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Acetyl-CoA and the Regulation of Metabolism: Mechanisms and Consequences

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Abstract

Acetyl-CoA represents a key node in metabolism due to its intersection with many metabolic pathways and transformations. Emerging evidence reveals that cells monitor the levels of acetyl-CoA as a key indicator of their metabolic state, through distinctive protein acetylation modifications dependent on this metabolite. We offer the following conceptual model for understanding the role of this sentinel metabolite in metabolic regulation. High nucleocytoplasmic acetyl-CoA amounts are a signature of a “growth” or “fed” state and promote its utilization for lipid synthesis and histone acetylation. In contrast, under “survival” or “fasted” states, acetyl-CoA is preferentially directed into the mitochondria to promote mitochondrial-dependent activities such as the synthesis of ATP and ketone bodies. Fluctuations in acetyl-CoA within these subcellular compartments enable the substrate-level regulation of acetylation modifications, but also necessitates the function of sirtuin deacetylases to catalyze removal of spontaneous modifications that might be unintended. Thus, understanding the sources, fates, and consequences of acetyl-CoA as a carrier of two-carbon units has started to reveal its underappreciated but profound influence on the regulation of numerous life processes.

Introduction

In response to a dynamic nutrient environment, cells must assess their metabolic state to decide whether to grow, survive, or die. It has become evident that metabolites themselves must feed back to regulate gene expression, signal transduction, and various protein activities in cellular decision-making processes [1,2]. These small molecule metabolites play critical roles in relaying metabolic information to their protein and nucleic acid counterparts. However, despite increased recognition of such reciprocal interplay, many aspects of the mechanisms through which metabolites exert their influence on cellular regulatory mechanisms are still being unraveled.

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Amongst the thousands of metabolites present in the cellular milieu at any given time, which might represent the “sentinel” metabolites that signify cellular metabolic state? One well-known signature of metabolic state is AMP, which indicates cellular energy charge and accumulates upon ATP insufficiency. AMP regulates the activity of the AMP-activated protein kinase (AMPK), which phosphorylates many proteins involved in cellular energy homeostasis [3]. Another example is NAD^+ , which indicates the cellular redox status as a ratio of NAD^+ to NADH [4,5]. Herein, we discuss the hypothesis that acetyl-CoA represents an additional prominent gauge of the cell’s metabolic state with substantial influence on numerous biological regulatory mechanisms.

Growth or Fed State - High acetyl-CoA in cytosol/nucleus

Acetyl-CoA is a metabolite derived from glucose, fatty acid, and amino acid catabolism. During glycolysis, glucose is broken down into two three-carbon molecules of pyruvate. The mitochondrial pyruvate dehydrogenase complex then catalyzes the oxidative decarboxylation of pyruvate to produce acetyl-CoA, a two-carbon acetyl unit that is ligated to the acyl-group carrier, CoA [6]. In the mitochondria, citrate synthase then catalyzes the condensation of the acetyl moiety of acetyl-CoA with oxaloacetate to yield a six-carbon citrate molecule. Citrate can proceed to be oxidized via the TCA cycle, or alternatively it can be transported to the cytosol as a substrate for the enzyme ATP citrate lyase, which cleaves citrate to regenerate acetyl-CoA and oxaloacetate [7] (Fig. 1). Under conditions of carbohydrate or glucose excess, the function of this pathway is to direct acetyl-CoA away from the mitochondria and back to the cytosol for the synthesis of fatty acids and sterols [8]. As such, cells can store excess carbohydrates as fat. Thus, the function of the ATP citrate lyase enzyme offers a clue to the logic and direction of carbon flow – acetyl-CoA units are shipped out of the mitochondria in the form of citrate when carbon sources are abundant, indicating a favorable nutrient state.

Nucleocytosolic pools of acetyl-CoA are also utilized for histone acetylation and the activation of gene expression. ATP citrate lyase was shown to provide a source of acetyl-CoA for histone acetylation in mammalian cells [9]. The budding yeast *Saccharomyces cerevisiae*, which lacks ATP citrate lyase, relies on acetyl-CoA synthetase enzymes to supply acetyl-CoA for histone acetylation [10]. Moreover, a special cohort of yeast genes important for growth, such as those required for ribosome biogenesis and the G1 cyclin *CLN3*, are especially dependent on histone acetylation for their activation [11,12]. As such, the expression of these growth genes is closely coupled to acetyl-CoA as an indicator of the cell’s nutritional state. Thus, when carbon sources are abundant, nucleocytosolic amounts of acetyl-CoA accumulate and facilitate the processes of lipid synthesis and histone acetylation (Fig. 1).

Survival or Fasted State - High acetyl-CoA in mitochondria

During starvation, cells must typically shift from growth to survival mode and alter metabolism towards functions important for viability. Instead of shipping acetyl units out to the cytosol, there is now a greater requirement for acetyl-CoA to be oxidized in the mitochondria for ATP synthesis (Fig. 1). Under such conditions, nucleocytosolic acetyl-CoA

levels therefore decrease. Fatty acids are a significant source of this mitochondrial acetyl-CoA pool [13]. CoA synthesis is induced to activate fatty acids as fatty acyl-CoAs [14,15], which can then be transported into mitochondria via the carnitine shuttle for β -oxidation. As a result, acetyl-CoA is generated in the mitochondria for oxidation or other possible fates. In the liver, mitochondrial acetyl-CoA is used to synthesize ketone bodies (acetoacetate and β -hydroxybutyrate) as alternative fuel sources for the brain and heart under conditions of carbohydrate scarcity [13,16]. Under such conditions, lower nucleocytosolic acetyl-CoA will also limit fatty acid synthesis, histone acetylation, and other growth-related processes. ATP citrate lyase is inhibited under these situations at both the transcriptional and post-translational levels [17,18].

Depletion of nucleocytosolic acetyl-CoA also represents a cue to induce autophagy [19,20]. In yeast, the expression of a core autophagy gene (*ATG7*) is repressed by acetyl-CoA [19]. During the yeast metabolic cycle (YMC), many *ATG* genes (including *ATG1* and *ATG8*) are repressed during growth phases when acetyl-CoA levels rise, and are activated only during the stress/survival phases when acetyl-CoA levels fall [21,22]. More generally, many other genes with functions involved in stress and survival are induced concomitantly with core autophagy genes [21,23,24]. These genes tend to be less dependent on histone acetylation for their activation [11,25,26], perhaps due to reduced availability of acetyl-CoA. In mammalian cells, autophagy regulation by acetyl-CoA occurs in a manner dependent on the p300 acetyltransferase [20]. Thus, the regulation of autophagy by acetyl-CoA may occur primarily at the level of transcriptional control of core autophagy genes.

Taken together, under fasted or carbon-poor states, nucleocytosolic amounts of acetyl-CoA decrease in cells, while mechanisms to channel acetyl-CoA into the mitochondria are engaged. These considerations support a model in which the subcellular compartmentalization of acetyl-CoA units undergoes a major shift during starvation, and the utilization of these acetyl units is re-purposed to support survival strategies (Fig. 1).

Sensing of acetyl-CoA through protein acetylation modifications

How might cells actually sense the abundance of acetyl-CoA? It is perhaps no coincidence that acetyl-CoA doubles as the acetyl donor for protein acetylation modifications (including histone acetylation) (Fig. 2). The abundance of protein acetylation modifications could therefore reflect the cell's metabolic state to regulate various protein activities. Studies performed under carbon-rich conditions where acetyl-CoA synthesis is not limiting may mask the contributions of this metabolite in cellular regulation. However, most organisms, as well as particular tissue microenvironments *in vivo* experience challenges in the nutrient environment that might limit acetyl-CoA biosynthesis or availability (e.g., carbon starvation or hypoxia). Recent studies have begun to provide compelling evidence that many protein acetylation modifications are indeed modulated by acetyl-CoA availability [27,28].

Besides histones, the acetyl-CoA synthetase family of enzymes was also identified to be regulated by reversible acetylation [29–31]. The acetylation of an active site lysine residue was observed to inhibit the activity of acetyl-CoA synthetase as a mechanism of feedback inhibition in response to high acetyl-CoA [32–34]. The deacetylation of these enzymes,

catalyzed by sirtuins, restores their activity [32–34]. Subsequent mass spectrometry surveys have now revealed that thousands of other proteins, including many other metabolic enzymes, can be acetylated [35–38]. In some cases, every enzyme in a particular biochemical pathway was found to be acetylated [39]. Although the majority of these modifications were found to be inhibitory, several were reported to be activating [40]. In some instances, the acetylation of particular metabolic enzymes was responsive to glucose levels in the media, suggesting that they could be linked to intracellular acetyl-CoA abundance. Whether specific acetyltransferase enzymes catalyze the majority of these acetylation modifications present on metabolic enzymes is not yet clear.

The yeast metabolic cycle (YMC) offers a system to investigate whether particular acetylation modifications might be coupled to acetyl-CoA itself. Studies of yeast cells undergoing the YMC during continuous, glucose-limited growth in a chemostat have revealed periodic changes in intracellular acetyl-CoA amounts as yeast cells alternate between growth and quiescent-like phases [22]. Several proteins are dynamically acetylated precisely in phase with the observed acetyl-CoA oscillations [11]. These include histones, several components of the transcriptional coactivator SAGA, a subunit of the SWI/SNF chromatin remodeling complex Snf2p, and a transcriptional coactivator of ribosomal subunit gene expression Ifh1p [11,41]. Interestingly, the dynamic acetylation of all of these proteins is dependent on the acetyltransferase Gcn5p, suggesting this enzyme has the capability of acetylating its substrates in tune with acetyl-CoA fluctuations *in vivo*. Consistent with this hypothesis, mutations within Gcn5p slow growth, disrupt the yeast metabolic cycle, or alter the cell's responsiveness to acetate [11,12]. Moreover, acetylation of SAGA subunits appears to aid its recruitment to growth genes [11]. A brief survey of other acetylated proteins that are not known to be Gcn5p substrates showed they are not dynamically acetylated across the YMC [11]. An analysis of the genomic regions bound by these acetylated histones revealed that several marks, in particular H3K9Ac, were present predominantly at growth genes, specifically during the growth phase of the YMC when acetyl-CoA levels rise [11,25]. These considerations suggest that the acetylation of these nuclear-localized proteins collectively functions to promote the activation of growth genes in response to a burst of nucleocytosolic acetyl-CoA.

Stoichiometry of acetylation modifications

Given the thousands of newly identified acetylated proteins, a pertinent question is what proportion of each protein is acetylated? Recent studies aiming to determine the stoichiometry of acetylated sites estimate that for many proteins, only a small fraction of the peptides are actually acetylated [42,43]. However, nuclear proteins, including histones and transcription factors, were estimated to be acetylated at much higher stoichiometry [43]. Conventional shotgun detection of peptides by mass spectrometry is biased towards abundant proteins, so perhaps it is unsurprising that a small fraction of a very abundant protein that is acetylated could be scored as a positive. Moreover, lysine residues on proteins can react spontaneously with thioesters such as acetyl-CoA or other acyl-CoA metabolites, resulting in non-enzymatic acetylation or acylation [44–48]. Non-enzymatic acetylation or acylation may be especially prominent within the mitochondria [43,46,48,49], which is thought to have higher acetyl-CoA concentrations and higher pH, thereby increasing the

nucleophilicity of lysyl side chains. Thus, while some non-enzymatic acetylation or acylation events could have evolved to be regulatory, the possibility also exists that many of these modifications could be spurious.

These considerations must be taken into account when determining the physiological significance of any detected acetylation site. Moreover, there are limitations to mutation of a lysine residue to either arginine or glutamine. These mutations are not always accurate acetylated or deacetylated lysine mimics, and could perturb protein function independent of site-specific acetylation. As such, it can be challenging to demonstrate whether a particular acetylation modification is functionally important *in vivo*. To help address these issues, methods for site-specific incorporation of acetyllysine [50], as well as better acetylated or deacetylated lysine mimics, have been developed [51,52]. The use of these and other methods will help clarify the extent through which protein acetylation modifications are responsive to acetyl-CoA fluctuations in a regulatory manner, either enzymatically or non-enzymatically.

Implications for sirtuin function

The accumulation of acetyl-CoA in subcellular compartments may also necessitate the activity of deacetylase enzymes to remove non-enzymatic acetylation modifications that could intentionally or unintentionally compromise protein function [28,53,54]. Such a “repair” or “detoxification” role may be fulfilled by the sirtuin family of protein deacylases (Fig. 2). Consistent with this idea, hyperacetylation of mitochondrial enzymes occurs in the absence of mitochondrial SIRT3 [55–57], and deacetylation of these enzymes typically increases their activity [53]. Moreover, the expression of SIRT3 is increased specifically under fasting states, in response to high-fat diets, or during exercise - conditions that all promote increased mitochondrial acetyl-CoA [53]. Likewise, the potential of proteins to be modified by other acyl-CoA metabolites besides acetyl-CoA is supported by the discovery of a wide variety of acylation modifications present on proteins, along with associated sirtuins that preferentially catalyze their removal [58–61]. Evidence that sirtuins evolved specifically to remove non-enzymatic protein acylation as a form of protein quality control has been summarized in a recent review [54]. In this model, failure of sirtuins to remove aberrant acylation modifications would hinder the function of effected proteins and consequently lead to dysfunctions in metabolism and susceptibility to disease [47,55,57].

The sirtuins utilize the cofactor NAD^+ to catalyze protein deacylation. However, deacylation of a lysine residue can also be executed using water as a nucleophile without a requirement for NAD^+ , a mechanism employed by many histone deacetylases (HDACs) [62]. The dependency of sirtuins on NAD^+ have led to the hypothesis that their activity could be regulated by fluctuations in NAD^+ concentrations [63,64]. However, such dependency on NAD^+ may serve an additional purpose and enable the removal of the acyl group via covalent attachment to ADP-ribose, to produce O-acyl-ADP-ribose metabolites, which themselves may have biological functions [65]. Moreover, if the acyl group were liberated as a free carboxylate, then the respective acyl-CoA synthetase enzymes could potentially convert these free carboxylates back to acyl-CoA metabolites, facilitating re-acylation and thus leading to a futile cycle (Fig. 2).

Summary and perspective

In summary, there is now compelling evidence that acetyl-CoA represents a fundamental gauge of cellular metabolic state that is monitored by the cell by way of distinctive protein acetylation modifications. Perhaps it is no coincidence that nature chose to carefully monitor the abundance of acetyl-CoA as a proxy for its metabolic state due to its requirement in the biosynthesis of cellular building blocks, two carbons at a time. Some of these acetyl-CoA-responsive modifications may be established by a delicate balance between the opposing activities of acetyltransferase and deacetylase enzymes, while others could be set in a non-enzymatic manner. Understanding the sources and fates, as well as the movement of acetyl groups between subcellular compartments reveals an underlying logic to metabolic strategies employed under growth versus survival, fed versus fasted, or normal versus tumorigenic metabolic states. Based on recent stoichiometry studies, it is tempting to speculate that acetylation originally evolved as a means to link nuclear activities with acetyl-CoA equivalents produced in the mitochondria. However, a consequence of using electrophilic acyl-CoA metabolites in cellular metabolism and regulation is their tendency to react spontaneously with nucleophilic moieties on proteins such as lysine residues. As such, the accumulation of particular acyl-CoA metabolites in various cellular compartments may have necessitated a mechanism to remove unintended acylation modifications on proteins. Future studies will continue to reveal the mechanisms and consequences of employing acetyl-CoA and other acyl-CoAs in cellular metabolism - their reciprocal influence on metabolism and cell regulation should no longer be overlooked.

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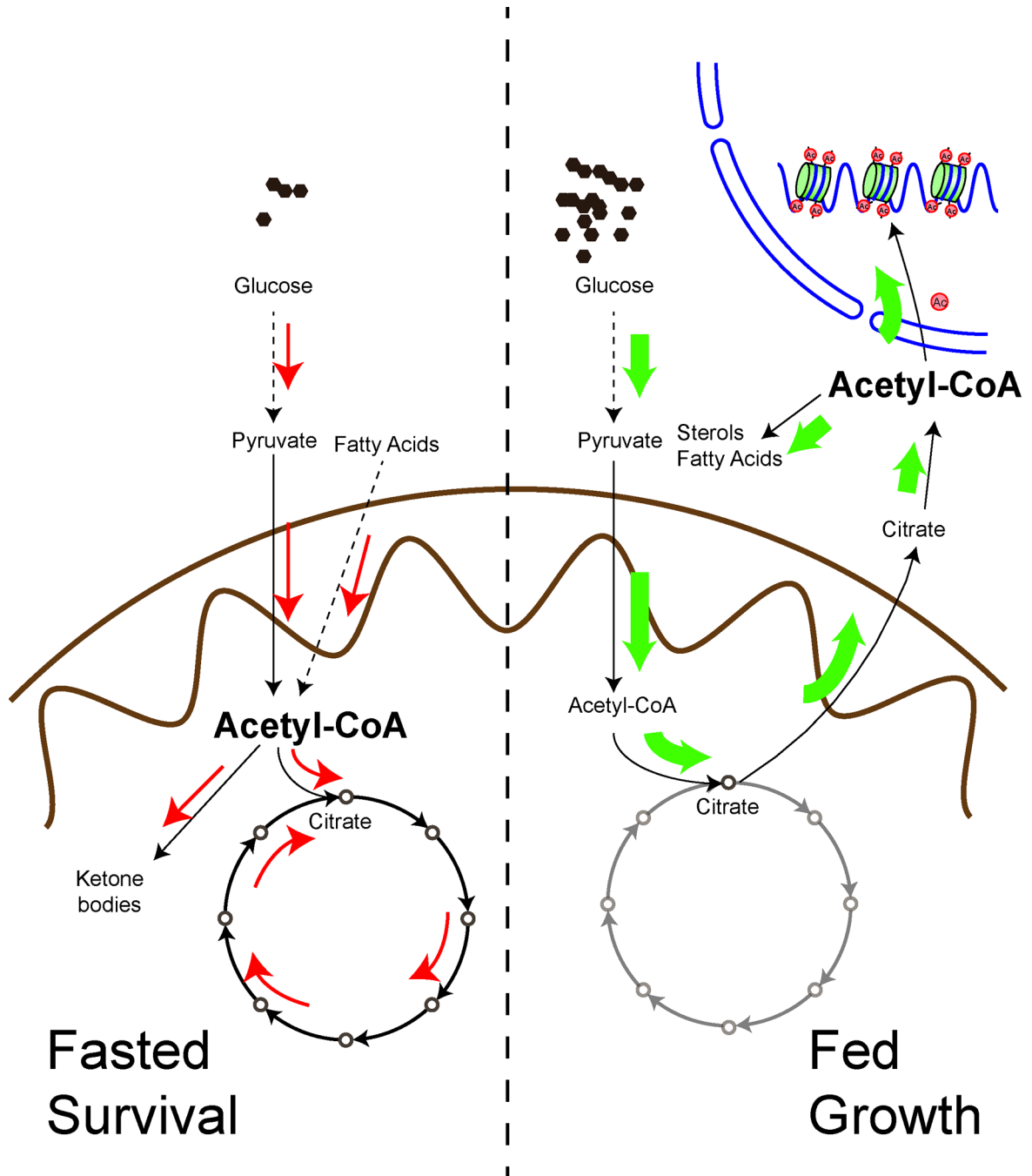
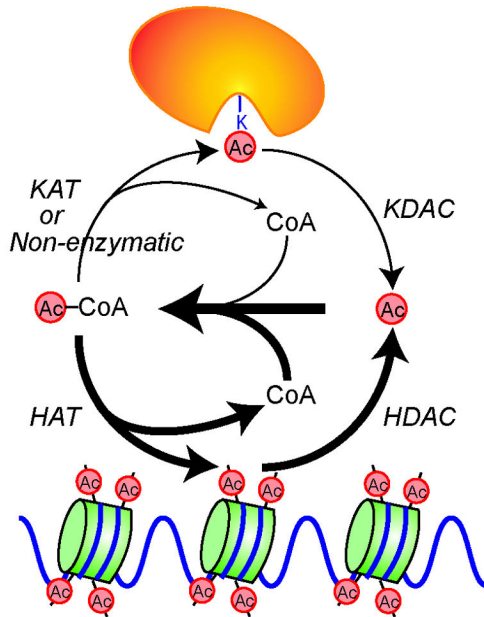


Figure 1. Schematic model proposing a general logic of acetyl-CoA utilization under fed versus fasted or growth versus survival states

Under fed or growth states, acetyl-CoA is directed out of the mitochondria and to the cytosol and nucleus for use in lipid synthesis or histone acetylation. Nucleocytosolic amounts of acetyl-CoA increase relative to mitochondrial amounts. Under fasted or survival states, acetyl-CoA is channeled into the mitochondria for synthesis of ATP and ketone bodies. Mitochondrial amounts of acetyl-CoA increase relative to nucleocytosolic amounts. Fatty acid oxidation significantly increases mitochondrial acetyl-CoA.

A



B

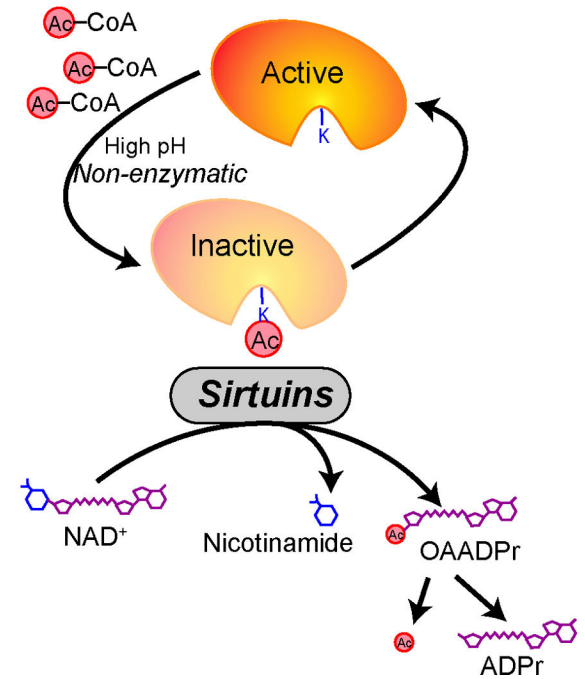


Figure 2. Dynamic acetylation and deacetylation of proteins

(A) The acetylation of proteins may be catalyzed by acetyltransferase enzymes or can occur spontaneously through reaction with acetyl-CoA directly. Deacetylase enzymes catalyze the removal of acetylation modifications. Liberated acetate can be converted back to acetyl-CoA. (B) Sirtuins utilize NAD⁺ to catalyze protein deacetylation, yielding nicotinamide and O-acetyl-ADP-ribose (OAADPr). The removal of aberrant acetylation or acylation modifications may restore protein function. Abbreviations: HAT (histone acetyltransferase), KAT (lysine acetyltransferase), HDAC (histone deacetylase), KDAC (lysine deacetylase), Ac (acetate), CoA (Coenzyme A).