

## IN FOCUS

## A Tale of Metabolites: The Cross-Talk between Chromatin and Energy Metabolism

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**Summary:** Mitochondrial metabolism influences histone and DNA modifications by retrograde signaling and activation of transcriptional programs. Considering the high number of putative sites for acetylation and methylation in chromatin, we propose in this perspective article that epigenetic modifications might impinge on cellular metabolism by affecting the pool of acetyl-CoA and S-adenosylmethionine. *Cancer Discov*; 3(5): 497-501. ©2013 AACR.

### INTRODUCTION

Metabolism can be defined as the sum of chemical reactions that occur within a cell to sustain life. It is also the way that a cell interacts with energy sources: In other words, it is the coordination of energy intake and its use and storage that ultimately allows growth and cell division. In animal cells, mitochondria have evolved to become the most efficient system to generate energy. This organelle consumes carbon sources via oxidative phosphorylation to produce ATP, the energy currency of the cell. In addition, the mitochondria produces intermediate metabolites for the biosynthesis of DNA, proteins, and lipids.

Under basic dividing conditions, uptake of nutrients is tightly regulated through growth signaling pathways, and thus differentiated cells engage in oxidative metabolism, the most efficient mechanism to produce energy from nutrients. Cells metabolize glucose to pyruvate through glycolysis in the cytoplasm, and this pyruvate is then oxidized into CO<sub>2</sub> through the mitochondrial tricarboxylic acid (TCA) cycle. The electrochemical gradient generated across the inner mitochondrial membrane facilitates ATP production in a highly efficient manner. Studies in recent years indicate that under conditions of nutrient excess, cells increase their nutrient uptake, adopting instead what is known as aerobic glycolysis, an adaptation that converts pyruvate into lactate, enabling cells to produce intermediate metabolites to sustain growth (anabolic metabolism; ref. 1). Interestingly, most cancer cells undergo the same metabolic switch (Warburg effect), a unique evolutionary trait that allows them to grow unabated. Although aerobic glycolysis generates much less ATP from glucose compared with oxidative phosphorylation, it provides critical intermediate metabolites that are used for anaplerotic reactions and therefore is an obligatory adaptation among highly proliferative cells. In response to variations in nutrient availability, cells regulate their metabolic output, coordinating biochemical reactions and mitochondrial activity by alter-

ing transcription of mitochondrial genes through activation of transcription factors such as PGC1 $\alpha$  and chromatin modulators that exert epigenetic changes on metabolic genes.

Mitochondrial dysfunction has been implicated in aging, degenerative diseases, and cancer. Proper mitochondrial function can be compromised by the accumulation of mutations in mitochondrial DNA that occur during aging. In addition, reactive oxygen species (ROS) produced during oxidative phosphorylation can promote oxidative damage to DNA, protein, and lipids, in turn adversely affecting global cellular functions. In recent years, several studies have illustrated a novel unexpected link between metabolism and gene activity: Fluctuations in mitochondrial and cytoplasmic metabolic reactions can reprogram global metabolism through their impact on epigenetic dynamics. These studies are briefly summarized in the first part of this article. In the second part, we propose a provocative novel hypothesis: The cross-talk between metabolism and epigenetics is a two-way street, and defects in chromatin modulators may affect availability of intermediate metabolites, in turn influencing energy metabolism.

### METABOLISM AFFECTS EPIGENETICS

A regulated cross-talk between metabolic pathways in the mitochondria and epigenetic mechanisms in the nucleus allows cellular adaptations to new environmental conditions. Fine-tuning of gene expression is achieved by changes in chromatin dynamics, including methylation of DNA and posttranslational modifications of histones: Acetyl, methyl, and phosphate groups can be added by acetyltransferases, methyltransferases, and kinases, respectively, to different residues on histones. Given the number of residues that can potentially undergo modifications in histone tails and in the DNA, it is reasonable to consider that metabolic changes affecting the availability of these metabolites will affect epigenetics (as discussed below).

Recently, acetylation of proteins was revealed to be as abundant as phosphorylation (2). This posttranslational modification involves the covalent binding of an acetyl group obtained from acetyl-CoA to a lysine. In histones, acetylation can modify higher-order chromatin structure and serve as a docking site for histone code readers. Recent mass spectrometry studies have uncovered the complete acetylome in human cells and revealed that protein acetylation occurs broadly in the nucleus, cytoplasm, and mitochondria, affecting more than 1,700 proteins (2). Acetylation of proteins depends on the availability of

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**doi:** 10.1158/2159-8290.CD-13-0059

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acetyl-CoA in each cellular compartment, but this metabolite is produced in the mitochondria and cannot cross the mitochondrial membrane. In single-cell eukaryotes, the pool of acetyl groups required for histone acetylation comes from the production of acetyl-CoA by the enzyme acetyl-CoA synthetase (Acs2p), which is responsible for converting acetate into acetyl-CoA. In mammalian cells, although they have a homolog enzyme to Acs2p, AceCS1, the majority of acetyl-CoA is produced from mitochondrion-derived citrate by the enzyme adenosine triphosphate (ATP)-citrate lyase (ACL; ref. 3). ACL is present in the cytoplasm and in the nucleus and is responsible for the production of acetyl-CoA from citrate in both compartments. Citrate is generated in the metabolism of glucose and glutamine in the TCA cycle. In contrast with acetyl-CoA, citrate can cross the mitochondrial membrane and diffuse through the nuclear pores into the nucleus, where it can be converted into acetyl-CoA by ACL. Wellen and colleagues (3) found that ACL is required for the acetylation of histones under normal growth conditions; knockdown of ACL decreases the pool of acetyl-CoA in the nucleus and reduces the level of histone acetylation. Strikingly, reduction in histone acetylation occurs preferentially around glycolytic genes, leading to the downregulation of their transcription and therefore to the inhibition of glycolysis. These observations reveal a process through which glucose metabolism dictates histone acetylation that, in a feedback mechanism, controls the rate of glycolysis.

Notably, deacetylation of histones also exhibits a metabolic influence. Deacetylation of histones is achieved by class I and class II histone deacetylases (HDAC) and by a separate class (class III), also known as sirtuins. Sirtuins use  $\text{NAD}^+$  as a cofactor for deacetylation, and the ratio of  $\text{NAD}^+/\text{NADH}$  regulates their activity. In diets that are rich in carbohydrates, growth factors stimulate cellular glucose uptake, and the production of energy is carried out through glycolysis. In this context, the  $\text{NAD}^+/\text{NADH}$  ratio decreases, in turn inhibiting, in theory, sirtuins in the cytoplasm (SIRT2) and nucleus (SIRT1, SIRT6, and SIRT7). In fact, low SIRT1 and SIRT6 activity generates a global increase in protein acetylation. Interestingly, SIRT6, which is exclusively nuclear, deacetylates histone H3 lysine 9 (H3K9) at HIF-1 $\alpha$  target genes, repressing their transcription. Because most of these genes are glycolytic, deacetylation of histones by SIRT6 modulates glycolysis. Indeed, *Sirt6*-deficient mice experience a dramatic increase in glucose uptake for glycolysis, triggering a fatal hypoglycemia in a few weeks (4).

In animal cells, both histone acetylation and deacetylation are under the control of glucose metabolism through the availability of acetyl-CoA and  $\text{NAD}^+$ , respectively. However, is this metabolic control restricted to acetylation, or can other reactions in the nucleus be influenced by the energy status of the cell?

Histone methyltransferases use *S*-adenosylmethionine (SAM) to transfer a methyl group onto lysine and arginine residues on histone tails. SAM is produced from methionine by the enzyme *S*-adenosyl methionine transferase (MAT) in a reaction that uses ATP. The recent finding of MAT in the nucleus suggests that the SAM pool could also be controlled locally in this compartment (5). The reverse reaction catalyzed by histone demethylases (HDM) uses flavin adenine dinucleotide ( $\text{FAD}^+$ ) and  $\alpha$ -ketoglutarate as coenzymes. FAD is a common redox coenzyme that exists in 2 different redox states. In its reduced state,  $\text{FADH}_2$  is a carrier of energy, and when oxidized,  $\text{FAD}^+$

is consumed in the oxidation of succinate to fumarate by the enzyme succinate dehydrogenase (complex II) in one of the last steps of the TCA cycle. On the other hand,  $\alpha$ -ketoglutarate is an intermediate in the TCA cycle. It is generated from isocitrate by the enzymes isocitrate dehydrogenase 1 and 2 (IDH1-cytosolic and IDH2-mitochondrial; Fig. 1A and B). On the basis of these findings, it is easy to infer that the amount of coenzymes used for histone methylation and demethylation could also be controlled by metabolic reactions. Moreover, the different cellular compartments compete for the same metabolites. Indeed, changes in diet that affect the biosynthesis of SAM, FAD, and  $\alpha$ -ketoglutarate in the mitochondria and cytoplasm have been shown to affect histone methylation (6).

More recently, some of the metabolic enzymes responsible for producing cofactors for nuclear biochemical reactions have been found to be mutated in cancer. For instance, *IDH1* and *IDH2* somatic mutations are recurrent in gliomas and acute myeloid leukemias. These mutations lead not only to a decreased production of  $\alpha$ -ketoglutarate but also to a new activity:  $\alpha$ -ketoglutarate is in fact converted into 2-hydroxyglutarate (2-HG), a metabolite rarely found in normal cells. The new metabolite is a competitive inhibitor of  $\alpha$ -ketoglutarate-dependent dioxygenase enzymes, including the Jumonji C (JmjC) domain containing HDM and the recently discovered TET family of 5-methylcytosine (5mC) hydroxylases involved in DNA demethylation (7). By inhibiting JmjC and TET enzymes, the aberrant production of 2-HG generates a genome-wide histone and DNA hypermethylation phenotype. This is considered to be, at least in part, at the origin of tumorigenesis in IDH1- and IDH2-mutated cells, and for this reason, 2-HG may earn its place as an oncometabolite. The discovery that mutations in metabolic enzymes may influence tumorigenesis by means of controlling genome-wide epigenetic changes caused a paradigm shift, indicating that such metabolic abnormalities may affect cancer beyond the Warburg effect.

## CAN THE NUCLEUS AFFECT METABOLISM BY UNBALANCING ACETYL-CoA AND SAM POOLS?

If changes in metabolic pathways such as the TCA cycle and one-carbon metabolism can modulate histone and DNA modifications due to the availability of acetyl and methyl group, is the reverse possible? In other words, can sustained changes in the activity of a chromatin modifier leading to retention of excessive acetyl or methyl moieties in chromatin cause the cellular pool of these metabolites to be threatened? In such a scenario (see Fig. 1A and B), the imbalance of acetyl or methyl groups would likely induce immediate changes in cellular metabolism before any transcriptional program is affected. For example, if histone acetylation is drastically increased, the concentration of acetyl-CoA might suddenly drop. Most likely, this would slow down those processes requiring acetyl-CoA (TCA cycle, lipogenesis, and 3-hydroxy-3-methylglutaryl-CoA synthesis), causing significant metabolic consequences much before gene transcription is affected. If the cause of the imbalance in the acetyl pool is eliminated, the cell will resume its normal metabolism, but if the original problem persists, the situation might evolve toward a chronic and severe defect in glucose and fat metabolism. Similarly, in cases in which



targets on chromatin, a number that could easily sequester acetate in the nucleus to a point where acetyl-CoA pools would be affected, hence impacting on cellular metabolism.

In this context, histone acetylation is a highly dynamic process and residues undergo transient modifications necessary to dictate adaptive changes in gene expression. This rhythm is maintained by the alternate action of histone acetyltransferases and deacetylases, the activities of which are coordinated in a highly regulated fashion. But what if this balance is disrupted? For example, H3K9 is a target of the HDAC SIRT6 (4), and only about 20% of this residue is most likely acetylated (8). *Sirt6*-null cells exhibit high glycolysis and low TCA cycle activity. Part of the mechanism is clearly transcription-dependent: SIRT6 deacetylation of H3K9 at the promoters of glycolytic genes is required to slow down the expression of these genes (4). However, it is emblematic how the absence of an HDAC has such a profound impact on the TCA cycle, a pathway that relies directly on acetyl-CoA availability. *Sirt6*-null cells accumulate 3- to 5-fold more acetyl groups on chromatin, as shown by changes in levels of H3K9 and H3K56 acetylation in bulk chromatin and not only at the promoters of glycolytic genes (4, 10, 11). With such an increase in acetyl molecules retained in the nucleus, it is possible that fewer acetyl moieties are available for acetyl-CoA synthesis, directly impinging on mitochondrial metabolism. Indeed, nuclear acetate is not used to directly replenish the acetyl-CoA pool but is rather recycled as citrate. Under conditions of nutrient limitations, in which cells are forced to recycle citrate to replenish the acetyl-CoA pool, a decrease in citrate metabolism might result in reduced acetate/acetyl-CoA availability. In this context, the changes in glycolytic gene transcription programs observed in *Sirt6*-deficient cells may not only be a response to nutrient stress but may also represent an important adaptive mechanism against a limiting acetyl-CoA pool. Although this represents an intriguing model, such a hypothesis remains to be experimentally tested.

### SAM Availability and Metabolism

Similar to acetylation, methylation of histones could also affect cellular metabolism by means other than transcriptional regulation; it might, in fact, alter cellular pools of SAM. In the case of methylation, the effect could actually be even more extreme: Each lysine or arginine on histones can potentially carry more than one methyl group and, most importantly, DNA can also be methylated. If we try to translate this statement to numbers, we could theorize that at least 22 residues per nucleosome can be methylated (i.e., R2, R3, K4, K9, R17, R26, K27, K36, and K72 on H3, plus R3 and K20 on H4; 2 H3 and 2 H4 molecules per nucleosome); with  $3.5 \times 10^7$  nucleosomes, there are  $6.6 \times 10^8$  potential targets for methylation, each of which can take up to 3 methyl moieties, leading to almost  $10^9$  methyl groups that could be retained on chromatin, similar to our calculation for acetyl moieties. To this estimate, we need to add the methyl groups retained by DNA itself. It has been determined that about 70% of the CpGs in our genome are methylated, accounting for  $4 \times 10^7$  methylated sites; this means that in our genome, at least  $2 \times 10^7$  CpG islands are still unmethylated (12). Overall, these numbers allow us to speculate that chromatin could retain a sufficient number of methyl moieties to clearly jeopardize methyl pools in other compartments. Furthermore, considering that SAM, the only methyl donor in the cell, is

produced exclusively in the one-carbon metabolic pathway, it is reasonable to speculate that mutations in chromatin modifiers could severely affect SAM-dependent metabolism. Moreover, one-carbon metabolism relies on dietary folate, which could be limiting, further indicating that pools of SAM could be easily affected by methyl availability. Overall, considering the large number of methylation targets in chromatin and the limited SAM production potential in cells, it is reasonable to speculate that drastic changes in enzymes regulating the methylation status of chromatin could cause a severe imbalance in SAM pools.

One-carbon metabolism plays an essential role in methyl group biosynthesis, amino acid metabolism, nucleotide biosynthesis, and mitochondrial protein biosynthesis (13). Carbon 3 of serine (derived from glycolytic intermediates) is the primary source of one-carbon metabolism in both the cytoplasm and mitochondria. In addition, mitochondrial oxidation of serine to formate and  $\text{CO}_2$  is dependent on the respiratory state of the cell. Finally, dimethylglycine, a product of phospholipid turnover and choline oxidation, can be converted to glycine through the action of the dimethylglycine dehydrogenase and the sarcosine dehydrogenase enzymes. The latter is FAD dependent, and it is connected to the respiratory chain through the electron-transferring flavoprotein (14). Together, these considerations imply that one-carbon metabolism does not function independently of other cellular metabolic pathways, and changes in this pathway may trigger a domino effect that could impair mitochondrial respiration, in turn impinging on the TCA cycle.

Although experiments to test this hypothesis are yet to be carried out, it is remarkable that mice deficient for the HDM *Jhdm2a*, a H3K9-specific demethylase, exhibit a severe metabolic imbalance with morbid obesity, in particular affecting fatty acid oxidation and mitochondrial oxygen consumption (15). Once more, as discussed for *Sirt6*-null cells, it is undeniable that gene transcription contributes to this phenotype. However, we propose that changes in methyl availability with a concomitant decrease in SAM might also play a role in the observed metabolic abnormalities, a possibility that remains to be tested.

*We can't solve problems by using the same kind of thinking we used when we created them.*

—Albert Einstein

Although the idea we propose might seem the obvious consequence of a tug-of-war between metabolites available in the cell and those already engaged in protein modification, unfortunately, the solution to this question is not as intuitive as the flow of thought that generated it. There are, first of all, knowledge barriers to overcome. The primary obstacle to prove (or disprove) this hypothesis is that we might need to establish a proper mathematical model defining how many acetyl/methyl moieties chromatin should “sequester” to induce an unbalanced pool of these metabolites. To do so, we should know the constants ( $K_m$ ) regulating each of the enzymes involved in such reactions, and those are values that remain for the vast majority yet unknown. To bypass this obstacle, we could empirically verify this hypothesis. Yet, to follow this approach, we ought to find the opportune stimulus, or most likely stress, to induce a massive change in acetylation or methylation of chromatin because, as we previously discussed, the mutation of a chromatin modifier by itself might not impair the cellular acetyl/methyl pool unless in the

presence of nutrient stress. Another element to keep in mind is that changes in chromatin modifications will affect transcription, and several reports have shown the tight link between epigenetic and metabolic gene expression. Therefore, to test this theory, any change in chromatin modifications should exert their measurable effect on metabolism before transcription is affected, otherwise the two components (transcription-dependent and -independent) might become undistinguishable.

Overall, there are several difficulties in verifying this hypothesis, yet before the studies on ACL by Wellen and colleagues (3), there was no notion in the field that a single enzyme could drastically affect acetate availability for histone modifications. Moreover, it has been convincingly shown in yeast that acetate concentrations do actually fluctuate, modulating the growth cycle of the cell: Low acetate concentration corresponds with low mitochondrial metabolism and low histone acetylation, all features of resting phase, whereas a spike in the acetate concentration triggers the acetylation of histones and a burst of mitochondrial activity signaling the entrance into the oxidative phase (which is an active growth phase; ref. 9). Indeed, if acetate levels within the cells were saturating, this oscillation could not be possible.

Considering these observations, the question we asked at the beginning still stands: If acetate availability affects histone acetylation, does acetate (or methyl) availability in the cytoplasm affect metabolism?

## CONCLUSIONS

Chromatin modifications and cellular metabolism are tightly connected. Thus far, the only aspects that have been considered are the retrograde signaling, with mitochondrial metabolites affecting histone modifications, and the anterograde transcriptional regulation of metabolism. A third aspect of the link between the nucleus and metabolism has been, in our opinion, omitted so far: a direct influence of chromatin on acetyl-CoA and SAM availability, which may also have an essential role in cancer establishment and development (Fig. 1A and B). Notably, a shift toward glycolytic metabolism is now considered a hallmark of cancer cells. It is also true that multiple tumors carry mutations in chromatin modifiers. However, new studies suggest that those two processes may be much more intertwined than previously appreciated, further blurring the limits on their respective roles in tumorigenesis. There is no doubt that changes in metabolite availability can have a drastic impact on chromatin modifications. We believe that the opposite may be true as well. At least in mouse models, deficiency in two chromatin modifiers, SIRT6 and JHMDM2A, causes drastic metabolic abnormalities. Even though some of those phenotypes depend on changes in gene expression, we would like to propose that severe attrition of metabolite pools might play a role as well, a possibility that awaits experimental proof.

## Disclosure of Potential Conflicts of Interest

R. Mostoslavsky is a consultant/advisory board member of Sirtris-GSK. No potential conflicts of interest were disclosed by the other authors.

## Authors' Contributions

**Conception and design:** B. Martinez-Pastor, C. Cosentino, R. Mostoslavsky

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## Acknowledgments

The authors thank John Denu for thoughtful discussions, Sita Kugel for critically reading the manuscript, and the members of the Mostoslavsky laboratory for helpful discussions.

## Grant Support

The work in the Mostoslavsky laboratory is supported in part by NIH grants GM093072-01 and DK088190-01A1. C. Cosentino is supported by a fellowship from the Fondazione Umberto Veronesi and the American-Italian Cancer Foundation. B. Martinez-Pastor is the recipient of a postdoctoral fellowship from the Spanish Ministry of Education.

Published online May 8, 2013.

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