coupling is based on chromatin remodeling at specific genomic sites. Although the specificity of action of these events is still unclear, it is however evident that the circadian clock is directly implicated in controlling the intracellular levels of critical metabolites, locking together the transcriptional feedback loop of the clock with the enzymatic feedback loop of the NAD^+ -salvage pathway. The extent to which the clock controls intracellular levels of other metabolites is not known, but the possibility is intriguing as a significant fraction of the genome is transcriptionally controlled by the circadian machinery.

Disease and Epigenetics—Is Metabolism the Link?

Many metabolites have been shown to have a direct effect on gene expression patterns through binding to nuclear receptors that in turn affect the transcription of the gene they bind to. Interestingly, even transient changes in the nutrition can have a long-lasting impact on gene expression patterns. This memory of former metabolic states may also be involved in disease progression. For example, patients suffering from diabetes mellitus are more prone to develop medical complications associated with hyperglycemia, even though their blood glucose is maintained at normal levels by standard therapies. Similar memory effects are also observed during embryonic development or cellular

differentiation, where genes "remember" their activity states with the help of the epigenetic machinery.

Several observations imply that variation in dietary composition can lead to increased disease susceptibility in subsequent generations, suggesting the transmission of a metabolically induced epigenetic signal to the next generation. For example, children whose mothers experienced the 1944 winter famine in the Netherlands late in their pregnancy have a smaller birth size and a higher risk of developing cardiovascular disease, obesity, and type 2 diabetes (Painter et al., 2005). Another study shows that the nutritional state during the slow growth phase in puberty has a fundamental effect on the mortality of the following generation (Kaati et al., 2002). The mechanisms that direct the inheritance of such predispositions to disease are unknown, but the fact that they are induced by metabolic changes and show a high level of variability underscores their epigenetic nature.

Heritable effects of metabolic disturbances have been mimicked by the ablation of key epigenetic enzymes. For example, the liver-specific ablation of the *Sirt1* gene leads to impaired metabolic signaling and causes hepatic steatosis and inflammation when the animals are fed a high-fat diet (Purushotham et al., 2009). Pharmacological activation of SIRT1, on the other hand, leads to improved insulin sensitivity under insulin-resistant conditions (Sun et al., 2007). These findings pave the way to the development of therapeutic strategies that would use SIRT1 activators as potential lead molecules for the treatment of type 2 diabetes. The central function of HDACs in modulating metabolic circuits is also evident in mice deficient in SIRT6, a nuclear sirtuin that is activated through its association with chromatin. These animals develop lethal hypoglycemia and die soon after birth (Zhong et al., 2010). Finally, histone demethylation is also critical for metabolic regulation. Mice carrying an inactive allele of the gene encoding the histone H3K9-specific demethylase KDM3a become obese in adulthood and have increased levels of circulating lipids. This metabolic alteration is very likely due to an increase in the abundance of repressive H3K9me modifications and a consequent downregulation of genes involved in fatty acid oxidation (Tateishi et al., 2009).

Major metabolic changes are frequently observed in cancers, in which cells switch to an anaerobic metabolism even in the presence of oxygen. This so-called "Warburg Effect" is accompanied by major alterations in gene expression profile whose causes are likely to be associated with specific chromatin-remodeling events. Recently, the genes encoding isoforms of the enzyme isocitrate dehydrogenase (IDH1 and IDH2) have been found to be mutated in a large variety of tumors. In addition to a reduction of the central metabolite 2-oxoglutarate, the mutated alleles of the *Idh1* and *Idh2* genes generate the oncometabolite 2-hydroxyglutarate, which acts as an inhibitor for several epigenetic modifiers, including demethylases containing the *jumonji* domain and the TET family of 5-methylcytosine hydroxylases (Xu et al., 2011). Thus, a defect in genes encoding metabolic enzymes could directly influence the enzymatic function of epigenetic regulators, leading to an increase in histone and DNA methylation. Causal connections between IDH mutations, changes in epigenetic modifications, and the altered patterns of gene expression observed when cancer cells switch to an anaerobic metabolism have not yet been demonstrated. However, it is tempting to speculate that the epigenetic reprogramming induced by an aberrant metabolite plays an important role in this process.

In addition to using multiple and varied cofactors, epigenetic modifiers also have vastly different kinetics (Barth and Imhof, 2010). This raises the question of the length and amplitude of the metabolic stimulus required to switch between metabolic states.

As we begin to connect the threads linking epigenetic modifications and metabolic pathways, an important challenge will be to determine how plastic the epigenome is to nutritional challenges and whether epigenetic changes triggered by altered metabolic states can be reversed. Despite the fact that we are only beginning to understand this dynamic relationship, accumulating data already give the German saying "man ist was man isst" (one is what one eats) a completely new meaning.

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