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Metabolic control of methylation and acetylation

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Abstract

Methylation and acetylation of DNA and histone proteins are the chemical basis for epigenetics. From bacteria to humans, methylation and acetylation are sensitive to cellular metabolic status. Modification rates depend on the availability of one-carbon and two-carbon substrates (S-adenosylmethionine, acetyl-CoA, and in bacteria also acetyl-phosphate). In addition, they are sensitive to demodification enzyme cofactors (α-ketoglutarate, NAD^+) and structural analog metabolites that function as epigenetic enzyme inhibitors (e.g., S-adenosylhomocysteine, 2-hydroxyglutarate). Methylation and acetylation likely initially evolved to tailor protein activities in microbes to their metabolic milieu. While the extracellular environment of mammals is more tightly controlled, the combined impact of nutrient abundance and metabolic enzyme expression impacts epigenetics in mammals sufficiently to drive important biological outcomes such as stem cell fate and cancer.

In classical kinase signal transduction cascades, a chemical trigger (such as hormone-receptor binding) leads to a rapid (~ 1 min) series of phosphorylation events that augment the signal and drive downstream effector functions such as gene transcription. While requiring ATP, such cascades are generally independent of metabolic status, as the cellular concentration of ATP (~ 10 mM) dwarfs the amount needed to saturate kinase active sites (~ 0.002-0.1 mM) and is also sufficient to outcompete related metabolites like ADP [1]. Thus, with the exception of kinases like AMPK and TOR that are specifically designed to sense metabolites, metabolism and kinase signaling can be reasonably viewed as distinct biochemical networks.

In contrast, other important protein covalent modifications occur on slower timescales and are tightly linked to cellular metabolite abundances. Foremost among these are methylation and acetylation. For these reactions, the physiological substrate concentrations are lower than ATP. Moreover, the reaction products, or other related endogenous metabolites, are

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often competitive inhibitors of substrate binding [2]. The tight binding (low K_i) of these inhibitors renders reaction rates sensitive to substrate concentration, even when substrate is nominally sufficient to saturate the enzyme ([substrate] > K_m):

$$V = \frac{V_{max}[S]}{K_m\left(1 + \frac{[I]}{K_i} + \frac{[S]}{K_m}\right)}$$

Although more work on the underlying enzymology is needed, much of metabolic control of methylation and acetylation seems to rely on such active site competition.

Acetylation and deacetylation

In bacteria, acetylation can be driven by acetyl-CoA or acetyl-phosphate. Based on recent experiments in *E. coli*, acetyl-phosphate, which reacts spontaneously with protein lysines, is thought to predominate. Manipulations that increase acetyl-phosphate, such as deletion of acetate kinase or nitrogen limitation, increase protein lysine acetylation. In contrast, knockout of phosphotransacetylase, which converts acetyl-CoA into acetyl-phosphate, decreases protein acetylation. While *E. coli* encodes a homolog of the classical eukaryotic histone acetylation enzyme Gcn5 (YfiQ), its knockout does not broadly alter protein acetylation. Thus, in bacteria, acetyl-phosphate levels are likely the primary determinant of protein acetylation rates [3].

Most eukaryotes are not known to make acetyl-phosphate and the only known substrate for acetylation is acetyl-CoA. Based on analysis of isolated mitochondria, their acetyl-CoA concentration is estimated to be 0.1-1.5 mM, [4]. The combination of abundant acetyl-CoA and high pH (which enhances the fraction of lysine residues in their neutral and thus nucleophilic form), results in substantial spontaneous mitochondrial protein lysine acetylation [5]. Such nonenzymatic protein acetylation may also happen outside mitochondria, facilitated by basic amino acid residues just upstream of the critical lysine in the protein sequence [6]. Nevertheless, due to lower acetyl-CoA levels (0.002-0.013 mM) [7] and pH, most acetylation outside mitochondria, including in the nucleus where histones reside, depends on specific modification enzymes such as Gcn5, MYST, and p300/CBP. Histone acetylation generally promotes associated gene transcription.

Acetyl-CoA can be made in mitochondria via catabolism of pyruvate, amino acids, or fatty acids (Figure 1). Transport of acetyl-CoA into the cytosol involves an ATP-driven metabolic cycle, where mitochondrial acetyl-CoA condenses with oxaloacetate to form citrate, which is transported into the cytosol and cleaved by ATP citrate lyase [8]. Activity of this cycle, which is induced by signals including insulin and Akt [7,9], impacts cytosolic acetyl-CoA levels. In hypoxia, pyruvate dehydrogenase is inhibited and acetate becomes a major source of cytosolic acetyl-CoA. The ligation of acetate and CoA, at the expense of ATP, is catalyzed by the enzyme acetyl-CoA synthetase 2 (ACSS2) in mammals. Hypoxic cancer cells in culture derive nearly half of cytosolic acetyl-CoA from acetate [10], and significant expression of ACSS2 has been found in certain breast, ovarian, and lung tumors [11]. Recently it has been reported that pyruvate dehydrogenase complex can be translocated

from the mitochondria to the nucleus [12]. This putatively enables direct conversion of nuclear pyruvate into acetyl-CoA for histone acetylation.

Although lysine acetylation is chemically stable, the removal of lysine acetylation can be achieved by straightforward amide hydrolysis by water. Three different phylogenetic classes of histone deacetylases (HDACs) carry out this reaction in a Zn^{2+}-dependent manner: Class I (human HDAC1–3 and HDAC8), II (human HDAC4–7 and HDAC9–10), and IV (human HDAC11). In contrast, Class III HDACs, also known as sirtuins (human SirT1-7), carry out an alternative reaction with NAD^+ as a co-substrate. Thus, NAD^+ levels, relative to NADH and nicotinamide, are important regulators of deacetylation. Although straightforward deacetylases are found in some eubacteria, the ties between NAD^+ and protein deacetylation are evolutionarily conserved, with deacetylation in *E. coli* carried out by the NAD^+-dependent deacetylase CobB [13]. Thus, protein acetylation and deacetylation depend on the concentrations of two of the most important central metabolic cofactors: acetyl-CoA and NAD^+.

Methylation and demethylation

The substrate for methylation is the activated one-carbon donor S-adenosylmethionine (SAM), which is transferred to DNA and protein targets via specific modification enzymes in both prokaryotes and eukaryotes (Figure 2). DNA methylation in prokaryotes occurs at the 6-position of adenosine residues (6mA), whereas in eukaryotes it typically occurs at the 5-position of cytosine residues (5mC). In both cases, DNA methylation is generally transcription inhibitory [14,15].

On histones, methylation can occur on arginines or lysines. Each individual lysine residue can be mono-, di-, or tri-methylated. While the residue is cationic in any of these forms, methylation enhances hydrophobicity and thereby alters binding of important gene-expression regulating proteins. Methyl-binding domains frequently recognize the modifications via aromatic cages. One class of histone methyltransferase, DOT1, targets the core histone body to regulate transcriptional activation and elongation. Chromosomal rearrangements that activate DOT1 are the leading causes of infant acute leukemias [16]. Another class, involving a highly conserved catalytic protein domain called SET, targets the histone tail where many regulatory proteins bind. While SET domain expressing proteins have the conserved chemical function of histone methylation, the gene expression outcome varies depending on the specific tail site. Typically, trimethylation of lysine 27 on histone H3 (H3K27me2/3) that is mediated by polycomb-group complex 2 (PRC2) represses gene expression, whereas trimethylation of lysine 4 of histone H3 (H3K4me3) that is mediated by Trithorax complex activates transcription.

While all methylation enzymes share the common substrate of SAM, the impact of SAM concentrations can be histone site specific. This can be achieved by differences in histone methyltransferase's SAM affinity. For example, in yeast, core histone methylation catalyzed by Dot1 is less sensitive to disrupted methionine and SAM biosynthesis than histone tail methylation catalyzed by SET enzymes. This reflects the lower K_m of Dot1 for SAM, and can be reversed by mutants that increase this K_m [17]. In general, H3K4me2/3, which is

methylated by the yeast enzyme Set1, is the most sensitive site to SAM [18-20]. Set1 was recently shown to interact with a metabolic enzyme complex comprising serine biosynthetic enzymes, serine hydroxymethyltransferase and methionine adenosyltransferases [21]. The higher sensitivity of H3K4 methylation to one-carbon status may arise due to this complex, the K_m of Set1 for SAM, and/or H3K4 being more exposed than other histone sites to demethylation. Consistent with complexes between metabolic enzymes and histone methyl transferases being functionally important, in *Caenorhabditis elegans*, different methionine adenosyltransferases appear to impact differentially histone methylation. Worms lacking methionine adenosyltransferase 1 (sams-1) are more sensitive to *Pseudomonas*, due to a reduction in H3K4me3, which is required to induce infection response gene transcription [22]. Knockdown of methionine adenosyltransferase 3 (sams-3) has no effect on H3K4me3, but decreases methylation of other sites (H3K9, H3K27, H3K36) [23]. Thus, histone methyltransferases may depend on local SAM production by specific associated methionine adenosyltransferase enzymes.

Removal of methylation is chemically challenging and involves diverse metabolic inputs. In bacteria, the removal of DNA methylation marks is usually achieved by two rounds of DNA replication. On the other hand, DNA alkylation damage including 1mA and 3mC can be removed by the enzyme AlkB. AlkB is a member of the chemically versatile class of enzymes known as Fe^{2+}/α-ketoglutarate-dependent oxygenases [24]. These enzymes couple iron-catalyzed oxidative decomposition of the co-substrate α-ketoglutarate (forming CO_2 and succinate) to the hydroxylation of the primary substrate. Their function in both DNA and protein demethylation is conserved from bacteria to humans. Although the closest eukaryotic homologs of AlkB function in DNA and RNA alkylation repair, the same catalytic strategy is used by the main family of eukaryotic DNA demethylases (TET) and histone demethylases (JMJC). Another family of histone demethylases, LSD1, while still consuming oxygen, uses FAD as a cofactor and does not require any co-substrate. Importantly, only the α-ketoglutarate-dependent JMJC enzymes can remove lysine tri-methylation.

The dependence of TET and JMJC enzymes on oxygen and α-ketoglutarate as co-substrates renders demethylation rates sensitive to both oxygenation and TCA cycle metabolism. Among TCA cycle metabolites, α-ketoglutarate promotes demethylation, whereas succinate and fumarate act as competitive inhibitors. In addition, α-ketoglutarate can undergo two-electron reduction to produce 2-hydroxyglutarate (2HG), which also competitively inhibits demethylation. Thus, methylation status is broadly sensitive to oxygen, one-carbon, and TCA-related metabolism.

Physiological function of acetylation and methylation in microorganisms

In *E. coli*, when carbon availability exceeds nitrogen availability, acetate and acetyl-phosphate accumulate, increasing protein acetylation. In complete media, deletion of the main enzymes making and consuming acetyl-phosphate (*ackA*Δ and *pta*Δ) result in 10-fold changes in overall protein acetylation, without markedly impacting growth rate. This suggests that any functional role of protein acetylation is subtle and/or context dependent [3]. In eukaryotes, metabolism is rewired to eliminate acetyl-phosphate, at the expense of

reducing the ATP yield of glycolysis from 4 ATP to 2 ATP per glucose. It is possible that such rewiring evolved in part to minimize spontaneous protein acetylation, which in turn allowed broader use of enzymatic acetylation for regulatory purposes.

Consistent with this, acetyl-CoA plays an important role in coordinating metabolism and transcription in yeast. When yeast are limited for glucose in continuous culture, they undergo rounds of synchronized metabolic cycling, which temporally separate expression of growth genes (such as ribosomes) from protective genes (such as antioxidant enzymes). A critical determinant of this metabolic cycling is intracellular acetyl-CoA concentration [25]. Acetyl-CoA levels are high coincident with the expression of growth genes, both in metabolically cycling yeast and in standard batch cultures. In glucose-limited metabolically cycling yeast, addition of acetate will induce growth gene expression. These effects are mediated through the histone acetyltransferase Gcn5, part of the SAGA complex. Gcn5 preferentially acetylates histone lysine residue H3K9 at growth genes [26]. Remarkably, roughly two-fold changes in acetyl-CoA are sufficient to alter histone acetylation and gene expression several-fold. This may reflect acetyl-CoA cooperatively regulating Gcn5 activity, both through acetylation of other SAGA subunits and through its direct role as the histone acetylation substrate. The net effect is that acetyl-CoA ties carbon status to growth gene transcription [27].

While histone acetylation is important for cell growth, deacetylation is important for transcriptional repression at certain loci. Yeast cells that fail to repress certain genes, including ribosomal RNA, have short replicative life span [28]. Overexpression of the sirtuin family histone deacetylate Sir2p extends the life span of yeast cells. Alternatively, the life span of yeast can be extended by mild carbon limitation, which activates Sir2p by decreasing NADH and increasing the NAD^+/NADH ratio [29]. Hypoxia is a powerful metabolic manipulation that markedly decreases the NAD^+/NADH ratio, and may accordingly be expected to inhibit sirtuin activity. Further investigation of the impact of hypoxia on microbial acetylation status is warranted.

Similarly, environmental factors can impact concentrations of metabolites involved in methylation and demethylation. While existing literature in microbes is limited, one can envision the environmental concentrations of folate, formate, serine, or methionine feeding into SAM levels and thus methylation rates. In terms of demethylation, in both *E. coli* and yeast, α-ketoglutarate responds the most strongly of any metabolite to nitrogen limitation. In *E. coli*, it coordinates carbon and nitrogen metabolism, suppressing glucose uptake and inhibiting transcription of alternative carbon source genes [30,31]. In yeast, it regulates the expression of nitrogen assimilation genes through the PII signal transduction proteins. It seems likely that the dependence of demethylases on α-ketoglutarate as a cofactor evolved in part to couple demethylation to nitrogen status. Thus, in addition to the well-established connection between acetyl-CoA, NAD^+, and growth gene transcription in yeast, there are likely other functionally important connections between metabolism, acetylation, and methylation in microbes.

Impact of metabolic milieu on mammalian epigenetics

In mammals, cells are fed through the circulation, with extracellular metabolite concentrations maintained in a relatively narrow range through systemic homeostatic mechanisms. Nevertheless, the metabolic environment can impact acetylation and methylation. Acetyl-CoA can be made from each major category of mammalian nutrients (carbohydrates, fats, protein). In mouse liver, free CoA is higher under fasting conditions, while acetyl-CoA is relatively stable [32]. To date, we are unaware of studies adequately investigating the link between dietary nutrient consumption, acetyl-CoA levels, and *in vivo* acetylation rates. Deacetylation by sirtuins, however, is known to be influenced by levels of their co-substrate NAD^+ (relative to competitive inhibitors). Moreover, even the deacetylases that use water as the sole substrate are subject to allosteric regulation by circulating β-hydroxybutyrate.

Both calorie restriction and high-fat diet may impact histone acetylation via NAD^+ levels, with calorie restriction potentially promoting high NAD^+/NADH and thus deacetylation [33]. High-fat diet has the converse effect, decreasing NAD^+ and thus sirtuin activity [34,35]. In high-fat diet-induced diabetic mice, the activity of nicotinamide phosphoribosyltransferase (NAMPT), a key enzyme in mammalian NAD^+ synthesis, is severely reduced. Administration of nicotinamide mononucleotide or riboside restores NAD^+, activates SirT1 and SirT3, and enhances insulin sensitivity [36,37]. The NAD^+-consuming enzyme nicotinamide N-methyltransferase (NNMT) is up-regulated in mouse models of obesity and insulin resistance [38] and is overexpressed in many tumors [39]. This enzyme has dual epigenetic effects, simultaneously depleting SAM and NAD^+, and thereby impairing methylation and deacetylation [38].

Although other HDACs have no co-factor requirement, their activity is nevertheless coupled to metabolism via the “ketone body” β-hydroxybutyrate, which inhibits class I HDACs, leading to increased H3K9 and H3K14 acetylation [40]. During fasting, the liver switches to fatty acid oxidation and circulating D-β-hydroxybutyrate levels can rise to above 1 mM. β-hydroxybutyrate increases H3K9 acetylation at the promoter of FOXO3A, a transcription factor for oxidative stress resistance genes [40], and diets that are low in carbohydrate promote ketogenesis and protect neurons from oxidative damage [41]. Altered acetylation does not, however, correlate with seizure control by ketogenic diet [42]. Thus, high calorie diets that include carbohydrates tend to inhibit deacetylation by sirtuins, whereas ketogenic conditions inhibit deacetylation by other HDACs.

The impact of diet on methylation rate is increasingly well established. Circulating homocysteine levels correlate inversely with cellular SAM:S-adenosylhomocysteine (SAH) ratio. Homocysteine levels tend to increase with age and disease, including atherosclerosis and thrombosis, although a causative role for homocysteine in these conditions has not been established [43]. Diet that is high in methionine and low in folate and cobalamin causes high circulating homocysteine [44]. In rodent studies, either methionine restriction (0.12% methionine diet in mice) or excess (regular diet plus 1% methionine in rats) has been reported to lower the SAM:SAH ratio and H3K4me3 [18,45]. In humans, vegetarians, who have a low intake of cobalamin (the cofactor for converting homocysteine into methionine),

tend to have high SAH correlated with low whole-genome methylation [46]. Overall, dietary factors have been estimated to account for 30% of serum methionine variation [18]. Thus, both amino acid and vitamin intake can propagate through metabolism to impact methylation rates.

A primary environmental factor impacting demethylation is hypoxia, which occurs in a variety of physiological and pathological settings, including intense exercise, high altitude, cancer, and atherosclerosis. Elevated NADH due to hypoxia may potentially inhibit sirtuin activity. Ties between low oxygen and demethylation are better established, with low oxygen inhibiting demethylation through two mechanisms: lack of oxygen as a substrate for demethylase enzymes and increased (likely via elevated NADH) synthesis of L-2HG, which competitively inhibits α-ketoglutarate-dependent demethylases [47]. The catalytic mechanism of α-ketoglutarate-dependent demethylases is similar to that of the classical oxygen sensors HIF prolyl hydroxylases, with human JMJC demethylase KDM4E having a saturating concentration of oxygen above the atmospheric concentration. Nevertheless, H3K9me3 enhancement in hypoxia is dependent on elevated L-2HG [48,49].

Collectively, these examples highlight the potential for physiological conditions to impact epigenetics via metabolism. Further work is needed, however, to establish the extent to which transcriptional adaptation to nutrient and oxygen availability occurs via acetylation and methylation versus other mechanisms. One appealing possibility is that metabolism-specific signaling machinery like insulin and HIF play a predominant role in short-term adaptation, whereas acetylation and methylation contribute substantially to long-term adaptation.

Control of stem cell fate by epigenetic regulatory metabolites

The maintaining of pluripotency requires an open and accessible chromatin structure. Histone acetylation contributes to the openness of chromatin and stem cell pluripotency. Human embryonic stem cells differentiate upon withdraw of basic fibroblast growth factor (bFGF), with concomitant decreases in the expression of pyruvate dehydrogenase, ATP citrate lyase, and ACCS2, and depletion of acetyl-CoA. Addition of acetate promoted H3K9/K27 acetylation, complementing bFGF in maintaining pluripotency [50]. While ties between deacetylation and stem cell maintenance are less well established, nicotinamide can facilitate human stem cell maintenance, possibly via sirtuins [51].

Open chromatin structure in stem cells is also dependent on both methylation and demethylation. H3K4me3 is transcriptionally activating and a crucial ESC self-renewal signal [52], and SAM production is important in maintaining the pluripotency of mouse embryonic stem cells (mESC). mESC specifically express threonine dehydrogenase [53], which oxidizes threonine into acetyl-CoA and glycine, a one-carbon donor. When the mouse embryonic fibroblasts are reprogrammed into induced pluripotent stem cells, SAM levels increase, along with H3K4 methylation, driven by threonine-derived methyl groups [19]. In humans, however, threonine dehydrogenase is a nonfunctional pseudogene, and SAM production relies on methionine. Methionine withdrawal or inhibition of methionine adenosyltransferase inhibits human stem cell growth [20]. On the demethylation side, mESC

differ from most cultured cells in having high flux from glucose to α-ketoglutarate. This both enables growth in the absence of glutamine, and maintains a high intracellular ratio of α-ketoglutarate to succinate, which facilitates demethylation. mESC cells have low levels of H3K27me3 and low DNA methylation. Supplementation with α-ketoglutarate favors mESC self-renewal, whereas succinate promotes differentiation [54]. Thus, in addition to responding to their nutrient environment, cells can modulate their metabolic network through changing enzyme levels to maintain particular epigenetic states.

Metabolism and epigenetics in cancer

Cancer cells resemble stem cells in many ways. Abnormal metabolism not only fuels cancer cell growth, but also shapes their epigenetics to favor proliferation. Oncogenic AKT and Myc promote ATP-citrate lyase-dependent histone acetylation [7,55], and pAKT(S473) correlates with histone acetylation in human gliomas and prostate tumors [7]. Butyrate is one of the bacterial fermentation products of dietary fiber in colon, and has been shown to reduce the risk of colorectal cancer [56]. In normal cells, butyrate goes through β-oxidation to generate acetyl-CoA. In cancerous colonocytes, butyrate functions as a HDAC inhibitor and is growth inhibitory. In both cases butyrate favors histone acetylation and activates transcription. For example, the upregulation of the tumor suppressor TES relies on butyrate-derived acetyl-CoA, whereas the upregulation of apoptosis related genes depends on the HDAC inhibition by butyrate [57].

Changes in methylation are prevalent in cancer genomes, with 2000-3000 promoters typically are aberrantly methylated per cancer cell and 70% of all the histone and DNA modification genes known to be mutated in human cancer [58]. In contrast, only a few of the ~ 2000 metabolic enzymes in the human genome are systematically mutated in cancer. Remarkably, each known oncogenic mutation in metabolic enzymes affects methylation. The most common and important of these are active site mutations in isocitrate dehydrogenase (IDH) 1 and 2, which lead to cancer through production of D-2HG [59-61]. Almost all cases of AML involve in activation of the DNA methylase TET2, which can occur through homozygous TET2 deletion or single-copy point mutation of IDH [62]. Loss-of-function mutations in fumarate hydratase and succinate dehydrogenase subunits cause the accumulation of fumarate or succinate and thereby also result in cancer through demethylase inhibition [63,64]. The discovery of these oncometabolites offers new therapeutic opportunities. A small molecule inhibitor of mutant IDH1 inhibitor reduces histone H3K9me3 and promotes glioma cells differentiation [65].

In addition to genetic lesions to metabolic enzymes, cancer epigenetics can be influenced by the tumor metabolic status. While the tumor environment is typically nutrient poor and low in serine, overall cancer genomes are hypermethylated. One contributor may be phosphoglycerate dehydrogenase, which diverts glycolytic intermediate 3-phosphglycerate into serine *de novo* synthesis and is genomically amplified in breast cancer and melanoma [66]. In addition, genes to convert serine into one-carbon units are strongly overexpressed in a broad spectrum of tumors and show correlation between expression and cancer progression [67]. On the demethylation side, one factor that could contribute to cancer genome hypermethylation is hypoxia.

Beyond the inhibition of specific metabolic oncogenes, it is intriguing to speculate that metabolic manipulations could, through epigenetic mechanisms, help to treat a larger subset of cancers. Methotrexate, an antifolate, can increases Fas death receptor expression by reducing promoter methylation [68]. The NAMPT inhibitor FK866 lowers cellular NAD^+ levels and increases p53 acetylation [69]. Further investigation is merited to identify the optimal combination of diet, metabolic inhibition, and epigenetic therapy to stop cancer.

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Highlights

- Epigenetic methylation and acetylation are sensitive to cellular metabolic status
- Diet and environment conditions shape epigenetics through metabolism
- Stem cells rewire metabolism to maintain open chromatin structure
- Oncometabolites cause cancer by interfering with DNA and histone demethylation

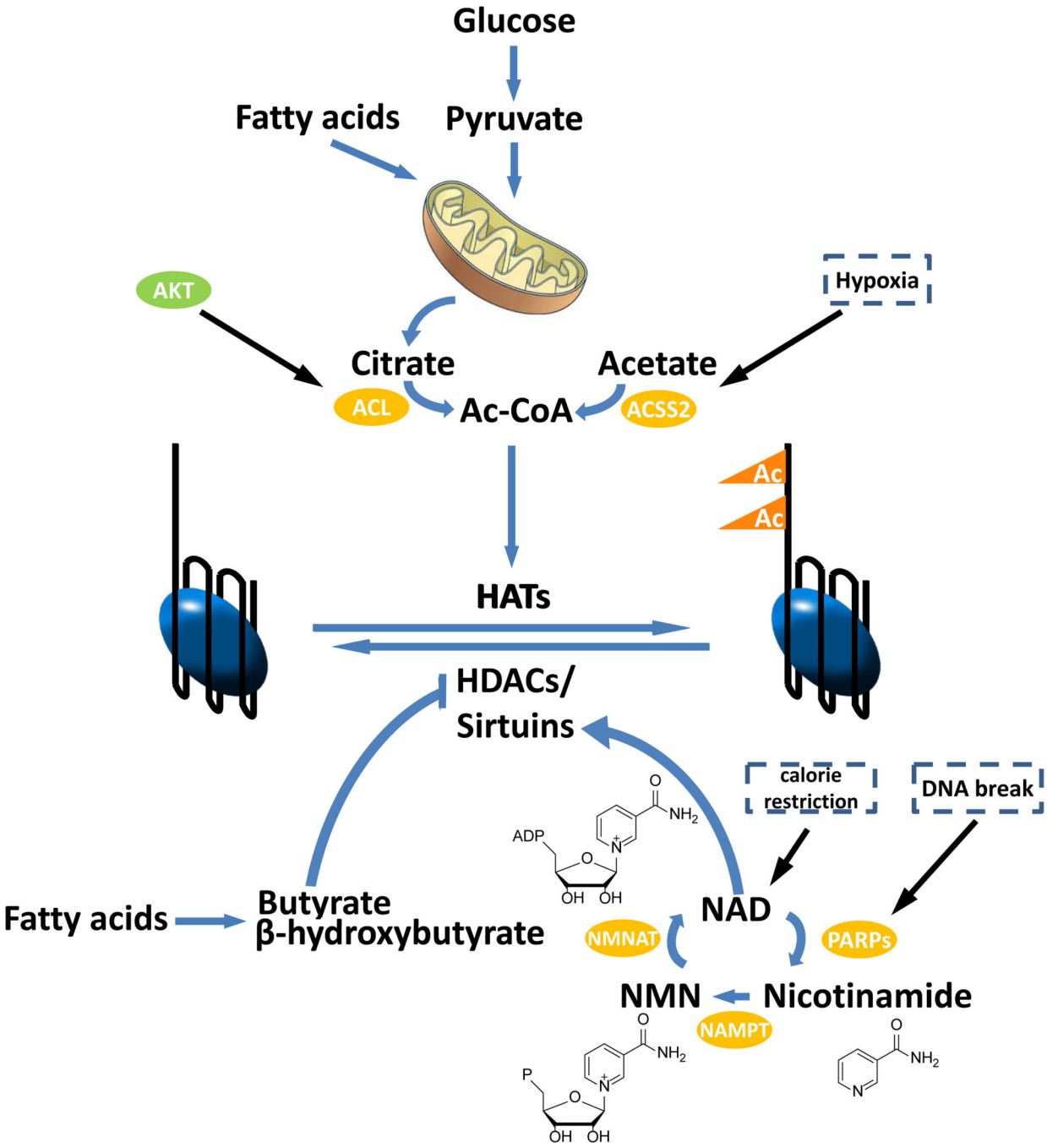

Figure 1.

Metabolic pathways contributing to histone acetylation and deacetylation. Acetyl-CoA is the substrate of histone acetyltransferase (HATs). Glucose derived pyruvate and fatty acids feed into mitochondria to produce acetyl-CoA and subsequently citrate. Mitochondrial citrate can be exported and converted to cytosolic acetyl-CoA by citrate-ATP lyase (ACL). AKT activates ACL by phosphorylation. Alternatively, cytosolic acetyl-CoA can be generated from acetate, which is the primary production route under hypoxia. Two classes of enzymes remove the histone acetylation marks, HDACs and sirtuins. Sirtuins use NAD^+ as the

substrate for deacetylation, generating nicotinamide and O-acetyl-ADP-ribose as the products. Nicotinamide is a sirtuin inhibitor. Calorie restriction or supplementation of NAD biosynthetic precursors enhance NAD^+ levels and thus sirtuin activity. Poly(ADP-ribose) polymerases (PARPs) use NAD^+ as substrate and deplete NAD under DNA damage conditions. Other HDACs have no co-substrate requirement, but can be inhibited by β-hydroxybutyrate.

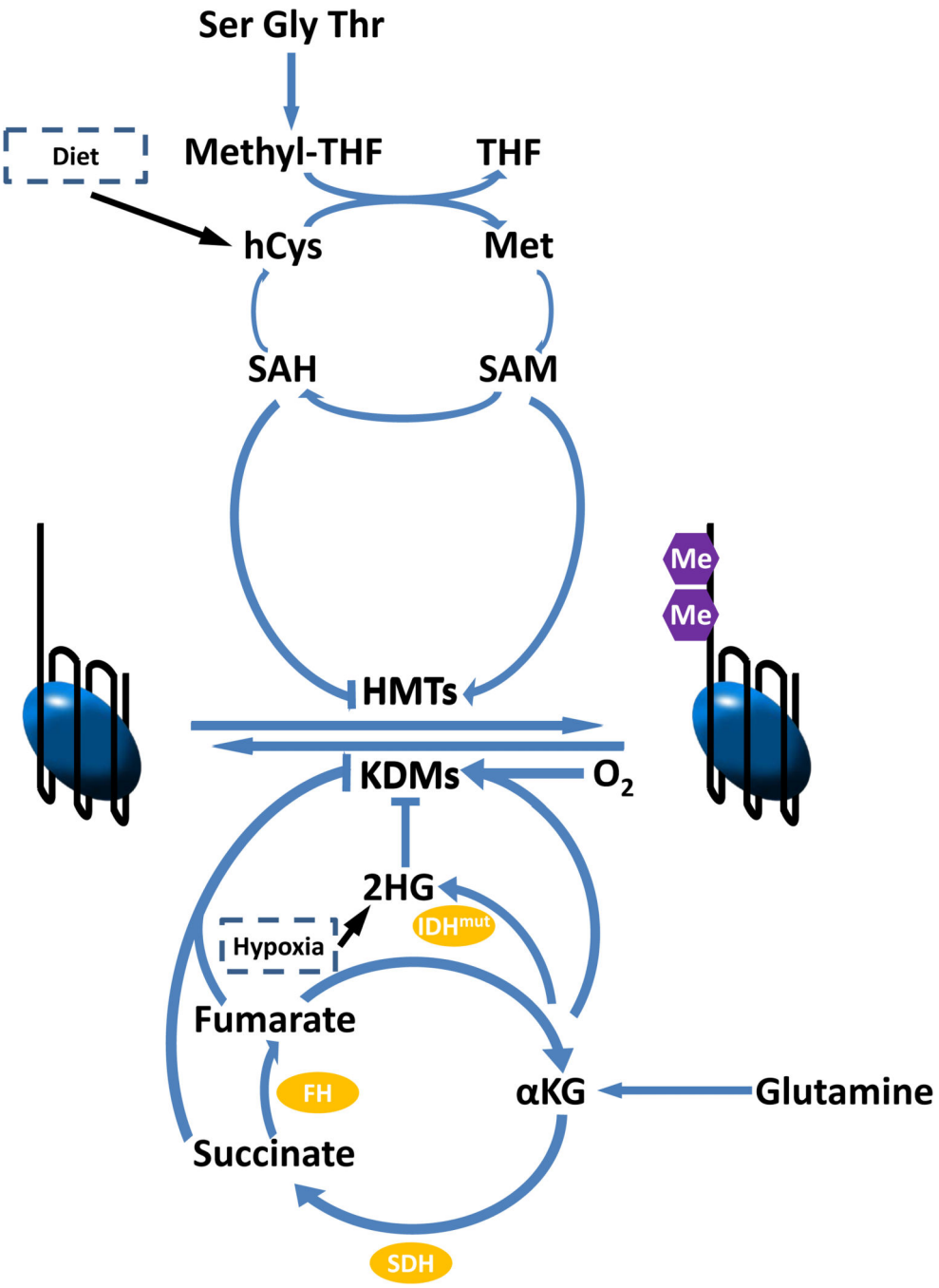

Figure 2.

Metabolic pathways contributing to histone and DNA methylation and demethylation. Histone and DNA methyltransferases use SAM as substrate and produce SAH as product. SAH is a competitive inhibitor of the methyltransferases, therefore the SAM:SAH ratio dictates the activity of the transferases. SAH can be removed by S-adenosylhomocysteine hydrolase, producing homocysteine, which can be remethylated to form methionine. This set of reactions is called the methionine cycle. The remethylation uses methyl-THF and vitamin B12. Serine, glycine and (in mouse but not human) threonine can all contribute to the

methyl-THF pool. S-adenosylmethionine synthetase converts methionine and ATP into SAM. DNA demethylation and most histone demethylation depend on both O_2 and α-ketoglutarate. Other dicarboxylic acids, such as succinate, fumarate and 2-hydroxyglutarate (2HG), are inhibitors of the α-ketoglutarate-dependent demethylases. Oncogenic mutations in isocitrate dehydrogenase (IDH1 R132H and IDH2 R172K) result in D-2HG production. Loss of succinate dehydrogenase or fumarase can similarly cause cancer due to accumulation of succinate and fumarate. All these mutations are oncogenic due to the inhibition of DNA and histone demethylation.