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Metabolic control of methylation and acetylation

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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.

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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 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):

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

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

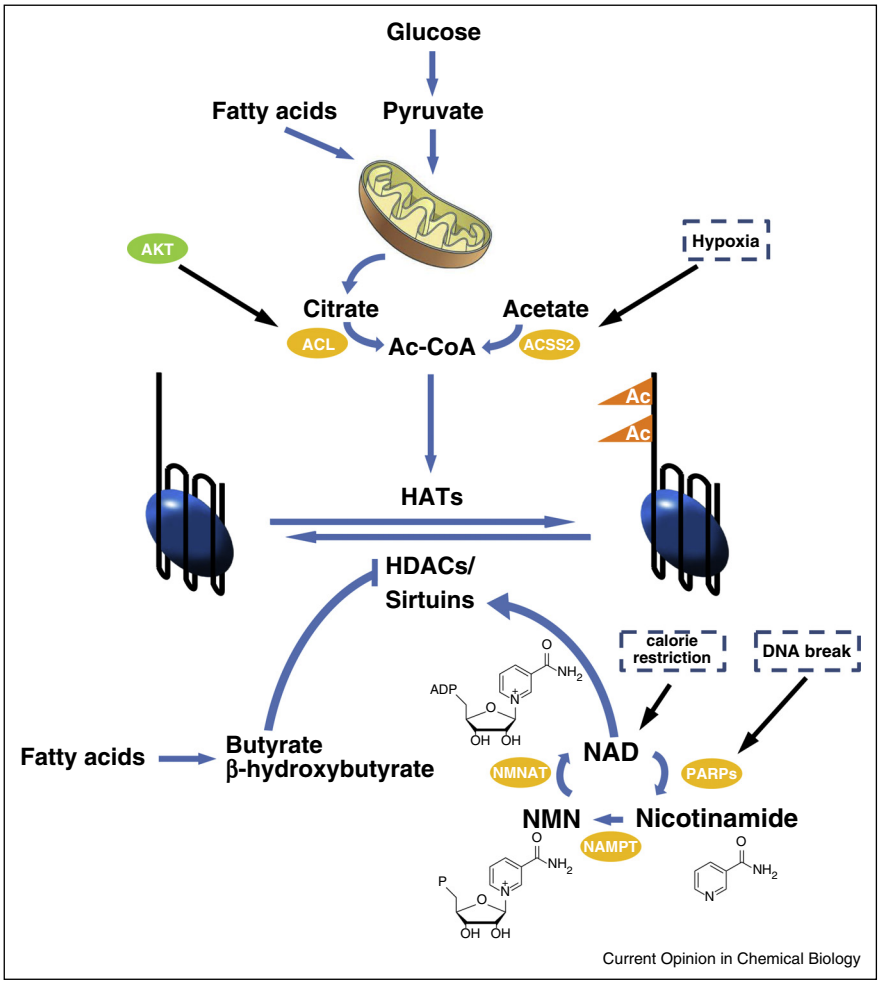
In bacteria, acetylation can be driven by acetyl-CoA or acetyl-phosphate. Based on recent experiments in *Escherichia coli*, acetyl-phosphate, which reacts spontaneously with protein lysines, is thought to predominate. Manipulations that increase acetyl-phosphate, such as deletion of acetate kinase or 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.

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 enhances 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.