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KAT(ching) Metabolism by the Tail: Insight into the links between lysine acetyltransferases and metabolism

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Abstract

Post-translational modifications of histones elicit structural and functional changes within chromatin that regulate various epigenetic processes. Epigenetic mechanisms rely on enzymes whose activities are driven by co-enzymes and metabolites from intermediary metabolism. Lysine acetyltransferases (KATs) catalyze the transfer of acetyl groups from acetyl-CoA to epsilon amine groups. Utilization of this critical metabolite suggests these enzymes are modulated by the metabolic status of the cell. This review highlights studies linking KATs to metabolism. We cover newly identified acyl-modifications (propionylation and butyrylation), discuss the control of KAT activity by cellular acetyl-CoA levels, and provide insights into how acetylation regulates metabolic proteins. Lastly, we conclude with a discussion of the current approaches for identifying novel KATs and their metabolic substrates.

Keywords

Histone acetyltransferase (HAT); Lysine acetyltransferase (KAT); metabolism; epigenetics; lysine acetylation; acetyl-CoA

Introduction: Chromatin organization and modification of histones

Genomic access in eukaryotic organisms is essential for gene expression and genome maintenance, and is dynamically regulated by the structure of chromatin. The nucleosome core particle represents the first level of chromatin organization, with higher ordered structures that progress from minimally folded nucleosome arrays to highly condensed metaphasic chromosomes.^[1] Higher order structures are repressive to active transcription, necessitating a chemical and physical means whereby DNA can be accessed for efficient expression. Histone residues can be reversibly modified on their N-terminal ‘tails’ as well as their globular domains. Covalent modifications of histones not only affect structural and intra-molecular interactions (i.e. octamer destabilization, tail-DNA association), but also serve as molecular signals that recruit proteins to specific chromatin regions. Histone modifications are read and propagated by a diverse set of enzymes that utilize histone reader motifs to recognize these marks.^[2] Collectively, these networks constitute an epigenetic code that coordinates gene expression levels resulting in phenotypic variation.^[3]

Numerous histone modifications have been identified and include acetylation, methylation, phosphorylation, ubiquitylation, sumoylation and ADP-ribosylation. These modifications require key intermediates common to diverse metabolic pathways, such as ATP,

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nicotinamide adenine dinucleotide (NAD⁺), and S-adenosylmethionine (SAM). Lysine acetylation requires the metabolite acetyl-coenzyme A (acetyl-CoA), and remains the most well studied post-translational modification on histones with broad effects on chromatin function and nuclear signaling pathways.^[4] A link between histone acetylation and active gene transcription was first noted by Allfrey *et al*, who showed that in vitro synthesis of RNA inhibited the acetylation of isolated histones.^[5] Since then, numerous studies have identified hyper-acetylated histone isoforms using chromatin immunoprecipitation assays^[6] and demonstrated a correlation between global acetylation and actively transcribed gene loci.^[7]

Various studies have demonstrated a correlation between global acetylation and actively transcribed gene loci.^[8] These studies provide compelling in vitro evidence that suggests lysine acetylation can destabilize chromatin structure leading to gene transcription. Histone acetylation and acetyltransferase activity are required for gene activation *in vivo*,^[9] while more recent microarray experiments have demonstrated that most lysines on H4 and H3 tails are acetylated in actively transcribed regions.^[10] Acetyltransferase activity has also been linked to other diverse cellular processes such as apoptosis, stress response and cancer (reviewed in ^[11]), while deacetylase activity, particularly from the NAD⁺-dependent sirtuin family, has been associated with aging, metabolism, cardiovascular disease and neurodegenerative disorders.^[12] Histone deacetylases and their metabolic implications have been reviewed in a number of published papers.^[13]

Given the significance of histone acetylation and the central role of acetyl-CoA in metabolism, it is important to understand the functional link between KAT activities and cellular metabolic status. This review summarizes the current knowledge on newly discovered protein acylation (propionylation and butyrylation) and its possible physiological relevance. The various pathways of acyl-CoA production and their impact on histone acylation are discussed. This review also highlights the implications of the cellular acetyl-CoA levels on KAT activity and the direct effect of acetylation on metabolic enzymes. Lastly, the current approaches for identifying novel KATs and their substrates are outlined.

Lysine acetyltransferases

Lysine acetyltransferases (KATs) are a class of enzymes that catalyze the transfer of the acetyl group from acetyl-CoA cofactor to the ε-amino group of lysine residues in histone and non-histone proteins. KATs and their associating complexes are commonly categorized into three distinct families according to the sequence homology of their catalytic domains and shared substrate specificity. These include the GNAT (Gcn5-related *N*-acetyltransferase), MYST (MOZ, Ybf2/Sas3, Sas2 and Tip60) and p300 (KAT3B) / CBP (KAT3A) co-activator KAT families. These KAT families all possess a structurally similar acetyl-CoA recognition domain, characterized by a conserved Arg/Gln-X-X-Gly-X-Gly/Ala segment.^[14]

Though KAT enzymes exhibit remarkable divergence in substrate specificity and cellular function,^[11a, 15] existing data suggests they utilize a common sequential (direct attack) mechanism of acetylation (Figure 1). GNAT and MYST KATs utilize an active-site glutamate/aspartate to deprotonate the ε-amine group of lysine, promoting nucleophilic attack on the carbonyl group of acetyl-CoA (Figure 1).^[16] Kinetic evidence suggests that human p300 and yeast Rtt109 KATs also employ a direct-attack mechanism, though no active-site base has been reported.^[17] Together, these studies provide convincing evidence for a unified mechanism of KAT catalysis.

Propionylation and butyrylation as novel lysine modifications

Recent studies have reported that propionylation and butyrylation are post-translational modifications that exist on histone and non-histone proteins. Chen *et al.* have conducted a mass spectrometry (MS) analysis of HeLa cells and identified propionylation and butyrylation on lysines 5, 8 and 12 of H4.^[18] Subsequent MS and chromatin-immunoprecipitation studies in yeast have shown that H3 and H2B are also propionylated and butyrylated, though these modifications have not been observed on H2A (see Figure 2).^[19] These modifications have also been discovered on several non-histone proteins, including the transcription factor p53 and the KATs p300 and CBP.^[18-20] When p300 was co-transfected with p53 in H1299 cells, the stoichiometry of p53 propionylation at K292 was at least 25-fold lower than acetylation at this site.^[20] Additionally, H3 K56 acetylation levels in yeast were ~85-fold higher compared to the propionylated counterpart.^[19] These studies indicate that propionylation and butyrylation are significantly less abundant than cellular acetylation levels. Given the low cellular abundance of these modifications, the functional roles of propionylation and butyrylation remain unclear. Liu *et al.* have shown that propionylation accounts for approximately 7% of the genome-wide H3 K23 modification in a myeloid precursor leukemia (U937) cell line.^[21] Additionally, propionylation at this site decreased during monocyte differentiation, while acetylation levels remained relatively constant.^[21] These findings suggest that propionylation and butyrylation are regulated post-translational modifications, and likely elicit specific cellular responses.

Enzymes that catalyze (de)propionylation and (de)butyrylation

To date no exclusive propionyltransferases or butyryltransferases have been identified, although a number of KATs are able to catalyze these C3 and C4 modifications. For example, p300 and CBP can propionylate and butyrylate core histones in cells and also readily undergo autopropionylation and autobutyrylation.^[18, 20-21] Interestingly, all MYST KATs tested (MOF, HBO1 and Tip60) do not display significant propionylation activity,^[18] though one study has shown that Esa1 in the picNuA4 KAT complex is able to transfer the propionyl group from propionyl-CoA to peptide substrates.^[16a] In these studies, rates of acetyl-transfer catalyzed by KATs are more efficient than propionyl- and butyryl-transfer.^[16a, 22] Additionally, sites of propionylation and butyrylation identified in yeast do not differ significantly from sites of acetylation, consistent with the notion that the length of the acyl chain does not alter site selection (Figure 2). In support of this, Leemhuis *et al.* have shown that the KAT PCAF is able to propionylate/ butyrylate H3 peptides at similar sites to those observed with acetyl-CoA.^[22]

Together, these findings suggest several possibilities for histone propionylation and butyrylation, one of which is the existence of exclusive propionyl-CoA and butyryl-CoA transferases. Alternatively, propionyl-CoA and butyryl-CoA could be accommodated by a few known KAT enzymes. Consistent with the latter, there appear to be no unique consensus sequences for existing sites of propionylation or butyrylation.^[18-20] Additionally, sites on chromatin that are propionylated and butyrylated, are also acetylated, with the exception of two sites (H4 K31, K44; see Figure 2). Lastly, propionylation and butyrylation may be an effect of “spurious” acyl-additions, in which propionylation or butyrylation may occur by non-enzymatic means. In this case, the cellular concentrations of acetyl-CoA, propionyl-CoA and butyryl-CoA would drive their abundance on proteins (described in the next section).

Several sirtuin deacetylases can catalyze the removal of propionyl- or butyryl-groups from modified peptides and proteins. Garrity *et al.* have demonstrated that the bacterial CobB

sirtuin depropionylates propionyl-CoA synthetase PrpE at K592,^[23] while yeast Sir2, human SIRT1, SIRT2 and SIRT3 exhibit robust depropionylase activity on non-histone substrates, including p300 and CBP.^[20-21] A critical factor in determining depropionylase or debutyrylase activity is the ability of the active site to accommodate altered acyl-lysine moieties. Hst2, a sirtuin found in yeast, binds propionyl-lysine and butyryl-lysine peptides with greater affinity than acetyl-lysine peptides.^[24] Interestingly, although Sirtuins are capable of removing these acyl groups, deacylase activity for Hst2, SIRT1, SIRT2 and SIRT3 decreased generally with increasing chain length in a similar trend to that observed for KATs.^[24] These studies support the possibility that existing histone deacetylases might be responsible for depropionylase and debutyrylase activities in cells, as many are able to accommodate larger acyl-groups.

Functional relevance of propionylation and butyrylation?

Similar to acetyl groups on lysine residues, propionyl- and butyryl-marks might be discriminated by histone code readers, akin to PHD (plant homeodomain) motifs that exhibit unique specificity for various methylated states of histone lysine residues.^[2] Bromodomains, which bind acetyl-lysine residues, are attractive candidates whose recognition of the modified lysine could be perturbed by acyl length.^[2] Additionally, these acyl-marks might elicit multi-site discrimination effects, in which the presence of propionylation or butyrylation at one site affects the binding affinity of a histone code reader at a nearby site.^[25] For example, Musselman *et al.* have demonstrated by combinatorial peptide library screening, tryptophan fluorescence and NMR that the binding of H3 tails by the PHD finger of CHD4 (chromodomain helicase DNA-binding protein 4) is potentiated by the presence of K9 methylation and acetylation, whereas methylation at K4 decreased affinity.^[26] Therefore, propionylation and butyrylation could mediate similar effects, given that these marks result in lysine charge neutralization and create a charge shielding effect that is preferred by PHD-CHD4 at K9.^[26] Because it is known that longer fatty-acid additions such as myristoylation (C14) and palmitoylation (C16) regulate protein-protein interactions,^[27] it is reasonable to speculate that propionylation and butyrylation may possess additional hydrophobic binding interactions compared to acetylation, and mediate diverse functions.^[28] Alternatively, propionylation and butyrylation may significantly alter the intrinsic dynamics of chromatin organization akin to acetylation.

Pathways for Acyl-CoA production

The utilization of acyl-CoA's by KAT enzymes suggests that the production of these metabolites is important for regulating KAT activity. These general pathways are summarized in Figure 3. In both yeast and humans, the enzymatic activation of acetate is a significant source of acetyl-CoA (reviewed in ^[29]). In yeast, mitochondrial acetyl-CoA is generated by acetyl-CoA synthetase 1 (Acs1), the pyruvate dehydrogenase complex and enzymes of β -oxidation.^[30] In yeast nucleus and cytoplasm, Acs2 is an important contributor of acetyl-CoA for histone acetylation, demonstrated by the fact that an *acs2-Ts* mutant strain results in a large decrease in histone acetylation and global changes in gene transcription.^[30]

In mammals, both ATP citrate lyase (ACL) and AceCS1 (acetyl-CoA synthetase 1) have important roles in the production of nuclear and cytosolic acetyl-CoA. The catabolism of glucose results in the formation of citrate, a small molecule capable of transport across the mitochondrial membrane (Figure 3). In the cytosol, citrate is cleaved by ACL in an ATP-dependent manner to form acetyl-CoA and oxaloacetate. Under normal growth and glucose rich conditions, siRNA knockdown of ACL or AceCS1 resulted in considerable reduction of histone acetylation, and there was an additive effect when both enzymes were knocked

down.^[31] Interestingly, with treatment of high levels of acetate (5 mM), AceCS1 was able to compensate for loss of ACL.^[31] Furthermore, serum treatment and adipocyte induced differentiation increased histone acetylation and the transcription of various metabolic genes in an ACL-dependent manner. These findings suggest acetyl-CoA production and epigenetic regulation are coordinated in response to nutrient signals.

In all eukaryotic systems, nuclear/cytosolic and mitochondrial acetyl-CoA appear to exist in distinct pools. In support of this, acetyl-CoA generated from the β -oxidation of exogenously added fatty acids did not increase histone acetylation under serum-starved conditions in.^[31] Additionally, in yeast, the loss of *ACS1*, *PDA1* (part of pyruvate dehydrogenase complex) and *POT1* (β -oxidation enzyme) genes did not affect bulk histone acetylation, providing compelling evidence that mitochondrial acetyl-CoA does not freely exchange with other cellular compartments.^[30] It is attractive to speculate that acetyl-CoA generation in the mitochondria might regulate the acetylation of proteins within this organelle. Support for this is provided by the fact that ~20% of mitochondrial proteins are acetylated.^[32]

Currently, there is no evidence for separate nuclear and cytoplasmic acetyl-CoA pools, as small metabolites such as acetyl-CoA are expected to freely diffuse through nuclear pores complexes.^[33] In support of this, radio-labeled acetate can be rapidly incorporated as an acetyl-donor on histones as measured by pulse-chase analysis.^[34] Currently, no specific nuclear transporter of acetyl-CoA or acetate is known to exist. However, it is intriguing that the proteins responsible for providing acetyl-CoA to KATs (yeast Acs2, human AceCS1 and human ACL) have been localized to both nuclear and cytoplasmic regions^[30-31]. Furthermore, immunoelectron microscopy has shown Acs2 localizes in distinct nuclear clusters^[30] and may increase the availability of acetyl-CoA for KATs within subnuclear regions. Such regulation might allow for targeted histone acetylation leading to transcriptional activation of specific genes.

It is unknown which pathway of propionyl-CoA and butyryl-CoA production provides substrates for the acylation of histone and non-histone substrates. However, these metabolites are derived from a number of metabolic sources. Propionyl-CoA and butyryl-CoA can be formed by the reaction of propionate or butyrate with CoA and ATP, catalyzed by short-chain acyl-CoA synthetases. These activities have been detected in both cytosolic and mitochondrial fractions in non-ruminant mammals (rats and guinea pigs), suggesting that roles for propionyl-CoA synthetase and butyryl-CoA synthetase could exist in humans.^[35] Additionally, both propionyl-CoA and butyryl-CoA are short chain-CoA intermediates of fatty acid synthesis and β -oxidation. The metabolism of amino acids, C5 ketone bodies, and odd chain fatty acids also provides propionyl-CoA.^[36] Unlike acetyl-CoA, which enters the TCA cycle via condensation with pyruvate, propionyl-CoA is metabolically distinct in that it is an anapleurotic precursor for the TCA cycle.^[36] Through a series of steps requiring carboxylation, rearrangement and thioester hydrolysis, propionyl-CoA is converted into succinate (Figure 3).

Few studies have sought to directly quantify propionyl-CoA and butyryl-CoA levels in vivo. Hosokawa *et al.* have shown that propionyl-CoA levels were 10-fold lower than acetyl-CoA in the livers of fed rats and decreased to 20-fold after 48 hours of fasting.^[37] However, propionyl-CoA levels may be higher, as another study has measured acetyl-CoA, propionyl-CoA and butyryl-CoA levels which existed in a ratio 4:2:1 from the livers of 48 hour-starved rats.^[38] Collectively, these findings are generally consistent with the low abundance of lysine propionylation and butyrylation observed on chromatin^[19-20]. Additional studies will be needed to elucidate how different metabolic status regulates co-enzyme concentrations and influences protein acylation.

Metabolic regulation of KAT activities

Cellular KAT activity may be regulated by co-enzyme abundance. Published acyl-CoA measurements are commonly expressed in moles of metabolite per tissue weight.^[37-39] Thus the exact molar concentration of acyl-CoAs, and how those concentrations may impact KAT activity, remain unclear. From a catalytic perspective, most kinetically characterized KATs have high catalytic efficiency ($k_{cat}/K_m \sim 10^5$ to $10^6 \text{ M}^{-1}\text{s}^{-1}$) and affinity (K_m or $K_d \sim 1 \text{ }\mu\text{M}$) for acetyl-CoA (see Table 1). Based on these kinetic and binding parameters, many KATs should respond similarly to fluctuations in acetyl-CoA levels. One interesting exception is yeast Gcn5, which has lower binding affinity for acetyl-CoA ($K_d = 8.5 \text{ }\mu\text{M}$) compared to other Gcn5 homologs in humans and *Tetrahymena* (K_d or $K_m \sim 1 \text{ }\mu\text{M}$, Table 1).^[16b, 16c, 40] Remarkably, mutation of active-site threonine 190 to alanine “rescued” this binding “defect” and improved acetyl-CoA binding 10-fold.^[40] This enhanced binding was predicted to increase histone acetylation and promote transcription activation. Surprisingly, introduction of this mutant into a yeast *gcn5Δ* strain did not result in a gain of function compared to wildtype when grown on rich media.^[40] These findings suggested that cellular acetyl-CoA levels are sufficiently high ($>8.5 \text{ }\mu\text{M}$) to saturate the KAT active site under nutrient rich conditions.

Another factor regulating KAT activity is product inhibition caused by the formation of Coenzyme A (CoA). Notably, Gcn5, picNuA4 and PCAF demonstrate tight binding affinities (K_d) or inhibition constants (K_i) of $\sim 1 \text{ }\mu\text{M}$, suggesting they might be sensitive to cellular levels of CoA (Table 1). In contrast, p300 and Rtt109 exhibit much poorer binding affinities ($K_d = 7 \text{ }\mu\text{M}$ and $40 \text{ }\mu\text{M}$, respectively) for CoA and would require higher concentrations for inhibition (Table 1). These observations suggest that p300 and Rtt109 may exist in a more constitutively active state compared to other more CoA-sensitive acyltransferases.

Because most KATs bind acetyl-CoA and CoA with similar affinity, the ratio of acetyl-CoA/CoA might be a more significant regulator of KAT activity than individual metabolite concentrations. In a recent HPLC-MS study, the measured ratios in rat liver of acetyl-CoA/CoA were 1 in standard diets, 0.7 in fasting diets, and 2 in fasting/ refeed diets.^[39] Therefore, KATs may be activated in nutrient rich environments, as increased levels of acetyl-CoA could out-compete CoA at KAT active sites and stimulate acetylation. Consistent with this hypothesis, Friss *et al.* have shown that the yeast SAGA and picNuA4 complexes are stimulated for global (i.e. non-targeted) H3 and H4 acetylation in response to glucose treatment after nutrient depletion.^[41] As both SAGA and picNuA4 have similar binding constants toward acetyl-CoA and CoA (Table 1), these findings suggest the activity of these essential enzymes is controlled by the relative amount of acetyl-CoA and CoA. Indeed, *cdc19Δ* (pyruvate kinase 1) and *acs1Δ/acs2-Ts* mutant strains are unable to induce histone acetylation in response to glucose, providing genetic evidence that the glycolytic fermentation pathway directly regulates chromatin acetylation by modulating acetyl-CoA levels.^[41] Collectively, these observations provide a functional connection between metabolic status and chromatin acetylation.

Considering the vital role of histone acetylation in the regulation of gene expression, an intimate link between metabolism and KAT induced-transcriptional activation is expected. Indeed, increased histone acetylation catalyzed by the picNuA4 and SAGA complexes is thought to accompany global transcriptional reprogramming in response to glucose treatment.^[41-42] Additionally, acetyl-CoA provided by ACS and ACL enzymes elicit transcriptional outcomes mediate by KAT activity. The transcriptional profile of an *acs-Ts1* yeast mutant revealed that $\sim 70\%$ of genes were significantly down-regulated, many of which were also regulated by KATs.^[30] Transcriptional down-regulation was also observed in an

ACL-dependent manner in mammals.^[31] Upon SiRNA knockdown of ACL, the expression of genes involved in metabolism such as glucose transporter (Glut4), hexokinase 2 (HK2), phosphofructokinase (PFK-1) and lactate dehydrogenase-A (LDH-A) were significantly decreased.^[31] Together, these studies implicate metabolism as a driving force for KAT mediated histone acetylation and transcriptional activation.

A functional connection between metabolism and transcriptional activation requires that the acetylation status of chromatin be highly dynamic. Pulse-chase experiments using radio-labeled acetate evaluated how quickly cells utilize metabolic precursors. Studies of human fibroblast and rat hepatoma cells identified two different rates of acetylation turnover for all four histones, with one faster half-life of 5-12 minutes and second slower rate.^[43] These findings may be consistent with the fastest species localized to transcriptionally active loci.^[44] More recently, these acetylation turnover rates were validated in *S. cerevisiae* using a continuous-label methodology that measured the rate of acetylation turnover under non-steady state conditions.^[45] H4 and H2B acetylation turnover rates were roughly equivalent to previously published reports under steady-state conditions (half-time = 3-24 minutes), and were remarkably similar to H2B and H4 protein turnover rates (half-time = 4 and 21 minutes, respectively).^[45] Taken collectively, these findings suggest that the cellular acetylation status is capable of rapid response to metabolic flux, and is functionally linked by the activities of ACS and KAT enzymes.

Other metabolic factors can influence KAT activity. For example, the mammalian master circadian regulator CLOCK is a MYST-family KAT that is regulated by NAD⁺ levels and SIRT1 deacetylase activity.^[46] In complex with its heterodimeric partner BMAL, CLOCK functions to acetylate H3 K9 and K14 at the promoter regions of other circadian genes and regulates their expression.^[46a, 47] Additionally, CLOCK acetylates K537 of BMAL1.^[47] Nakahata *et al.* have reported that once SIRT1 forms a complex with CLOCK-BMAL1, SIRT1 deacetylates BMAL and histones at CLOCK controlled genes, resulting in the silencing of CLOCK activities.^[46c] Control of SIRT1 deacetylation is mediated by the oscillation of NAD⁺, which in turn is regulated by the oscillating transcription of NAMPT (nicotinamide phosphoribosyltransferase), an enzyme that catalyzes the rate-limiting step of NAD⁺ synthesis.^[46b, 46d] Since the CLOCK/BMAL-SIRT1 complex controls the expression of NAMPT, this transcriptional system forms an elegant feedback loop in which NAMPT controls its own expression.^[46c, 46d, 48] Potentially, acetyl-CoA levels might oscillate to provide another layer of CLOCK regulation in mammals. Using GC-TOF-MS and LC-MS/MS techniques, Tu *et al.* have shown that acetyl-CoA levels oscillate during yeast metabolic cycles with a periodicity reminiscent of circadian rhythms.^[49] Thus, existing data suggests KAT activity may be regulated by acetyl-CoA oscillation in mammals.

Lastly, it is interesting to speculate on the influence of butyrate on KAT activities. Butyrate is a metabolic inhibitor of class I and II HDACs, and treatment of cells with millimolar levels of butyrate increases bulk histone acetylation.^[13b, 50] It should be noted that butyrate can serve as a precursor to the generation of acetyl-CoA (via β -oxidation) or butyryl-CoA (via a synthetase) for KAT enzymes. Thus, the observed increase in histone acetylation due to butyrate treatment may be a result of simultaneous KAT activation and HDAC inhibition.

KAT acetylation and regulation of metabolic proteins

Recent proteomic surveys have identified a large number of acetylated proteins in bacteria and mammalian systems.^[32, 51] Many of these proteins have metabolic functions and/or reside in mitochondria. In fact, most enzymes in glycolysis, gluconeogenesis, the TCA cycle, the urea cycle, fatty acid metabolism, and glycogen metabolism are found acetylated in human liver tissues.^[32, 51] Additionally, protein acetylation appears to be regulated by

nutrient status, as distinct protein acetylation patterns have been observed in the liver mitochondria of fed, fasted, and calorically restricted mice.^[32, 52] Thus, protein acetylation might be important for regulating the flux of metabolic pathways in response to various nutrients. In support of this, a number of metabolic enzymes in *Salmonella enterica* were shown as differentially acetylated when grown on glucose or citrate as the carbon source, and these acetylations were dependent on Pat and CobB reversible acetylation activities.^[51d] Moreover, the flux ratio of glycolysis over gluconeogenesis, as measured by ¹³C labeling of glucose, increased in *cobBA* strains and decreased in *patA* strain.^[51d] Concomitantly, the flux ratio of the glyoxylate bypass over the TCA cycle measured by ¹³C labeling of citrate resulted in opposite effects. Together, this study provides evidence to support the idea that protein acetylation allows for metabolic adaptation, so that diverse energy sources can be more efficiently utilized.

While several metabolic targets of specific deacetylases have been identified (reviewed in ^[53]), there are currently only two examples where the identity has been established for KATs regulating acetylation of metabolic enzymes. Acetyl-CoA synthetase provides unique example of a central metabolic enzyme that is regulated by reversible acetylation. In *S. enterica*, ACS (acetyl-CoA synthetase) is reversibly acetylated at lysine 609 by the GNAT-related acetyltransferase Pat, and is deacetylated by a sirtuin homolog, CobB.^[54] Acetylation of ACS results in severe impairment of the adenylation half reaction.^[54b] Similar regulation of ACS homologs has been identified in other bacterium such as *Bacillus subtilis* and *Rhodospseudomonas palustris*.^[55] In mammals, AceCS1 and AceCS2 are acetylated at conserved lysines and deacetylated by SIRT1 and SIRT3 respectively.^[56] The identification of acetylated AceCS1/2 proteins represent the first finding of a mammalian metabolic protein regulated by reversible acetylation.^[56] Deacetylation results in enzyme activation, though AceCS1 and AceCS2 have distinct metabolic functions. AceCS1 deacetylation increases acetyl-CoA utilized for fatty acid synthesis and histone acetylation, whereas AceCS2 deacetylation results in acetyl-CoA production for utilization in the TCA cycle. While reversible acetylation of Acs1 and Acs2 has not been directly observed in yeast, the deletion of all sirtuins (*sir2hst1hst2hst3hst4Δ*) result in yeast that are unable to grow solely on acetate or propionate, suggesting that sirtuins are required for activating acyl-CoA synthetases in yeast.^[57] The identity of the acetyltransferase that acetylates acetyl-CoA synthetases in mammalian and yeast model systems remain unclear. However, one study has shown that mammalian acetyl-CoA synthetases can be acetylated and inactivated by bacteria Pat.^[56a] Given that acetyl-CoA synthetases provide acetyl-CoA for KATs, the post-translational modification of these enzymes provide a unique feedback circuit in which KATs are able to control the production of their own substrates.

The gluconeogenic regulator phosphoenolpyruvate carboxykinase (Pck1 in yeast and PEPCK in humans) serves as an interesting example in which acetylation state regulates enzyme activity. Pck1/PEPCK catalyzes the rate-limiting step of gluconeogenesis by converting oxaloacetate (OAA) into phosphoenolpyruvate (PEP) and carbon dioxide. In yeast, Pck1 is reversibly acetylated at K514 by the opposing activities of Esa1 in the NuA4 KAT complex and the Sir2 deacetylase.^[58] Kinetic analysis of Pck1 purified from an *esa1-Ts* mutant yeast strain revealed a ~6-fold V_{max} defect in the ability to convert PEP, ADP and HCO_3^- into OAA, suggesting that acetylation at K514 activates Pck1.^[58] Further, a K514Q mutant demonstrated efficient growth on ethanol and glycerol, while the K514R mutant exhibited robust growth defects. These findings suggest acetylated Pck1 is required for the utilization of alternative carbon substrates via gluconeogenesis.^[58] This mechanism of control appears to be conserved, as Tip60 acetylates human PEPCK, while silencing of Tip60 results in decreased glucose secretion from HepG2 cells.^[58]

In contrast to catalytic activation, other reports of PEPCK acetylation suggested a negative effect on activity. Zhao *et al.* have found that acetylation (Lys 70, 71 and 594) of human PEPCK increases with glucose treatment in HEK 293T cells.^[51b] Cyclohexamide (a translation inhibitor) treatment led to decreased protein abundance of a triple glutamine mutant (K70/71/594Q) relative to the corresponding arginine mutant, suggesting that acetylation acts to destabilize PEPCK and hinder intracellular glucose production.^[51b] Although these two acetylation studies appear to be contradictory, they might reveal that different sites of acetylation have distinct regulatory roles for PEPCK function.

In addition to direct acetylation, PEPCK transcriptional expression is controlled by reversible acetylation of the transcription factor PGC1- α (Peroxisome proliferator-activated receptor gamma coactivator 1- α). In fact, PGC1- α acetylation is one example in which a given metabolic status regulates KAT and HDAC activity. In the fasted state (high [NAD⁺]), SIRT1 deacetylates and activates PGC1- α , which then promotes the transcriptional activation of gluconeogenic genes, including PEPCK.^[59] In the fed state (presumably high [acetyl-CoA]), Gcn5 acetylates and sequesters PGC1- α away from the promoters of gluconeogenic genes in cultured hepatocytes and in mouse liver.^[59b, 60] As a result, the expression of most gluconeogenic enzymes, including PEPCK, are repressed and glucose production decreases.^[60] Thus acetylation acts by a multi-faceted mechanism to regulate PEPCK enzymatic activity, stability, and expression based on metabolic status.

Approaches for identifying novel KATs and their substrates

Recent MS-based technologies have catalogued the existence of hundreds of acetylated proteins, which appear throughout the cell and suggests that reversible acetylation might regulate the function of nearly every facet of cell physiology. Prior to high throughput MS approaches, detecting protein acetylation relied heavily on western blot analysis with pan or site-specific acetyl-lysine antibodies and radioactive detection from the use of ¹⁴C or ³H labeled acetyl-CoA.^[32, 61] The first proteomic study identifying acetylated proteins by MS has been pivotal in understanding the extent to which acetylation might regulate diverse cellular processes, including central metabolic enzymes. Kim *et al.* have identified 388 sites of acetylation on 195 proteins, with acetyl-lysine found in >20% of mitochondrial proteins.^[32] Since then, further development of MS-based techniques has allowed for better identification and quantification of novel acetylated proteins. These methods include iTRAQ (isobaric tag for relative and absolute quantitation) MS, as well as SILAC (stable isotope labeling with amino acids in cell culture) MS.^[51b, 51c] Anti-acetyl-lysine antibodies were used to enrich acetylated peptides from trypsin-digested samples, and resolved by chromatographic steps prior to identification by MS.^[51b, 51c] However, the exclusive use of antibody enrichment has limitations, as the extent of sequence-specific effects of the anti-lysine antibodies has not been evaluated in detail. Thus, it is possible that a large number of acetylated proteins have yet to be identified. Some of these limitations might be overcome by the development of novel chemical approaches, such as the utilization of bioorthogonal alkyne-acetyl-CoA analogs that act as acyl-donors in KAT-catalyzed reactions.^[62] Other high-throughput formats might facilitate identification of acetylated proteins. Lin *et al.* have utilized protein microarrays to find non-histone protein targets for the NuA4 KAT complex.^[58] In this case, transfer of ¹⁴C acetyl-CoA onto a nitrocellulose slide containing 5800 yeast proteins was monitored in the presence of NuA4. Of these, 91 proteins were acetylated by NuA4 and 13 proteins were validated as *bona fide* substrates in vivo.^[58] Using peptide arrays, similar unbiased approaches could be utilized to identify potential targets of known KATs. This approach has the capacity to identify new acetylation sites that can be validated in cells using quantitative MS methods.

Despite significant advances identifying novel acetylated proteins, the KAT(s) responsible for non-histone acetyltransferase activity, especially in the mitochondria, remain unknown. Bioinformatic homology searches on known mitochondria proteins have not revealed candidate proteins with obvious sequence similarity to known KATs. Lack of sensitive and appropriate activity-based methodologies have hindered the identification of KATs responsible for the acetylation of metabolic proteins. One strategy for trapping acetyltransferases is based on CoA affinity and the use of fluoroacetyl-CoA or sulfoxide-CoA probes.^[63] Here, a nucleophile within the KAT is proposed to attack the carbonyl-moiety of the probe resulting in a probe-KAT covalent adduct that is subsequently purified by an affinity-based technique. Unfortunately, this method requires a catalytic mechanism in which a strong nucleophile or covalent enzyme intermediate is involved. The observation that KATs appear to utilize a sequential (direct attack) mechanism (Figure 1), suggests that these compounds have limited utility. Therefore additional activity-based methodologies should be developed to facilitate the identification of KATs involved in metabolic processes.

Outlook

Epigenetic mechanisms rely on enzymes whose activities are driven by co-enzymes and metabolites from intermediary metabolism. These include methyltransferases that use SAM, protein demethylases that use α -ketoglutarate, chromatin remodeling enzymes that utilize ATP, Sirtuin deacetylases that require NAD⁺, and KATs that utilize acyl-CoA. In retrospect, it should not be surprising that there should be an exquisite link between chromatin function and metabolism, however, there have been few studies that clearly establish this link. Here we have explored this theme, using KATs as an example of this intricate connection.

Cellular acyl-CoAs are impacted by numerous metabolic pathways, in which the enzymes responsible for acyl-CoA production are themselves regulated by the opposing activities of KATs and deacetylases. In particular, acetyl-CoA synthetases (ACSSs) provide acetyl-CoA to KAT enzymes for histone acetylation, resulting in global transcriptional reprogramming. Remarkably, KAT enzymes acetylate and inactivate ACS, thereby providing a potential feedback mechanism in which KATs can self-regulate acetyltransferase activities. Such regulation is unlikely limited to ACS, as recent proteomic surveys have revealed the existence of hundreds of acetylated proteins, many of which are key enzymes in metabolic processes. Future work is necessary to understand the extent to which metabolic pathways are influenced by acetylation and other forms of acylation such as propionylation and butyrylation. To fully elucidate the mechanisms of protein acylation will require the identification of the enzymes responsible for catalyzing these marks, including novel propionyltransferases, butyryltransferase and the full complement of KATs responsible for the acetylation of metabolic proteins.

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Figure 1.

KATs utilize sequential (direct attack) mechanism of acetylation. “R” denotes various alkyl chains that can be accommodated by KATS. Carboxylate group is a general base (aspartate or glutamate) that facilitates abstraction of proton on ϵ -amine of lysine. Following attack of acyl-group on acyl-CoA by unprotonated lysine, a putative tetrahedral intermediate forms. Collapse of this intermediate results in the formation of acylated lysine on protein and CoA products.

Figure 3.

Metabolism and protein acylation are intimately linked through metabolites acetyl-CoA, propionyl-CoA and butyryl-CoA. ACL- ATP citrate lyase; ACS- acetyl-CoA synthetase; KAT- lysine acyltransferase; PDH-pyruvate dehydrogenase complex. Pathways leading to the production of propionyl-CoA and butyryl-CoA production are not shown.

Table 1

Kinetic constants for select KAT enzymes

KAT enzyme	k_{cat}/K_m for AcCoA ($\text{M}^{-1} \text{s}^{-1}$)	K_m or K_d for AcCoA (μM)	K_d or K_i for CoA (μM)	Reference
Esa1 in picNuA4 complex	$(6.4 \pm 0.9) \times 10^5$	$K_m = 2.5 \pm 0.3$	$K_i = 1.8 \pm 0.8$	[16a]
Gcn5	6.8×10^5	$K_d = 8.5 \pm 2.6$	$K_i = 6.7 \pm 5.1$	[16c]
PCAF	$(2.3 \pm 0.8) \times 10^5$	$K_d = 0.64 \pm 0.12$	$K_i = 0.44 \pm 0.12$	[16b]
p300 ^[a]	2.1×10^5	$K_d = 0.740 \pm 0.60$	$K_d = 7.3 \pm 1.4$	[17a, 64]
Rtt109 in complex with Vps75	$(3.9 \pm 0.5) \times 10^5$	$K_d = 1.4 \pm 0.3$	$K_d = 43 \pm 5$	[17b]

^[a] K_d of acetyl-CoA with p300, as binding measurements without turnover was not possible with acetyl-CoA