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Review

# Influence of Metabolism on Epigenetics and Disease

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**Chemical modifications of histones and DNA, such as histone methylation, histone acetylation, and DNA methylation, play critical roles in epigenetic gene regulation. Many of the enzymes that add or remove such chemical modifications are known, or might be suspected, to be sensitive to changes in intracellular metabolism. This knowledge provides a conceptual foundation for understanding how mutations in the metabolic enzymes SDH, FH, and IDH can result in cancer and, more broadly, for how alterations in metabolism and nutrition might contribute to disease. Here, we review literature pertinent to hypothetical connections between metabolic and epigenetic states in eukaryotic cells.**

## Introduction

Central to the many definitions of "epigenetics" is the knowledge that genes contain regulatory information beyond their nucleotide sequences. This information can either be dynamic and transitory in nature or be relatively stable, capable of being passed on to somatic daughter cells, as occurs during lineage commitment, and in some cases to offspring via the germline, as occurs with parentally imprinted genes. The most thoroughly understood epigenetic mechanisms influence gene expression and do so as a result of changes in chemical modifications of the DNA (for example, methylation of CpG dinucleotides within gene promoters) or the physical accessibility of the DNA by virtue of its association with histones, nonhistone proteins, or noncoding RNAs (for example, *XIST*).

The basic building block of chromatin is the nucleosome core particle, consisting of approximately 147 base pairs of DNA wrapped around a histone octamer that contains two copies each of histones 2A, 2B, 3, and 4. The tails of histones H3 and H4 are subject to a variety of posttranslational modifications including acetylation, methylation, phosphorylation, sumoylation, and ubiquitylation. In general, histone acetylation is associated with a more open chromatin configuration (euchromatin) that is permissive for transcription. Histone deacetylation is usually associated with condensed, compacted chromatin (heterochromatin) and transcriptional repression. The positions of nucleosomes relative to the DNA strand also influence which genes are capable of being transcribed and are regulated by chromatin remodeling complexes such as SWI/SNF complexes.

Histone acetylation leads to an increased negative charge, which loosens the interaction between the histone and the negatively charged DNA. In addition, acetylated histones recruit specific chromatin-associated proteins that contain bromodomains. Histone methylation, by contrast, does not alter histone charge but instead creates a docking site for chromatin-associated proteins that contain specific methyl histone-binding domains, such as plant homeodomain (PHD) domains, tudor domains, or chromodomains. These chromatin-associated "reader" proteins often recruit other proteins that contain additional chromatin-modifying activities (including "writers" and "erasers" that add or remove specific histone posttranslational modifications, respectively). The consequences of histone methylation are influenced by the specific histone residue that is modified, the number of methyl groups added (mono-, di-, or trimethylation), and other contextual factors. Methylation of H3K4, H3K36, and H3K79 is often associated with transcriptionally active euchromatin. By contrast, methylation of H3K9, H3K27, and H4K20 helps specify transcriptionally repressed heterochromatin.

Histone methylation can also influence DNA methylation and vice versa. Specific methyltransferase enzymes are involved in de novo and maintenance DNA methylation. Methylation of CpG dinucleotides in promoter regions typically inhibits transcription. DNA methylation tends to be a more stable modification than histone methylation but can undergo changes during embryogenesis and aging. It has been appreciated for many years that cancers can display global DNA hypomethylation while, at the same time, exhibiting hypermethylation of genomic regions responsible for the expression of tumor suppressor genes.

Many enzymes that play important roles in epigenetic gene regulation utilize cosubstrates generated by cellular metabolism, thereby providing a potential link between nutrition, metabolism, and gene regulation. In this review, we describe examples of such enzymes as well as evidence that altered metabolism, through altered epigenetics, can contribute to disease. As this topic has recently also been reviewed by others (e.g., Dawson

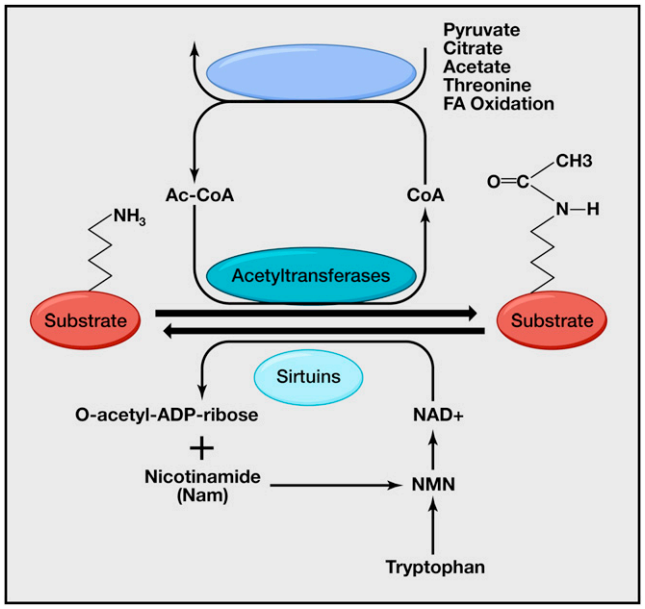


**Figure 1. Metabolism and Acetylation/Deacetylation**
Histone acetylases use acetyl-CoA (Ac-CoA) as an acetyl donor, whose synthesis requires coenzyme A (CoA). Ac-CoA can be regenerated in chemical reactions involving pyruvate, citrate, acetate, and various amino acids such as threonine and by fatty acid beta oxidation. Deacetylation by Sirtuin family histone deacetylases requires NAD+, leading to the generation of O-acetyl-ADP ribose and nicotinamide (NAM). NAD+ is produced from NMN (nicotinamide mononucleotide), which can be salvaged from NAM or produced de novo from tryptophan. For simplicity, enzymes catalyzing the various reactions are not shown.

and Kouzarides, 2012; Lu and Thompson, 2012; Teperino et al., 2010) we focus particular attention on pertinent studies that might have been overlooked. We have also purposely been provocative by raising questions and, in some cases, challenging existing dogma in the field.

## Acetyl-CoA: "Activated Acetate" and Histone Acetylation

Acetyl-CoA fuels the TCA cycle for the production of ATP under aerobic conditions and is a critical building block for cholesterol, lipids, amino acids, and other components required for cell growth. It was discovered as the "activated" form of acetate, so named because of its favorable energetic state for two-carbon donation in anabolic biochemistry (Lipmann and Kaplan, 1946). Acetyl-CoA is also the substrate used by histone acetyl transferase (HAT) enzymes to modify histone tails as an integral determinant of the epigenetic state of chromatin in eukaryotic cells (Figure 1) (Lee and Workman, 2007; Shahbazian and Grunstein, 2007).

### *Acetyl-CoA Generation*

Acetyl-CoA can be produced through a variety of metabolic pathways, both catabolic and anabolic (Figure 1). Principal among these is the conversion of pyruvate into acetyl-CoA via the mitochondrial pyruvate dehydrogenase complex late during the oxidation of glucose and the β-oxidation of fatty acids. Given its polarity and relative chemical complexity, acetyl-CoA does not readily diffuse across membranes. As such, metazoan cells have evolved the malate-citrate antiporter system to move mitochondrial citrate to the cytoplasm, where it can combine with ATP and CoA to be converted to acetyl-CoA and oxaloacetate via the ATP citrate lyase enzyme, thereby affording a cytoplasmic pool of acetyl-CoA for lipid biosynthesis. Acetyl-CoA can also be produced catabolically from threonine via a mitochondrial threonine dehydrogenase enzyme uniquely expressed in mouse embryonic stem cells; anabolically from acetate, ATP, and CoA via acetyl-CoA synthase enzymes localized in mitochondrial, cytoplasmic, or nuclear compartments of mammalian cells; and via an anaplerotic pathway through reductive carboxylation of α-ketoglutarate.

### *Acetyl-CoA Fluctuation*

A recurring theme woven throughout this review asks the question of how the levels of consumable nutrients, enzyme substrates, and even molecular oxygen are sensed by cells as a means of adaptation. More specifically, we focus on the possibility that gene expression is modulated via epigenetic modification of chromosomal proteins in accord with the abundance of essential metabolites. The activity of almost all enzymes involved in intermediary metabolism is regulated as a function of the abundance of both enzyme substrate and product. By contrast, many enzymes involved in key aspects of intracellular signaling are not regulated in this way. Take, for example, the hundreds of protein kinase enzymes that, using ATP as a substrate, modify target proteins by phosphorylation. With the exception of the adenosine monophosphate-regulated protein kinase (Hardie, 2011), almost no protein kinase enzymes are built to sense the level of cellular ATP in the context of their regulatory function. Unless a cell is in a deathly sick state of ATP under abundance, protein kinase enzymes are capable of functioning perfectly well irrespective of ATP levels. This obviously results from the fact that protein kinases are endowed with affinity for substrate that is considerably more avid than the ambient levels of intracellular ATP.

Turning to enzymes involved in epigenetic regulation, starting with HATs, we ask the following: are we to consider them as being analogous to substrate-limited metabolic enzymes, or are they instead protein kinase-like in having evolved properties that shield themselves from fluctuation in the level of intracellular acetyl-CoA? Before considering whether acetylase enzymes might be substrate regulated, it is first important to ask whether the intracellular levels of acetyl-CoA fluctuate in biological settings. Studies of two different eukaryotic cells have indeed given evidence of significant fluctuation in acetyl-CoA. For example, prototrophic (wild-type) strains of yeast grown in the nutrient-limiting environment of a chemostat spontaneously enter a synchronous and highly robust metabolic cycle (Klevecz et al., 2004; Tu et al., 2005; Tu and McKnight, 2006, 2009; Tu et al., 2007). Over a 4–5 hr cycle the cells rhythmically oscillate between oxidative and reductive growth (Figure 2). Mitochondrial respiration during the oxidative phase of this yeast metabolic cycle (YMC) helps accumulate appropriate levels of energetically valuable building blocks required for transition into a reductive, glycolytic phase wherein the cells commit to DNA synthesis and cell division. Acetyl-CoA levels fluctuate dramatically as a function of the YMC. Acetyl-CoA levels peak at a 6-fold-higher level at the transition of the oxidative (Ox)

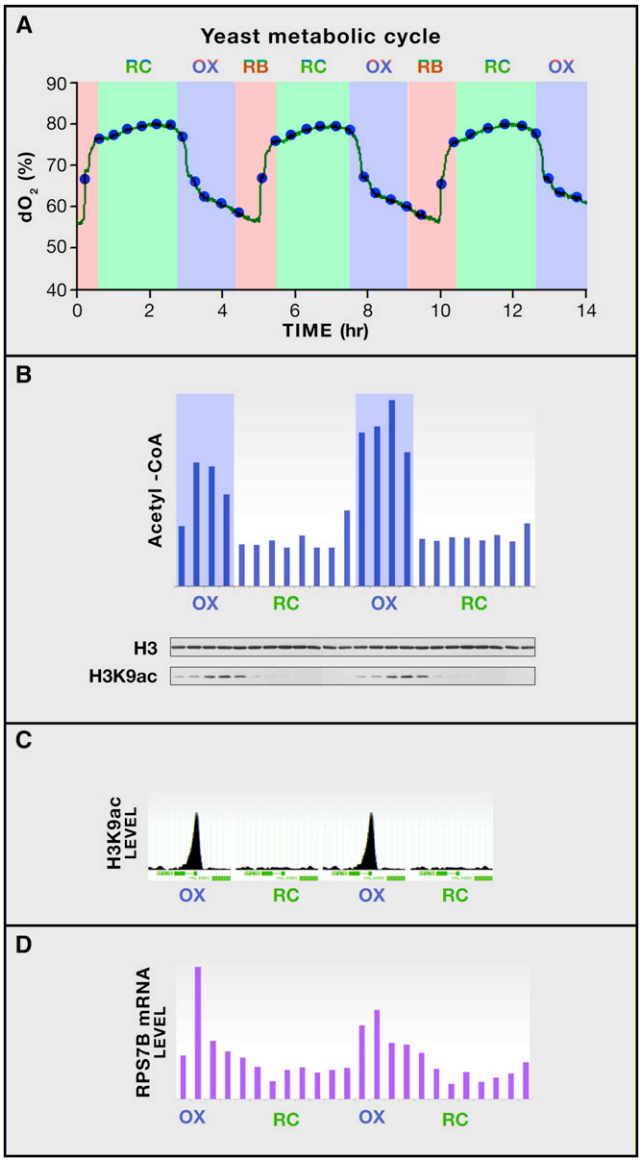


**Figure 2. Evidence of Transient Acetylation of Histone H3 Only during the Oxidative Phase of the Yeast Metabolic Cycle**

(A) Periodic fluctuation in oxygen levels in a chemostat growing prototrophic yeast. The yeast metabolic cycle (YMC) is roughly 5 hours in duration and defined by sequentially repeating oxidative (Ox), reductive building (RB), and reductive charging (RC) metabolic phases (adapted from Tu et al., 2005).

(B) Quantitative measurement of acetyl-CoA levels over the YMC reveal elevated levels of the metabolite during the Ox phase of the YMC. Western blot measurements of H3K9 acetylation over the YMC reveal dynamic acetylation temporally correlate with the peak abundance of acetyl-CoA.

(C) ChIP-seq analysis of H3K9 acetylation on the promoter of the gene encoding the RPS7B ribosomal protein reveals modification limited to the Ox phase of the YMC.

(D) Transcript abundance of the RPS7B mRNA peaks during the Ox phase of the YMC precisely when acetyl-CoA levels are of highest abundance and when the promoter of the RPS7B gene is modified by H3K9 acetylation.

phase to the reductive building (RB) phase relative to the metabolically quiescent, reductive charging (RC) phase of the YMC (Cai et al., 2011; Tu et al., 2007). One likely means by which intracellular levels of acetyl-CoA peak at the Ox/RB boundary is the coordinated induction of all enzymes required to convert ethanol into acetylaldehyde, acetylaldehyde into acetate, and acetate into acetyl-CoA at this precise temporal window of the YMC. In this way, ethanol fermented via the consumption of glucose during the RB phase of the YMC and accumulated in the extracellular reservoir of the chemostat can be retrieved and rebuilt into a valuable cellular building block. That the "recycled" hydrocarbon of ethanol enzymatically converted into acetyl-CoA might be directly relevant to the epigenetic state of yeast cells is strongly hinted by the fact that the terminal enzyme in the pathway, acetyl-CoA synthase, has been shown to be localized to the nucleus (Takahashi et al., 2006).

Significant fluctuation in the abundance of acetyl-CoA has also been observed as a function of the differentiation of mouse embryonic stem cells (ESCs). Undifferentiated ESCs contain significantly higher levels of acetyl-CoA than the embryoid body (EB) cells induced to differentiate by the combined withdrawal of leukemia-inhibitory factor (LIF) and application of retinoic acid (RA) (Wang et al., 2009a, 2011). The observed fluctuation of acetyl-CoA levels correlates with dramatic changes in the expression of the threonine dehydrogenase (TDH) enzyme, which is rate limiting for the conversion of threonine into glycine and acetyl-CoA. Undifferentiated mouse ESCs express levels of TDH upward of 1,000-fold higher relative to any other source of mouse cells or tissues, and the gene encoding TDH is stringently repressed immediately upon induction of ESC differentiation in response to LIF withdrawal and RA administration. When undifferentiated ESCs are exposed to a specific chemical inhibitor of the TDH enzyme, intracellular levels of acetyl-CoA drop precipitously (Alexander et al., 2011). As will be discussed subsequently, it has been hypothesized that the ability of the TDH enzyme to convert threonine into glycine and acetyl-CoA may not only fuel mouse ESCs in a specialized manner but also help dictate an equally specialized epigenetic state.

### *Control of Gene Expression by Acetyl-CoA*

Do the unusually high levels of acetyl-CoA present in undifferentiated mouse ESCs, or yeast cells poised at the Ox/RB boundary of the YMC, play a determinative role in epigenetic regulation of gene expression? Where this has only been hypothesized for mouse ESCs, data have been gathered to affirmatively answer this question in yeast cells (Figure 2).

As described by Tu and colleagues (Cai et al., 2011; Cai and Tu, 2011, 2012), numerous acetylation marks on the K9, K14, K23, and K27 residues of histone H3 and the K5, K8, and K12 residues on histone H4 only appear over a 30–45 min window corresponding exactly with the Ox/RB boundary that is coincident with the peak abundance of intracellular acetyl-CoA. The fact that these acetylation marks peak at the Ox/RB boundary gives evidence that this form of epigenetic regulation is unusually dynamic, consistent with reports that the half-life of histone acetylation may be as short as 3 min (Waterborg, 2002). Further DNA microarray and ChIP-seq experiments led to the identification of roughly 1,000 growth genes selectively acetylated and activated only when intracellular levels of acetyl-CoA peak (Cai et al., 2011, Tu et al., 2005). These include genes encoding ribosomal components, translation factors, and the regulatory D1 cyclin, corresponding precisely to the set of genes known to gate entry

of yeast cells into the cell division cycle (Jorgensen et al., 2002). The precision of temporal induction of these 1,000+ genes is astounding; the entire gene set is coordinately induced within a single-digit number of minutes within the 4–5 hr YMC (Rowicka et al., 2007).

### *Acetyl-CoA and Histone Acetyltransferase Enzymes*

The GCN5 histone acetylase enzyme of the SAGA complex has been identified as the critical enzyme responsible for transient acetylation of growth genes at the Ox/RB boundary (Cai et al., 2011). This conclusion derives from ChIP-seq experiments showing the selective association of the SAGA complex with the promoters of the entire battery of growth genes only during the window of peak acetyl-CoA accumulation, along with genetic experiments wherein it has been demonstrated that catalytically active GCN5 is critically required for YMC oscillation (Cai et al., 2011). What properties of the SAGA complex and GCN5 might uniquely qualify it as an acetyl-CoA sensor? Several regulatory subunits of the SAGA complex are themselves transiently acetylated only during the Ox/RB window, raising the possibility that a complex pathway of allosteric regulation is at the heart of the sensing ability of SAGA (Cai et al., 2011). More simplistically, it is possible that GCN5 requires high levels of acetyl-CoA and that the enzyme is less active in other phases of the YMC relative to the Ox/RB window wherein acetyl-CoA levels peak. In this regard it may be notable that the off-rate of acetyl-CoA binding to the yeast GCN5 enzyme is more than an order of magnitude more rapid than that of human p300/CBP HAT enzyme, human GCN5, or Tetrahymena GCN5 (Langer et al., 2002).

### *Cancer Connection*

The genes whose promoter regions and chromatin are differentially acetylated exactly when acetyl-CoA levels peak during the YMC encode precisely those protein and RNA products required to enable cell growth. This yeast growth gene battery matches closely with the genes induced by the c-Myc oncoprotein in mammalian cells (Ji et al., 2011), which have been reported to be codependent upon c-Myc and GCN5/SAGA (McMahon et al., 1998, 2000). This precisely orchestrated pattern of yeast growth gene induction in response to ambient levels of intracellular acetyl-CoA probably represents an evolutionarily ancient regulatory pathway allowing cells to properly link the commitment of cell growth and division to nutritional state. Future studies will help assess whether this same pathway is employed by mammalian cells, especially the nutrient-limited cells of solid tumors. In this vein, it is noteworthy that the production of acetyl-CoA in HeLa cells necessary to drive histone acetylation has been attributed to the enzymatic conversion of citrate into oxaloacetate and acetyl-CoA via the ATP citrate lyase enzyme (Wellen et al., 2009). Whereas mammalian cells contain three paralogous enzymes capable of converting acetate into acetyl-CoA, the latter study provides evidence that cancer cells make acetyl-CoA via a fundamentally different pathway than prototrophic yeast. The observations of Wellen and colleagues do conclude, however, that the GCN5 histone acetyltransferase enzyme of the SAGA complex is of critical importance for histone acetylation in response to the combined provision of glucose and growth factors to otherwise quiescent cells. As such, both yeast and human cancer cells may employ similar strategies to couple nutrient availability to the control of gene expression.

## NAD+ and Deacetylation

The burning of metabolic fuels uses molecular oxygen as the ultimate electron acceptor. Instead of being directly transferred to $O_2$, electrons evolving from oxidative reactions use pyridine nucleotides as specialized carriers, with the reduced forms of these carriers then being able to transfer electrons to molecular oxygen. Nicotinamide adenine dinucleotide (NAD) is a key electron carrier in the oxidation of hydrocarbon fuels. The nicotinate moiety of NAD (niacin or vitamin B6) is derived from tryptophan and combines with 5-phosphoribosyl-1-pyrophosphate (PRPP) to yield nicotinate ribonucleotide and inorganic pyrophosphate. Desamido-NAD is then formed via the transfer of an AMP moiety from ATP to nicotinate ribonucleotide, with the final step in the synthesis of NAD involving the transfer of the ammonia generated from the amide group of glutamine to the nicotinate carboxyl group. NADP, the related, phosphorylated derivative of NAD, is made via the transfer of a phosphoryl group from ATP to the 2′-hydroxyl group via an NAD kinase enzyme. Upon electron acceptance, NAD+ and NADP+ are converted to the reduced forms of these pyridine nucleotides. The ambient intracellular ratio of NAD+/NADH is roughly 100:1, whereas the ratio of NADP+/NADPH is 1:100. These ratios reflect the evolved necessity for NAD+ to function primarily as an electron acceptor in the burning of hydrocarbon fuels and the necessity for NADPH to fulfill anabolic biosynthetic reactions including the synthesis of cholesterol, bile acids, steroid hormones, and triglycerides.

Considerable attention has been paid to the hypothetical role of fluctuating NAD+ levels as a function of nutritional state and the activity of deacetylase enzymes. These enzymes come in two flavors, those that catalyze deacetylation in an NAD+-independent manner, yielding the deacetylated substrate and free acetate as products; and those that are NAD+-dependent, yielding O-acetyl-ADP-ribose, the deacetylated substrate, and nicotinamide as products (Denu, 2005; Feldman et al., 2012; Haberland et al., 2009; Sauve et al., 2006). The latter proteins are members of the sirtuin family of deacetylases (Figure 1), which include two isoforms that are primarily housed in the nuclei of mammalian cells (SIRT6 and SIRT7), three that are localized to mitochondria (SIRT3, SIRT4, and SIRT5), and two that are found in both cytoplasmic and nuclear compartments (SIRT1 and SIRT2) (Finkel et al., 2009; Guarente, 2011a; Haigis and Sinclair, 2010; Verdin et al., 2010).

### *NAD+ and Sirtuin Deacetylases*

For the purpose of simplicity, one can consider the action of sirtuin deacetylase enzymes as being a counterbalance to nutrient-driven protein acetylation. On a more microscopic level, the involvement of the mitochondrial SIRT3 enzyme in the deacetylation of the acetyl-CoA synthase enzyme AceCS2 is revealing (Hallows et al., 2006; Schwer et al., 2006). Under appropriate nutritional conditions, AceCS2 is acetylated on a specific lysine residue that inhibits the ability of the enzyme to convert acetate into acetyl-CoA, a regulatory scheme conserved from *Salmonella* to mammals (Hirschey et al., 2011; Starai et al., 2002). SIRT3-mediated deacetylation of AceCS2 reactivates the enzyme. One potential reason for justifying why AceCS2 is

deacetylated by a sirtuin enzyme is that the product of the reaction is not acetate, which might create a futile cycle, but instead O-acetyl-ADP-ribose and nicotinamide. Alternatively, sirtuin-mediated production of the latter metabolite might avail it for biosynthetic or regulatory purposes (Hassa et al., 2006). On a more macroscopic level, one can consider the ability of SIRT1 to deacetylate the PGC1α transcriptional coactivator. PGC1α coordinately regulates many genes whose products conspire to control intermediary metabolism in many tissues of the body. When heavily acetylated, PGC1α is inactive (Lerin et al., 2006; Rodgers et al., 2005). SIRT1-mediated deacetylation reactivates PGC1α (Lerin et al., 2006). In the cases of both AceCS2 and PGC1α, access to ample nutrients can simplistically be understood to inhibit the activities of the two proteins via acetyl-CoA-mediated acetylation. This inhibition, in turn, can be respectively counterbalanced by the mitochondrial SIRT3 and nuclear SIRT1 enzymes.

Caloric restriction would logically be expected to demand the activity of the sirtuin family of deacetylase enzymes. For example, SIRT3-mediated deacetylation of AceCS2 would be desired to maximize production of acetyl-CoA from acetate under conditions of caloric restriction, and SIRT1-mediated deacetylation of PGC1α would help activate transcription of the appropriate battery of nuclear genes important for adaptation to starvation or caloric restriction. Evidence has been reported that the levels of expression of sirtuin enzymes can adapt to metabolic state (Hirschey et al., 2011). It has likewise been reported that NAD+ levels may increase upon caloric restriction, thereby offering an alternative means of sirtuin activation. Although it is counterintuitive to consider that cells or tissues would produce higher levels of NAD+ under conditions of caloric restriction, where the need of the cofactor as an electron acceptor for oxidation of hydrocarbons should be diminished, this interpretation has gained widespread acceptance (Cantó and Auwerx, 2011; Guarente, 2011b). Such interpretations contradict classical studies showing that NAD+ levels do not increase as a function of starvation. The collective work of Krebs and Veech exhaustively demonstrated that NAD+/NADH levels do not change as a function of starvation, irrespective of whether one measures bound or free fractions of the cofactors (Krebs and Veech, 1969; Veech et al., 1969).

It has also been reported that NAD+ levels fluctuate as a function of the circadian cycle, thereby instructing nuclear sirtuin enzymes to control the epigenetic state of chromatin in an NAD+-regulated manner. Mouse embryo fibroblast (MEF) cells deficient in the CLOCK transcription factor were reported to contain only 4%–5% as much NAD+ as wild-type MEF cells (Nakahata et al., 2009). When NAD+ levels were measured in liver tissue of wild-type mice, two ultradian sets of peaks and troughs of NAD+ abundance were observed per 24 hr cycle (Ramsey et al., 2009). The peak-to-trough fluctuation in NAD+ abundance varied by 20%–30%, as reported in the latter study. By contrast, when NAD+ levels were measured as a function of the YMC, which is far more robust in amplitude than metabolic fluctuation taking place as a function of the circadian cycle, no changes in NAD+ levels were observed (Tu et al., 2007). Likewise, extensive studies of yeast cells exposed to a variety of nutritional states, including caloric restriction, have shown no alteration in NAD+ or nicotinamide levels that could be interpreted to increase the activity of sirtuin enzymes upon glucose restriction (Evans et al., 2010). Thus it remains unclear whether sirtuin activity is operatively linked to metabolic state via fluctuations in the intracellular levels of NAD+.

What is clear, however, is that sirtuin enzymes sit in diametric opposition to protein acetylating and that protein acetylation can be influenced by intracellular levels of acetyl-CoA. In the case of the AceCS2 enzyme that uses acetate to produce mitochondrial acetyl-CoA, SIRT3 serves to induce the catalytic activity of the enzyme by removing an inhibitory acetylation mark. In the case of PGC1α, the nuclear SIRT1 enzyme serves to deacetylate this transcriptional coactivator, thereby liberating its capacity to induce the expression of genes whose products are required in energy-depleted cells. Recent studies have provided evidence that the genes encoding SIRT3 and SIRT6 are tumor suppressors (Kim et al., 2010; Sebastián et al., 2012). In this regard, it is particularly intriguing that the battery of SIRT6 target genes (Sebastián et al., 2012) has been reported to overlap significantly with genes codependent on the c-Myc oncoprotein and the SAGA/GCN5 histone acetylase complex (Cai et. al., 2011; Ji et al., 2011; McMahon et al., 1998, 2000).

### *NAD+ Independent Histone Deacetylases*

A recent study has raised the possibility of a different sort of connection between an abundant metabolite and the NAD+-independent class of histone deacetylase enzyme. β-hydroxybutyrate, which is one of the three ketone bodies, is produced at mM quantities after prolonged exercise or starvation (Candido et al., 1978). Like sodium butyrate, β-hydroxybutyrate inhibits the activities of many NAD+-independent histone deacetylase enzymes (Shimazu et al., 2013). By administering β-hydroxybutyrate to laboratory mice via an intraperitoneal pump, Shimazu and colleagues were able to demonstrate enhancement of H3K9 and H3K14 acetylation, induced expression of FOXO3A-regulated genes, and resistance to oxidative stress. These data provide evidence that a distinct metabolite, β-hydroxybutyrate, is able not only to fuel metabolic adaptation to starvation but also to help sustain a protective epigenetic state by inhibiting the activities of NAD+-independent histone deacetylase enzymes.

## Methylation of DNA and Histones: SAM and the "Activated Methyl Cycle"

S-adenosylmethionine (SAM) contains the active methyl donor group utilized by most methyltransferase enzymes (Figure 3). Tetrahydrofolate (THF), when methylated on its N-5 atom ($N^5$-MTHF), also acts as a methyl donor. Unlike SAM, the transfer potential of the methyl donor group of $N^5$-MTHF is not sufficiently high for most biosynthetic methylation reactions. SAM is produced by the condensation of methionine and ATP during the first of nine steps required for the conversion of methionine to succinyl-CoA. The methyl group of SAM is chemically activated via the positive charge on the adjacent sulfur atom, which causes the SAM methyl group to be considerably more reactive than the methyl group on $N^5$-MTHF. Enzyme-catalyzed donation of the methyl group of SAM to an acceptor macromolecule yields S-adenosylhomocysteine (SAH), which, in turn, is hydrolyzed to homocysteine and adenosine. The activated methyl

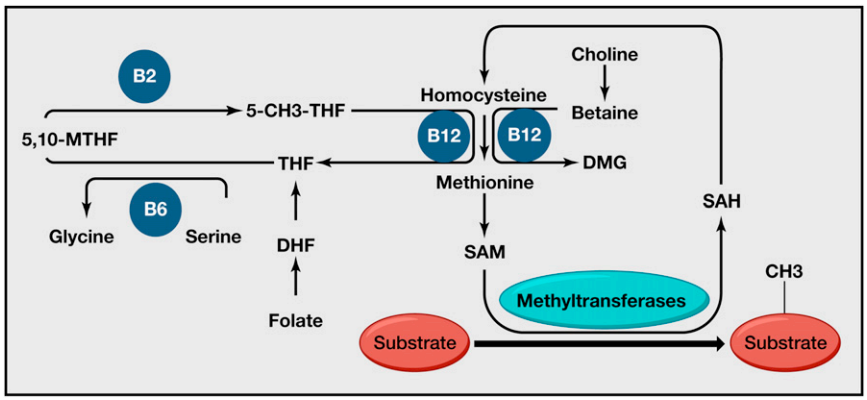


**Figure 3. Metabolism and Methyltransferases**

DNA and histone methyltransferases use S-adenosylmethionine (SAM), derived from methionine, as a methyl donor, resulting in the generation of S-adenosylhomocysteine (SAH). SAH is converted to homocysteine, which is then converted back to methionine in a vitamin B12-dependent reaction that utilizes carbons derived from either choline or folate. DHF, dihydrofolate; THF, tetrahydrofolate; 5,10-MTHF, 5,10-methylene THF; CH3, methyl. Also shown are steps requiring vitamin B6 and B2. For simplicity, enzymes catalyzing the various reactions are not shown.

cycle can then be looped back via the transfer of a methyl group from $N^5$-MTHF to homocysteine, regenerating methionine.

### *SAM and Histone/DNA Methylation*

Histone methylation represents a covalent modification that is of equal importance to histone acetylation in defining the epigenetic state of chromatin (Kouzarides, 2002; Zhang and Reinberg, 2001). DNA itself is also subject to methylation on the C5 atom of cytosine (Bird, 2002). Intense studies reported over the past decade have led to the identification of a multitude of enzymes that afford the methylation and demethylation of both histone and DNA substrates. Both histone and DNA methylation require SAM as the high-energy methyl donor (Figure 3). Parallel with the aforementioned thinking concerning the possibility that acetyl-CoA levels might specify epigenetic state, the question can be raised as to whether ambient levels of intracellular or intranuclear SAM might help drive histone methylation. The conversion of methionine to SAM is catalyzed in an ATP-dependent manner by methionine adenosyltransferase (MAT) enzymes (Sakata et al., 1993). A recent study has given evidence that one of the MAT isoforms, MATIIα, associates with a gene-specific transcription factor designated MafK. The latter protein is a small bZip transcription factor that, depending upon its heterodimeric partner, can either repress or activate gene expression (Hintze et al., 2007; Igarashi and Sun, 2006; Muto et al., 1998; Ochiai et al., 2006; Zhang et al., 2006). Affinity chromatography experiments employing a tagged version of the MafK protein led to the discovery of its interaction with MATIIα, raising the possibility that the MafK transcription factor might recruit this enzyme directly to its target genes (Katoh et al., 2011). Cytological experiments revealed the nuclear localization of the MATIIα enzyme, and the results of a variety of molecular biological experiments were interpreted to indicate that the association of the MATIIα methyltransferase enzyme with MafK target genes may be required for transcriptional repression. If correct, these observations offer the possibility that a localized increase in the production of SAM might help establish the epigenetic state of cells.

### *Threonine Dehydrogenase and SAM*

Related interpretations have evolved from studies of mouse ESCs. As mentioned earlier in this review, mouse ESCs express exceptionally high levels of the TDH enzyme, which catabolizes threonine into glycine and acetyl-CoA (Figure 4). When ESCs are exposed to a selective chemical inhibitor of the TDH enzyme, intracellular levels of acetyl-CoA drop significantly (Alexander et al., 2011). Simultaneous increases in the intracellular abundance of threonine and 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) were observed upon TDH inhibition in ESCs. The increase in threonine can logically be attributed to diminution in the activity of the enzyme that degrades it. Increases in AICAR, a biosynthetic intermediate that awaits $N^5$-MTHF-mediated one-carbon donation to continue along the pathway of purine biosynthesis, could likewise be attributed to attenuation in the production of mitochondrial glycine. The latter catabolite of TDH-mediated degradation of threonine is known to feed the mitochondrial glycine cleavage enzyme complex responsible for converting tetrahydrofolate to $N^5$-MTHF (Figure 4). Not surprisingly, chemical inhibition of the TDH enzyme in mouse ESCs causes a precipitous drop in the level of intracellular $N^5$-MTHF (Alexander et al., 2011). Having observed reduced levels of $N^5$-MTHF and increased levels of the AICAR intermediate in purine biosynthesis upon chemical inhibition of the TDH enzyme, it was straightforward to predict that catabolism of threonine is vitally important for ESCs to biosynthesize the required building blocks for DNA synthesis. Given that the cell division cycle for mouse ESCs (4–5 hr in length) is more rapid than even the fastest growing of cultured cancer cell lines, it may reasonably be concluded that these cells require the specialized features of the TDH catabolic pathway to convert threonine into both acetyl-CoA and the glycine-dependent methylation capacity essential for the biosynthesis of nucleotides. Perplexingly, unique among all primates, mammals, and—perhaps—all metazoan species, humans do not encode a functional TDH enzyme (Edgar, 2002). As such, the unique metabolic properties engendered by copious expression of the TDH enzyme in mouse ESCs cannot apply to human stem cells.

More recent work on mouse ESCs has led to the conclusion that TDH-mediated catabolism of threonine might also be important for maintaining high levels of SAM (Shyh-Chang et al., 2013). Knowing that $N^5$-MTHF is required for the regeneration of methionine from homocysteine, it was logical to predict that intracellular levels of SAM might drop when ESCs are deprived of threonine.

Intriguingly, threonine restriction was observed to dramatically impede deposition of the H3K4me2 and H3K4me3 marks on chromatin. These impediments were selective; threonine

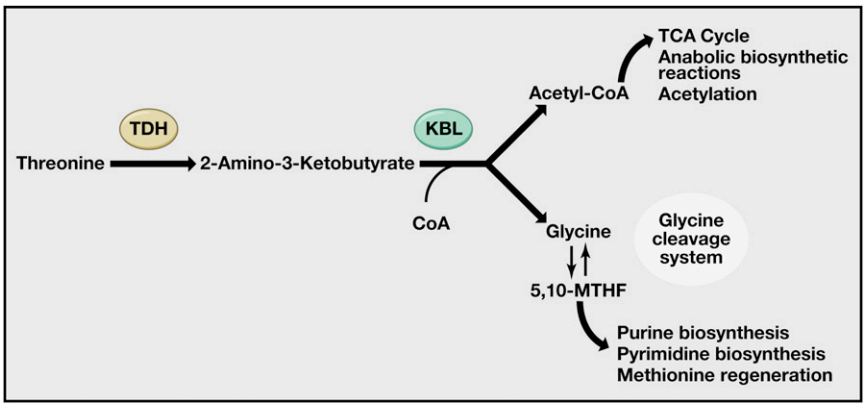


**Figure 4. TDH-Mediated Catabolism of Threonine**

Threonine is catabolized to acetyl-CoA and glycine via a two-step process, first involving the rate-limiting threonine dehydrogenase (TDH) enzyme yielding the short-lived intermediate 2-amino-3-ketobutyrate. This intermediate is subsequently subject to the 2-amino-3-ketobutyrate ligase (KBL) enzyme that, supplemented by coenzyme A (CoA), yields the final products of the reaction, acetyl-CoA and glycine. Both steps of the catabolic reaction take place in the mitochondria of eukaryotic cells. The former product, acetyl-CoA, can be fed into the TCA cycle or used as an anabolic building block for other metabolites. The latter product, glycine, is used to feed the mitochondrial glycine cleavage system for the conversion of tetrahydrofolate (THF) into $N^5$, $N^{10}$-methylene-tetrahydrofolate (MTHF). MTHF, in turn, is capable of one-carbon donation in biosynthetic reactions involving purine and pyrimidine synthesis, as well as the regeneration of methionine from homocysteine.

deprivation had no effect on the deposition of H3K4me1, H3K9me3, H3K27me3, H3K36me3, or H3K79me3 marks on histone tails. If correct, these observations offer a logical interpretation of the connection between nutritional and epigenetic states analogous to what has been learned from studies of acetyl-CoA levels driving the epigenetic state of prototrophic yeast cells growing under nutrient-limited conditions (Cai et al., 2011).

The H3K4me2 and H3K3me3 marks that disappear when mouse ESCs are deprived of threonine (Shyh-Chang et al., 2013) are part of a ''bivalent'' epigenetic state believed to be uniquely important for keeping the chromatin structure of genes encoding developmentally important transcription factors in a properly repressed/poised state for subsequent induction as a function of embryogenesis (Bernstein et al., 2006). It is therefore possible that, by catabolizing threonine along pathways important for the production of both acetyl-CoA necessary for histone acetylation and methylation capacity via the generation of $N^5$-MTHF and SAM, the TDH enzyme may be of fundamental importance in controlling two unique properties of mouse ESCs—their incredibly rapid rate of cell division and their unique retention of developmental pluripotency.

## FAD and Histone Demethylation

Flavin adenine dinucleotide (FAD) is derived from the vitamin riboflavin (vitamin B2) and functions as the prosthetic group for certain oxidation-reduction enzymes. Riboflavin is phosphorylated by riboflavin kinase to generate riboflavin 5′-phosphate (sometimes called flavin monucleotide or FMN), which is then converted to FAD by FAD synthetase (also called FMN adenyltransferase). The former enzyme appears to be rate limiting for FAD production.

In a landmark paper, Yang Shi and coworkers showed that LSD1 (also called KDM1A or AOF2) is a nuclear FAD-dependent enzyme capable of demethylating methylated H3K4 both in vitro and in vivo, thereby establishing that histone methylation is a dynamic, reversible process (Shi et al., 2004). The chemical reaction catalyzed by LSD1 requires a protonated lysine epsilon amino group, thereby limiting its activity to monomethylated and dimethylated H3K4 (Figure 5). LSD1 and its paralog LSD2 (also called KDM1B or AOF1) can also influence H3K9 methylation and DNA methylation, either due to their participation in multiprotein complexes that contain additional chromatin-modifying enzymes, including deacetylases and methyltransferases, or due to indirect effects of H3K4 methylation on recruitment of such enzymes (Ciccone et al., 2009; Lan et al., 2008; Wang et al., 2009b). It has also been argued that the reactive oxygen species produced by the LSD1 histone demethylation reaction can react with neighboring DNA and other macromolecules and thereby affect transcription (Perillo et al., 2008).

FAD is produced in mitochondria. It has been suggested that the nuclear location of LSD1 might render it particularly sensitive to changes in FAD availability (and the ratio of FAD to FADH2) arising from the activities of other flavin-linked dehydrogenases and oxidases, including those associated with fatty acid β-oxidation and the TCA cycle (Hino et al., 2012). LSD1, in turn, regulates mitochondrial respiration and energy expenditure. Specifically, LSD1 binds directly to genes such as *PPARγ coactivator-1α* (*PGC1α*), *PDK4*, *FATP1*, and *ATGL* and represses their transcription associated with loss of H3K4 methylation (Hino et al., 2012).

## 2-Oxoglutarate-Dependent Dioxygenases

### *JmjC and TET Demethylases*

A number of chromatin-modifying enzymes, including the approximately 30 JmjC domain-containing histone demethylases and the three TET (ten-eleven translocation) proteins, are 2-oxoglutarate-dependent dioxygenases (Loenarz and Schofield, 2011) (Figure 5). The JmjC histone demethylases, in contrast to LSD proteins, are capable of demethylating trimethylated lysines and arginines. Different JmjC histone demethylases exhibit preferences for different histone methylation marks. For example, KDM5A (also called RBP2 or JARID1A) specifically recognizes methylated H3K4. TET proteins can oxidize 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), 5-formyl cytosine (5fC), and 5-carboxylcytosine (5caC) (He et al., 2011; Ito et al., 2011; Tahiliani et al., 2009). 5hmC in the nervous system

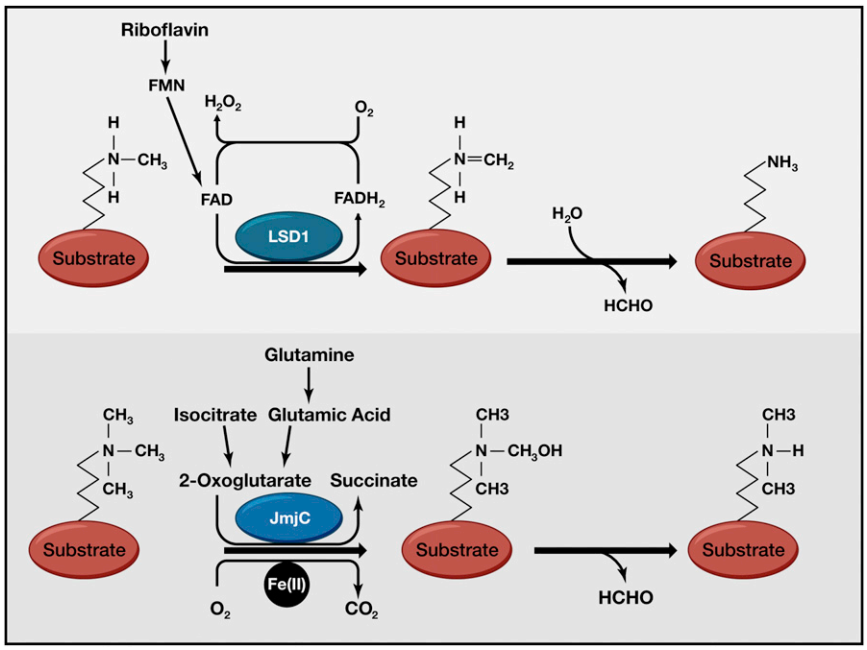


**Figure 5. Histone Demethylase Reactions**
LSD demethylases oxidize monomethylated and dimethylated histones using FAD (flavin adenine dinucleotide) as a cofactor. The oxidized methyl group is unstable and, after attack by water, given off as formaldehyde (HCHO). FAD is derived from FMN (flavin mononucleotide), which in turn is derived from riboflavin. JmjC demethylases hydroxylate methylated histones in a reaction coupled to decarboxylation of 2-oxoglutarate to succinate. 2-oxoglutarate (also called α-ketoglutarate) can be derived from several sources including isocitrate and glutamic acid. Spontaneous release of the hydroxylated methyl group results in demethylation.

is associated with actively transcribed genes and is recognized by the histone-reader protein MeCP2, which is mutationally inactivated in the neurological disorder of Rett syndrome (Mellén et al., 2012). TET-catalyzed oxidation of 5mC followed by decarboxylation or base excision is strongly suspected of contributing to DNA demethylation (Cortellino et al., 2011; He et al., 2011; Ito et al., 2011), yet there are currently conflicting reports regarding the effect of TET inactivation on global DNA methylation patterns in somatic cells (Figueroa et al., 2010; Ko et al., 2010; Yamazaki et al., 2012).

### *Iron and Oxygen*

2-oxoglutarate-dependent dioxygenases require oxygen and Fe (II) in addition to 2-oxoglutarate (also called α-ketoglutarate). The latter metabolite is decarboxylated to succinate during the oxidation reaction (Figure 5). These enzymes are also sensitive to reactive oxygen species and their activity is enhanced in the presence of ascorbic acid. These biochemical attributes render these enzymes potentially susceptible to carcinogenic metals such as nickel, arsenic, and chromium, which displace iron, contribute to oxidative stress, or do both (Chervona and Costa, 2012).

Certain 2-oxoglutarate-dependent dioxygenases, such as the EglN prolyl hydroxylases that mark the HIF transcription factor for destruction, have $O_2$ $K_m$ values at or near atmospheric oxygen levels and hence their activity is sensitive to changes in oxygen availability within a physiologically relevant range (Hirsilä et al., 2003). By contrast, the collagen prolyl hydroxylases have very low $O_2$ $K_m$, presumably so that they can function in environments such as poorly vascularized wounds (Hirsilä et al., 2003).

The $O_2$ $K_m$ values for the JmjC histone demethylases and TET proteins are not known, although indirect evidence suggests that at least some of the former could be oxygen sensitive. Specifically, the messenger RNAs (mRNAs) for multiple JmjC histone demethylase genes, including *JMJD1A* (an H3K9 demethylase), *JMJD2B* (an H3K9 demethylase), and *JARIDC* (an H3K4 demethylase), are induced by hypoxia and HIF, conceivably to compensate for their diminished catalytic activity under low-oxygen conditions (Chervona and Costa, 2012; Xia et al., 2009) (Figure 6A). Moreover, increased H3K4 and H3K9 histone methylation has been documented in cells treated with hypoxia in vitro (Johnson et al., 2008; Tausendschön et al., 2011; Zhou et al., 2010).

HIF synthesis is influenced by the PI3K-AKT-mTOR pathway, which senses nutrients such as glucose and amino acids, while its degradation is under the control of the EglN prolyl hydroxylases, which respond to changes in oxygen and different Krebs cycle intermediates (Figure 6A). Therefore, HIF provides another link between metabolism and epigenetics through its transcriptional regulation of JmjC histone demethylases and other modifiers of epigenetic state.

### *2-Oxoglutarate*

2-oxoglutarate is produced from isocitrate in the mitochondria by the action of isocitrate dehydrogenases 2 and 3 (IDH2 and IDH3) in the TCA cycle and can also be generated from several amino acids including arginine, proline, histidine, and glutamine, which can be converted to glutamic acid and transaminated to produce 2-oxoglutarate. 2-oxoglutarate generated in the course of the TCA cycle is converted to succinyl CoA by the action of the 2-oxoglutarate dehydrogenase complex and is also consumed during the conversion of cysteine and lysine to pyruvate and acetyl-CoA, respectively. 2-oxoglutarate generated in the mitochondria can enter the cytosol, either as 2-oxoglutarate or after transamination to produce glutamic acid, via specific transporter proteins such as the malate-2-oxoglutarate antiporter, which participates in the malate-aspartate shuttle. A third IDH paralog, IDH1, resides in the cytosol and peroxisomes and provides an alternative source for nonmitochondrial 2-oxoglutarate.

Intracellular 2-oxoglutarate levels are estimated to be in the low millimolar range, well above the 2-oxoglutarate $K_m$ values of the JmjC histone demethylases and TET proteins determined to date (Chowdhury et al., 2011; Pritchard, 1995). A caveat, however, is that such $K_m$ values are typically determined under idealized in vitro conditions using purified enzymes in the

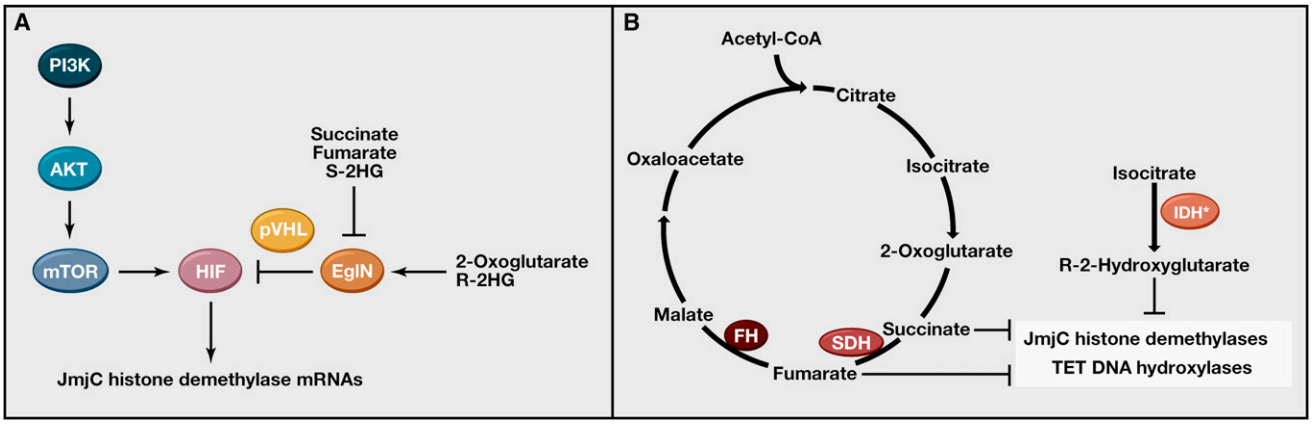


**Figure 6. Mutant Metabolic Enzymes, Epigenetics, and Cancer**

(A) The abundance of the HIF transcription factor is regulated by the 2-oxoglutarate-dependent EglN dioxygenases, which are sensitive to changes in oxygen and metabolism, and by the PI3K-AKT-mTOR pathway, which is involved in nutrient sensing and frequently mutationally activated in cancer. HIF induces the transcription of a number of JmjC histone demethylases.

(B) Mutational inactivation of the Krebs Cycle Enzymes SDH and FH leads to the accumulation of succinate and fumarate, respectively, whereas tumor-derived IDH1 and IDH2 mutants produce high levels of R-2-hydroxyglutarate (R-2HG). Succinate, fumarate, and R-2HG can inhibit 2-oxoglutarate-dependent dioxygenases, including JmjC histone demethylases and TET DNA hydroxylases.

absence of endogenous inhibitory molecules such as fumarate, succinate, and reactive oxygen species. In this regard, intracellular fumarate and succinate concentrations are estimated to be in the high micromolar and low millimolar range, respectively (Johnson et al., 2008). In model systems, many metabolic enzymes display $K_m$ values in vivo that are higher than their corresponding in vitro values and certainly closer to the concentrations of their cosubstrates (Bennett et al., 2009; Yuan et al., 2009). This presumably allows such enzymes to respond to changes in the concentrations of available agonists and antagonists. Moreover, the concentrations of 2-oxoglutarate in specific subcellular compartments such as the nucleus are not known, nor is it known how much 2-oxoglutarate is free versus tightly bound to other proteins. It thus remains possible, but not proven, that JmjC proteins and TET proteins can respond under physiological conditions to changes in agonists such as 2-oxoglutarate or antagonists such as succinate and fumarate. The functions of these proteins do, however, appear to be deregulated as a result of altered metabolism under the pathological conditions found in the specific cancers described below.

## SDH, FH, and IDH Mutations and Cancer

Inactivating mutations affecting the mitochondrial succinate dehydrogenase (SDH) complex subunits and fumarate hydratase (FH) have been identified in cancers, particularly in familial paragangliomas and papillary renal cancers, respectively (Kaelin, 2009). These mutations lead to the marked accumulation of succinate and fumarate, respectively (Figure 6B). Somatic mutations affecting cytosolic IDH1 and mitochondrial IDH2 have been identified in gliomas, acute myelogenous leukemia, chondrosarcomas, and cholangiosarcomas. Somatic mosaicism for such mutations causes Ollier Disease syndrome and Maffucci syndrome, which are characterized by the development of endochondromas and spindle cell hemangiomas (Amary et al., 2011; Pansuriya et al., 2011). Tumor-associated IDH mutations unmask a latent ability of these enzymes to produce the R enantiomer of 2-hydroxyglutarate, which accumulates to low millimolar levels in IDH mutant tumors (Dang et al., 2009; Gross et al., 2010; Ward et al., 2010).

R-2HG, succinate, and fumarate are all capable of inhibiting multiple 2-oxoglutarate-dependent dioxygenases, including the JmjC histone demethylases and TET proteins, when present at sufficiently high concentrations (Figure 6B) (Cervera et al., 2009; Chowdhury et al., 2011; Figueroa et al., 2010; Koivunen et al., 2012; Lu et al., 2012; Smith et al., 2007; Turcan et al., 2012; Xiao et al., 2012; Xu et al., 2011). The challenge for the field is to determine which of these enzymes are actually inhibited by these metabolites in vivo in tumors bearing the appropriate mutations and which of these enzymes are causally linked to the transformed phenotype. For example, the HIF1α transcription factor accumulates in SDH and FH mutant tumors, presumably due to inhibition of the EglN prolyl hydroxylases (Dahia et al., 2005; Isaacs et al., 2005; Pollard et al., 2005, 2007; Selak et al., 2005). Moreover, it is plausible that HIF1α promotes the formation of such tumors based on its repertoire of downstream targets. Deletion of *HIF1α*, however, worsened the pathological changes observed in mice engineered to lack FH, suggesting that it normally constrains, rather than promotes, the emergence of FH-defective tumors (Adam et al., 2011).

A number of 2-oxoglutarate-dependent enzymes that act on chromatin are themselves targets of inactivating mutations in cancer, including the UTX H3K27 demethylase (also called KDM6A), JARID1C H3K4 demethylase (KDM5C), and TET2, making them attractive candidates for pathogenically relevant inhibition by succinate, fumarate, and R-2HG (Abdel-Wahab et al., 2009; Dalgliesh et al., 2010; Tefferi et al., 2009; van Haaften et al., 2009). In this regard, TET2 and IDH mutations are both found in acute myelogenous leukemia and are mutually exclusive, consistent with the idea that R-2HG produced by mutant IDH provides an alterative means of inactivating TET2 (Figueroa

et al., 2010). Moreover, IDH mutant tumors have been reported to display DNA hypermethylation changes consistent with TET2 inactivation. In addition, TET2 inactivation is sufficient to promote hematopoietic stem cell self-renewal and to block differentiation (Figueroa et al., 2010; Li et al., 2011; Moran-Crusio et al., 2011; Noushmehr et al., 2010; Turcan et al., 2012; Losman et al., 2013). Therefore, inactivation of TET2 probably contributes to leukemic transformation at the hands of mutant IDH and the R-2HG "oncometabolite" it produces. Interestingly, R-2HG is sufficient to promote cytokine independence and block differentiation of hematopoietic cells in vitro and these effects are fairly rapidly reversed upon R-2HG withdrawal (Losman et al., 2013; K. Yen, personal communication). This suggests either that these phenotypes are not due to epigenetic targets of R-2HG or that R-2HG-induced epigenetic changes are surprisingly dynamic.

In summary, *SDH*, *FH*, and *IDH* mutations cause the accumulation of succinate, fumarate, and R-2HG, respectively. These metabolites appear to cause cancer by affecting the behavior of various 2-oxoglutarate-dependent dioxygenases, including dioxygenases linked to DNA and histone methylation, and therefore have the potential to alter the epigenome.

## Nutrition, Epigenetics, and Disease

Altered epigenetics is believed to play a part in a variety of diseases in addition to cancer, including diabetes, obesity, dyslipidemia, hypertension, and neurodegeneration. The biochemical considerations outlined herein provide a conceptual framework for understanding how environmental changes, including changes in nutrition, could affect epigenetics and therefore diseases where epigenetic alterations play a role.

In this regard, particular attention has been paid to the influence of diet on one-carbon metabolism, which plays an important role in DNA and histone methylation and in risk of disease. Studies in rodents and sheep have confirmed that the availability of nutrients required for one-carbon metabolism (for example, folate, choline, methionine, and betaine, as well as selected B vitamins) at the time of conception and during pregnancy can induce epigenetically driven phenotypes in their offspring (Dolinoy et al., 2007; Sinclair et al., 2007; Waterland et al., 2006; Wolff et al., 1998). There is also evidence that changes in glucose and glucose metabolism can leave lasting epigenetic marks (Park et al., 2012; Pirola et al., 2010).

Observational studies in humans are also consistent with a possible role of nutrition in epigenetics and disease. For example, studies of individuals conceived during famine conditions have revealed decreased DNA methylation of specific loci, such as the *IGF2* gene, associated with an increased risk of obesity, dyslipidemia, and insulin resistance later in life (Dominguez-Salas et al., 2012). Genetic variation in a number of genes linked to one-carbon metabolism, including *methylenetetrahydrofolate dehydrogenase 1*, *FTHF dehydrogenase*, *5–10 methylene-THF reductase*, *methionine synthase*, and *glycine-N-methyltransferase*, has been linked to a variety of disease phenotypes including cancer and developmental defects (Stover, 2011).

Biomarkers or dietary histories indicative of nutritional deficiencies associated with defects in one-carbon metabolism (for example, folate deficiency) have frequently been associated with an increased risk of cancer in epidemiological studies. Disappointingly, however, intervention trials have failed to demonstrate a reduction of cancer risk in individuals randomized to receive folic acid supplements compared to controls (Andreeva et al., 2012; Cole et al., 2007; Song et al., 2012; Zhang et al., 2008). This might suggest that folate deficiency correlates with, but does not cause, cancer or that folate is important during an early time window, perhaps occurring at an early age and prior to or during tumor initiation, but not thereafter. In this regard, animal studies suggest that folate might actually increase cancer progression if given to nascent tumors in the colon (Kim, 2004).

There are many bioactive molecules present in food and herbs, in addition to folate, that are capable of influencing epigenetics and that have been touted as potential chemopreventative agents. Examples include various B vitamins, retinoic acid, vitamin D3, reservatrol, genistein and daidzein, epigallocatechin-3-gallate (EGCG), and curcumin (Gerhauser, 2013; Stefanska et al., 2012). Moving forward, it will be important to determine whether these agents influence epigenetic enzymes at concentrations that can be achieved in vivo (as opposed to cell culture studies), to understand their dose-response relationships, and to determine when, during the lifetime of an individual, they can exert their salutary effects.

## Conclusions and Future Questions

Many epigenetic enzymes are potentially susceptible to changes in the levels of cosubstrates and cofactors such as oxygen, ATP, acetyl-CoA, MTHF, S-adenosylmethionine, NAD, FAD, and 2-oxoglutarate and are hence poised to respond to changes in nutrient intake and metabolism. We need to learn more, however, about how much the levels of some of these cosubstrates and cofactors can vary in space (for example, in different cellular subcompartments and in different tissues) and how much they can fluctuate over time (for example, in response to changes in nutrition or as a function of age). If they do not vary significantly, how are they sensed and how are they buffered? If they do vary significantly, do they actually become limiting for specific epigenetic enzymes in vivo? If so, does this contribute to disease?

There is increasing evidence that early exposures, including intrauterine exposures, can lead to lasting epigenetic changes and that epigenetic differences can influence many phenotypes, including the risk of disease. Among the relevant exposures are exposures related to nutrition. This has led to the hypothesis that "we are what we eat but also what our parents ate" (Dominguez-Salas et al., 2012). We need better biomarkers for assessing variability in nutrition and metabolism in the population and its effects on epigenetics. From a public health perspective we need to better understand which alterations in metabolism and epigenetics cause, rather than correlate with, disease and when—and how—it might be possible to intervene.

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