Mitochondrial protein acetylation regulates metabolism

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Abstract

Changes in cellular nutrient availability or energy status induce global changes in mitochondrial protein acetylation. Over one-third of all proteins in the mitochondria are acetylated, of which the majority are involved in some aspect of energy metabolism. Mitochondrial protein acetylation is regulated by SIRT3 (sirtuin 3), a member of the sirtuin family of NAD^+-dependent protein deacetylases that has recently been identified as a key modulator of energy homoeostasis. In the absence of SIRT3, mitochondrial proteins become hyperacetylated, have altered function, and contribute to mitochondrial dysfunction. This chapter presents a review of the functional impact of mitochondrial protein acetylation, and its regulation by SIRT3.

Introduction

Post-translational acetylation of proteins is the covalent addition of an acetyl group to the ε-amino group of lysine residues that occurs on a wide array of proteins. This simple modification neutralizes the positive charge of the lysine residue, potentially altering its propensity to interact with nearby amino acids or other proteins. In this way, acetylation can influence multiple protein functions, including DNA–protein interactions, transcriptional activity, subcellular localization, protein stability and enzymatic activity [1]. The acetylation state of a given protein results from the balanced action of HATs (histone acetyltransferases) and HDACs (histone deacetylases), enzymes that catalyse the addition and removal, respectively, of an acetyl group from a lysine residue.

Although initially discovered on histones, lysine acetylation also occurs on several classes of non-histone proteins [1]. An extensive proteomic survey of cellular proteins revealed that a large number of mitochondrial proteins are subject to reversible lysine acetylation [2]. In this study, mouse liver mitochondria were collected from fed and fasted mice, purified and digested, and the resulting lysate was subjected to immunoaffinity purification of lysine-acetylated peptides. Proteomic analysis of the acetylated peptides identified 277 lysine acetylation sites in 133 mitochondrial proteins, and conclusively established lysine acetylation as a common PTM (post-translational modification) in the mitochondrion. Most lysine-acetylated proteins identified in mitochondrial fractions were metabolic enzymes. Lysine acetylation was also identified on the mitochondrial DNA-encoded ATP synthase F_o subunit 8, implying that proteins can become acetylated within mitochondria.

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The acetylation status of several proteins is regulated by a large family of NAD^+-dependent protein deacetylases called the sirtuins. They are named after the yeast Sir2 (silent information regulator 2), and regulate important biological pathways in eubacteria, archaea and eukaryotes. Bacteria and archaea encode one or two sirtuins, but mice and humans have seven sirtuins, named SIRT1–7. The seven mammalian sirtuins occupy different subcellular compartments, such as the nucleus (SIRT1, −2, −3, −6 and −7), the cytoplasm (SIRT1 and −2) and mitochondria (SIRT3, −4 and −5) [3–8]. The sirtuins are assigned to five subclasses (I–IV and U) on the basis of the phylogenetic conservation of a ~250 amino acid core domain [9,10]. Among mammalian sirtuins, SIRT1, −2 and −3 are class I sirtuins, and have high homology with the yeast sirtuins Sir2, Hst1 and Hst2, and exhibit robust deacetylase activity. Class II sirtuins, including mammalian SIRT4, have no detectable deacetylase activity and instead show weak ADP-ribosyltransferase activity [6,11]. Class III sirtuins, including mammalian SIRT5, have weak deacetylase activity on histone substrates [12,13]. Class IV sirtuins have ADP-ribosyltransferase and deacetylase activity (SIRT6) or unknown activity (SIRT7) [14,15]. Class U sirtuins are intermediate between classes I and IV and have only been observed in bacteria.

The sirtuins mediate a deacetylation reaction that uses NAD^+ as a cofactor, yielding *O*-acetyl-ADP-ribose, the deacetylated substrate, and NAM (nicotinamide) (reviewed in [16,17]). The dependence of the sirtuins on NAD^+ suggests that their enzymatic activity is directly linked to the energy status of the cell, either via the cellular NAD^+/NADH ratio, the absolute levels of NAD^+, NADH or NAM, or a combination of these variables [18–22]. Indeed, the sirtuins have important roles in controlling metabolism in a variety of organisms (reviewed in [23]).

Thus the three sirtuins located in the mitochondria (SIRT3, SIRT4 and SIRT5) are poised to mediate mitochondrial protein acetylation levels. However, deacetylase activity has not been detected for SIRT4, whereas SIRT5 only displays low levels of deacetylase activity. Mice lacking SIRT3 exhibit high levels of mitochondrial protein acetylation, whereas mice lacking either SIRT4 or SIRT5 showed no obvious change [24]. These observations demonstrated that SIRT3 is a soluble protein in the mitochondrial matrix [25,26], and is the major mitochondrial protein deacetylase (Figure 1).

Interestingly, MATs (mitochondrial acetyltransferases) have not been identified, raising the question as to how mitochondrial proteins become acetylated. Because non-enzymatic acetylation of histones with acetyl-CoA occurs *in vitro* on the ε-amino group of lysine residues under physiological conditions [27], high acetyl-CoA levels in the mitochondria could facilitate a similar non-enzymatic acetylation mechanism. Alternatively, MATs could mediate the acetylation reaction. Acetylated proteins in mitochondria show a preference for a histidine or tyrosine residue at the +1 position flanking the acetylated lysine residue, which suggests that this unique acetylation consensus site could be recognized by a MAT. In contrast, histones favour lysine or acetylated lysine at the −4 or +4 position, whereas non-histone cytosolic proteins prefer an asparagine residue at the −1 position and non-histone nuclear proteins prefer a histidine residue at the +1 position. Furthermore, because of the difference in motif preference of mitochondrial proteins compared with nuclear or cytosolic proteins, MATs could form a class of acetyltransferases different from the known nuclear and cytosolic enzymes [2], and could be awaiting discovery.

Acetyl-CoA is the end-product of glucose-derived pyruvate oxidation, amino acid catabolism and fatty acid oxidation, and therefore mitochondrial protein acetylation could be a convergence point for carbohydrate, amino acid and fat metabolism. Because acetyl-CoA is an indicator of cellular energy status, acetylation could have evolved to couple metabolic enzyme activity to fluctuating levels of this key metabolite. Acetylation has clearly emerged

as a common PTM in mitochondria, and SIRT3 plays a major role in regulating mitochondrial protein acetylation. The field has advanced rapidly in recent years, both in terms of identification of new acetylated mitochondrial proteins, and with regard to the functional significance of some of these acetylation events. This chapter highlights some of these key findings.

Mitochondrial acetylated protein landscape

Mitochondrial protein acetylation is sensitive to metabolic perturbations. For example, mitochondrial protein acetylation increases in the liver during fasting. In mice, 62% of acetylated mitochondrial proteins were identified in mitochondrial fractions isolated from both fed and fasted animals, whereas 14% of acetylated mitochondrial proteins were unique to fed mice, and 24% of acetylated mitochondrial proteins were unique to fasted mice [2]. Mice fed a calorie-restricted diet also show increases in mitochondrial protein acetylation [28], similar to the acetylation patterns observed during fasting.

Paradoxically, mitochondrial protein acetylation increases in mice during high-fat diet feeding [29,30]. Such changes are observed with long-term, but not short-term, high-fat diet feeding [29]. Furthermore, mitochondrial protein hyperacetylation is also observed with dietary ethanol supplementation [31]. Thus an altered metabolic state, such as nutrient deprivation or nutrient excess, or ethanol exposure, all lead to increases in mitochondrial protein acetylation.

To determine which proteins become acetylated under different dietary and metabolic conditions, several proteomic studies have been performed (D. Lombard, personal communication and [2,28,32–34]). In the first study of mitochondrial protein acetylation, performed in liver collected from fed and fasted mice (as described above), an estimated 20% of all mitochondrial proteins were acetylated [2]. More recently, two studies found that virtually every major metabolic enzyme is acetylated, both inside and outside the mitochondria [33,34]. Thus key enzymes involved in glycolysis, the TCA (tricarboxylic acid) cycle, the urea cycle, fatty acid metabolism and glycogen metabolism were all acetylated.

To gain a better understanding of the prevalence of mitochondrial protein acetylation, we integrated the acetylation sites identified in several major proteomic studies [2,28,32,34], coupled with unpublished proteomics data (D. Lombard, personal communication) (Figure 2A). We then compared these acetylated proteins with all proteins annotated with mitochondrial subcellular localizations via GO (gene ontology) (compendium in [35]). Taken together, we estimate approximately 35% of all mitochondrial proteins have at least one acetylation site (Supplementary Table S1 at http://essays.biochemistry.org/bses-says/052/bse0520023add.htm).

Furthermore, the majority (53%) of acetylated proteins in the mitochondria have only one or two acetylation sites (33% and 20% respectively; Figure 2B). Remarkably, 11% of all acetylated mitochondrial proteins contained more than ten unique acetylation sites. The ten mitochondrial proteins with the highest number of acetylation sites all had >20 unique acetylation sites [Idh2, 22 sites; Hadh, 23 sites; Acat1, 24 sites; Slc25a5, 25 sites; Got2, 25 sites; Acaa2, 25 sites; Atp5a1, 26 sites; Aco2, 29 sites; Hadha, 47 sites; and Cps1, 53 sites (Supplementary Table S1)].

To better understand the mitochondrial processes that could be regulated by mitochondrial protein acetylation, we used DAVID (database for annotation, visualization and integrated discovery) 6.7 [36]. First, we determined which mitochondrial pathways were enriched in protein acetylation. By assigning a single, primary GO term to each mitochondrial protein,

we measured which mitochondrial processes contained acetylated proteins. In agreement with previously described studies, pathways involved in the generation of energy, fatty acid metabolism, sugar metabolism and amino acid metabolism contained several acetylated proteins. We found over 50% of the proteins in these pathways were acetylated (Figure 3A, dark blue, and Supplementary Table S1). In contrast, mitochondrial DNA maintenance, transcription, RNA processing and translation had fewer than 25% acetylated proteins in these pathways (Figure 3A, light blue).

To gain further insight into the mitochondrial pathways that could be regulated by protein acetylation, we entered all acetylated mitochondrial proteins into DAVID, and identified the functional annotations of these mitochondrial acetylated proteins on the basis of KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways, PANTHER (protein analysis through evolutionary relationships) classifications [37], BioCarta pathway analysis, and the EC number for chemical reactions. From this comprehensive analysis, the metabolic pathway with the highest enrichment score was oxidative phosphorylation, with 49 acetylated proteins (Figure 3B). Indeed, protein acetylation regulates ATP production both directly by deacetylation of one subunit of Complex I (NDUFA9) [38] and Complex II (SdhA) [39,40], and indirectly by reducing the activity of the fatty acid oxidation pathway upstream of oxidative phosphorylation [41]. Surprisingly, several other metabolic pathways were enriched for mitochondrial protein acetylation, for which the role of acetylation has not yet been reported; these pathways include tryptophan metabolism, arginine and proline metabolism, lysine degradation, β-alanine metabolism, limonene and pinene degradation, ascorbate and aldarate metabolism, and histidine metabolism. In addition to metabolic pathways, pathways known to influence development of Parkinson's disease, Huntington's disease and Alzheimer's disease were also identified, by functional annotation clustering, as highly enriched for mitochondrial protein acetylation. These findings support the role of mitochondrial dysfunction as a contributor to neurodegenerative disease [15,42–44], and suggest that perturbed regulation of mitochondrial protein acetylation could play an important role in these disease states.

Regulation of mitochondrial proteins by reversible acetylation

Even though a large number of acetylated proteins in the mitochondria have been identified, the effect of acetylation on most of these proteins is unknown. The first report of a functional role for acetylation on a mitochondrial protein described the activation of AceCS2 (acetyl-CoA synthetase) by mitochondrial SIRT3-catalysed deacetylation of a single lysine residue (Lys^{642}) which lies near the active site [45,46]. AceCS2 activation occurs in extrahepatic tissues during prolonged starvation to convert acetate into acetyl-CoA for energy production, and deacetylation by SIRT3 is coincident with the response to metabolic stress.

In a more recent study, SIRT3 was shown to stimulate fatty acid oxidation by deacetylating one acetylation site on LCAD (long-chain acyl-CoA dehydrogenase) out of eight total acetylation sites, thereby activating the fatty acid oxidation pathway [41]. Liver, heart, skeletal muscle and brown adipose tissue from fasted SIRT3-knockout mice showed reduced fat oxidation, and the fasted mutant animals exhibited fatty liver, reduced hepatic ATP production, hypoglycaemia and cold intolerance, all predicted consequences of defective fatty acid oxidation. From the eight acetylated lysine residues, Lys^{42} was identified as the major physiological site of acetylation and target of SIRT3, and was critical for the catalytic activity of LCAD. The physiological role of acetylation on lysine residues not targeted by SIRT3 is unknown. Defects in fatty acid oxidation are associated with metabolic diseases, including diabetes, cardiovascular disease and liver steatosis. Indeed, a follow-up study identified SIRT3 as a critical regulator of mitochondrial function, and suppression by high-

fat diet feeding or reduction in enzymatic activity by a point-mutation both contribute to the metabolic syndrome [29]. LCAD hyperacetylation was induced by high-fat diet feeding and was sufficient to reduce enzymatic activity in the livers of wild-type mice, in a manner similar to that observed in SIRT3-knockout mice. These data suggest that mitochondrial protein acetylation and/or SIRT3 activity is a potential therapeutic target for the treatment of metabolic disorders.

Another study showed that SIRT3 deacetylates and stimulates the catalytic activity of HMGCS2 (3-hydroxy-3-methylglutaryl-CoA synthase 2), a mitochondrial liver enzyme that catalyses the rate-limiting step in ketone body synthesis, a critical pathway up-regulated during the starvation response [47]. Ketone bodies are an essential energy source for a number of tissues, particularly the brain, in place of glucose when glucose is low, and are synthesized from acetyl-CoA that has been diverted from the TCA cycle. HMGCS2 catalyses one step in this pathway, the conversion of acetoacetyl-CoA and acetyl-CoA into HMG-CoA (3-hydroxy-3-methylglutaryl-CoA). Fasted SIRT3-knockout mice failed to deacetylate and activate HMGCS2, and therefore exhibited diminished levels of hepatic and serum ketone bodies. The study also presents one mechanism by which SIRT3 deacetylation of HMGCS2 modulates activity of the enzyme. Of the eleven HMGCS2 acetylated sites identified by proteomic analysis, only three are targeted by SIRT3 (Lys^{310}, Lys^{447} and Lys^{473}). Acetylation of the three sites was inversely correlated with HMGCS2 activity and their deacetylation increased enzymatic activity by increasing the V_{max}, but not the K_m, for the substrates. Molecular dynamic simulations comparing unacetylated and acetylated HMGCS2 revealed acetylation-induced changes in protein conformation. When unacetylated, the ε-amino group of Lys^{310} forms electrostatic interactions with nearby aspartate residues of the enzyme and with acetyl-CoA. These interactions are abrogated by acetylation, which eliminates the positive charge on the Lys^{310} side chain, producing conformational changes that propogate through the enzyme to critical catalytic residues distant from the site of acetylation. Thus acetylation of some, but not all, lysine residues can change the overall structure of the enzyme, leading to changes in enzymatic activity.

In addition to AceCS2, LCAD and HMGCS2, acetylation has been shown to control the enzymatic activity of several additional mitochondrial metabolic enzymes, such as malate dehydrogenase [34] and isocitrate dehydrogenase [48] in the TCA cycle, glutamate dehydrogenase [24] in amino acid catabolism, enoyl-coA hydratase/3-hydroxyacyl-CoA dehydrogenase [34] in the fatty acid oxidation pathway, carbamoyl phosphate synthetase 1 [13] and ornithine transcarbamoylase [49] in the urea cycle, and manganese superoxide dismutase [50,51] in the antioxidant system.

Future perspectives

Sufficient evidence now exists to conclude that reversible acetylation is critical for mitochondrial function. However, the full regulatory programme of acetylation is unknown. Hundreds of acetylated mitochondrial proteins identified by MS (mass spectrometry)-based proteomics approaches remain to be validated. Even after rigorous validation of acetylation sites on mitochondrial proteins, the presence of acetylation does not necessarily correlate with effects on protein function. For example, acetylation of lysine residues on HMGCS2 not targeted by SIRT3 induces no protein conformational changes [47]. However, electrostatic surface potential will continue to drop as positively charged ε-amine groups become acetylated, which could disrupt protein–protein interactions or substrate/cofactor binding. Thus, in order to uncover the specificity of mitochondrial protein function regulated by acetylation, additional studies describing the effects of specific acetylation/deacetylation sites are needed. Furthermore, because protein identity among mitochondria from different tissues is highly variable (only ~50% conservation) [52], a better understanding of tissue-

specific changes in mitochondrial protein acetylation will be needed for a range of physiological and pathophysiological conditions.

Conclusions

Mitochondrial protein acetylation is an emerging and fundamental mechanism for regulating the activities of mitochondrial proteins and overall mitochondrial function. Although more work needs to be done to fully understand this complex regulatory mechanism, mitochondrial protein acetylation is an important part of the adaptive metabolic response, where dramatic changes in energy metabolism must co-ordinate to ensure survival. Mitochondria are crucial for several cellular processes, including the production of more than 90% of cellular ATP, apoptosis, cell-cycle progression, proliferation and aging, and their dysfunction has been implicated in a wide range of human metabolic and neuro-degenerative diseases [53–56]. Mitochondrial protein acetylation should now be considered as a potentially critical component of the mitochondrial metabolic regulatory network.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Summary

- Protein acetylation is a common PTM in the mitochondria, and approximately 35% of all mitochondrial proteins are acetylated.

- The majority of acetylated mitochondrial proteins that have been identified are involved in some aspect of energy metabolism.

- Mitochondrial protein acetylation levels are regulated by SIRT3, a member of the sirtuin family of NAD^+-dependent protein deacetylases.

- In the absence of SIRT3, mitochondrial proteins become hyperacetylated, have reduced activity, and lead to mitochondrial dysfunction.
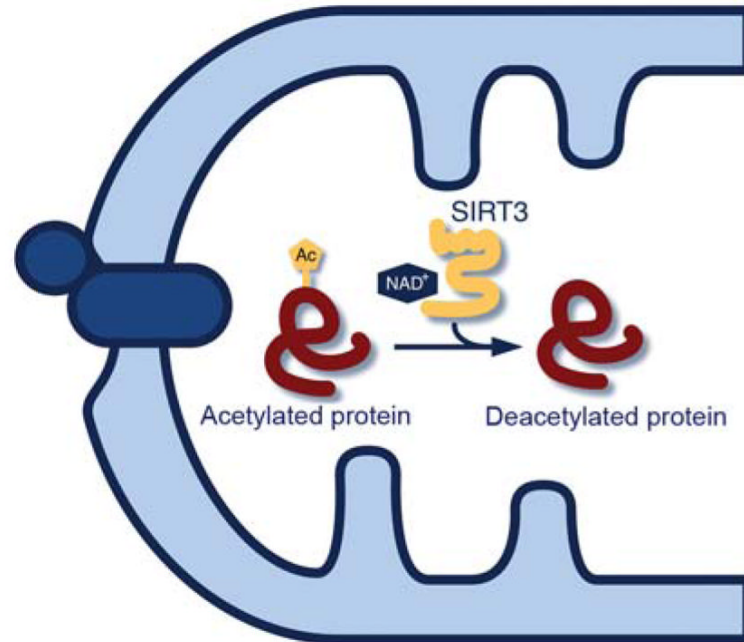
Figure 1. SIRT3 is a mitochondrial NAD$^+$-dependent protein deacetylase
SIRT3 is encoded in the nucleus and imported into the mitochondrial matrix. SIRT3 uses NAD$^+$ as a cofactor and removes acetyl groups from protein lysine residues within mitochondrial proteins, rendering the protein deacetylated. Ac, acetyl group.
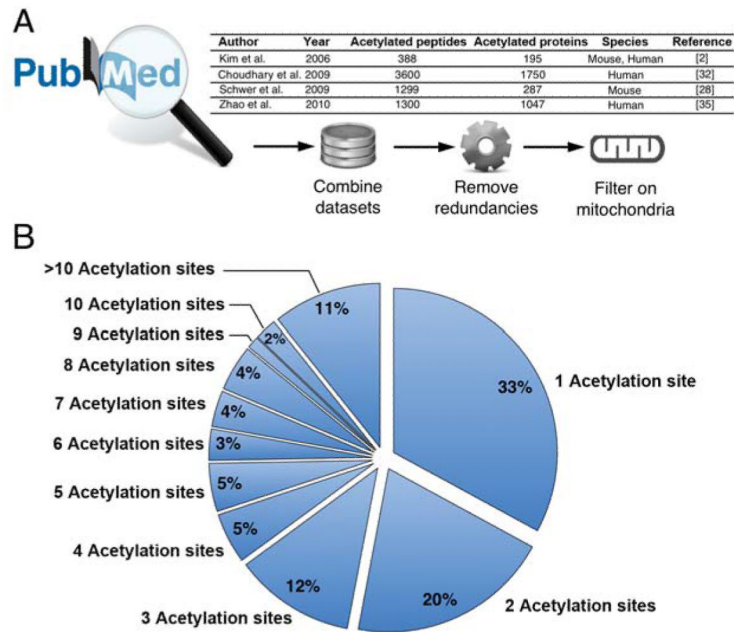
Figure 2. Protein acetylation is abundant in the mitochondria
(**A**) Several proteomic datasets were integrated to identify all acetylated proteins. Redundant acetylated proteins were removed and datasets were filtered on mitochondrial proteins in mice and humans. The PubMed logo is reproduced with the permission of the National Library of Medicine. (**B**) Acetylated mitochondrial proteins were counted and tallied for unique sites of acetylation (percentage of mitochondrial proteins containing a number of acetylated sites compared with all acetylated mitochondrial proteins).

Figure 3. Metabolic pathways are highly acetylated
(**A**) Summary of all annotated mitochondrial functions and their levels of acetylation; each box represents one annotated function by GO term; the size of the box indicates the number of proteins labelled with common annotation; the colour of the box indicates the percentage of acetylated proteins in the annotated pathway (light blue, 25%; medium blue, 26–50%; dark blue, 51–75%; and very dark blue, 76%). (**B**) Representative schematic diagram of oxidative phosphorylation (Complex I–V) showing unacetylated (blue) and acetylated (orange) proteins. ROS, reactive oxygen species.