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Review

Are sirtuin deacylase enzymes important modulators of mitochondrial energy metabolism?☆

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ABSTRACT

Background: In recent years, reversible lysine acylation of proteins has emerged as a major post-translational modification across the cell, and importantly has been shown to regulate many proteins in the mitochondria. One key family of deacylase enzymes is the sirtuins, of which SIRT3, SIRT4, and SIRT5 are localised to the mitochondria and regulate acyl modifications in this organelle.
Scope of review: In this review we discuss the emerging role of lysine acylation in the mitochondrion and summarise the evidence that proposes mitochondrial sirtuins are important players in the modulation of mitochondrial energy metabolism in response to external nutrient cues, via their action as lysine deacylases. We also highlight some key areas of mitochondrial sirtuin biology where future research efforts are required.
Major conclusions: Lysine deacetylation appears to play some role in regulating mitochondrial metabolism. Recent discoveries of new enzymatic capabilities of mitochondrial sirtuins, including desuccinylation and demalonylation activities, as well as an increasing list of novel protein substrates have identified many new questions regarding the role of mitochondrial sirtuins in the regulation of energy metabolism.
General significance: Dynamic changes in the regulation of mitochondrial metabolism may have far-reaching consequences for many diseases, and despite promising initial findings in knockout animals and cell models, the role of the mitochondrial sirtuins requires further exploration in this context. This article is part of a Special Issue entitled Frontiers of mitochondrial research.

1. Introduction

1.1. Mitochondria and protein acylation

Mitochondria are key organelles that play a central role in major cellular processes, including energy transduction, intracellular signalling, and apoptosis. Mitochondria are not static organelles, but exist largely as a reticular network, with inherent morphological and metabolic plasticity to allow for functional adjustments in response to the prevailing cellular stresses and metabolic requirements. For example in response to physiological changes in nutrient availability, cold exposure or disease states, mitochondria can change their number, shape, activity and preferred fuel substrates to appropriately sustain the bioenergetics needs of the cell. The critical role of the mitochondria in regulating cellular homeostasis is highlighted by the fact that defects in mitochondrial function (e.g. impaired oxidative phosphorylation, excess reactive oxygen species (ROS) production, altered mitochondrial dynamics) are implicated in many diseases including diabetes, cancer and neurodegeneration, as well as the ageing process [1–4].

Mitochondrial metabolism must therefore be tightly regulated to maintain normal cellular functions, and recently it has been shown that post-translational modifications of mitochondrial proteins are a

Abbreviations: AceCS2, acetyl-CoA synthetase 2; ADP, adenosine diphosphate; CPS1, carbamoyl phosphate synthetase 1; CPT1, carnitine palmitoyltransferase I; CR, calorie restriction; FOXO3a, forkhead box 03a; GDH, glutamate dehydrogenase; HeLa, human cervical carcinoma cells; HIF-1α, hypoxia inducible factor 1α; HMGCS2, 3-hydroxy-3-methylglutaryl CoA synthase 2; IDH2, isocitrate dehydrogenase 2; LCAD, long chain acyl-CoA dehydrogenase; MCAD, medium chain acyl coenzyme A dehydrogenase; MCD, malonyl-CoA decarboxylase; MDH, malate dehydrogenase; MEFs, murine embryonic fibroblasts; mPTP, mitochondrial permeability transition pore; MRPL10, mitochondrial ribosomal protein L10; NAD^+, nicotinamide adenine dinucleotide; NADPH, nicotinamide adenine dinucleotide phosphate (reduced); NDUFA9, NADH dehydrogenase (ubiquinone) 1α subunit 9; OTC, ornithine carbamylase; PDH, pyruvate dehydrogenase; PDH E1α, E1α subunit of PDH; PGC1α, peroxisome proliferator-activated receptor gamma coactivator 1α; PTM, post-translational modification; ROS, reactive oxygen species; SDHa, succinate dehydrogenase subunit a; SOD2, superoxide dismutase 2; TCA, tricarboxylic acid cycle

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key feature of this regulation. Lysines are amino acid residues within proteins, that are susceptible to a wide range of post-translational modifications. One important form of post-translational modification is lysine acylation, which is the addition of specific acyl groups to the lysine residue. There are a large number of reported acyl modifications, such as acetylation, malonylation, succinylation, propionylation, butyrylation, and crotonylation [5–7]. Of these acyl modifications, reversible lysine acetylation has been the most extensively studied and a number of reports in the last decade have shown that acetylation is a highly prevalent and functionally relevant post-translational modification in the mitochondria [8–12].

Several of the acyl modifications mentioned above are directly regulated by the sirtuin family of enzymes. Sirtuins are highly conserved nicotinamide adenine dinucleotide $(NAD)^+$-dependant deacylases and mono-ADP-ribosyl transferases [13,14]. Mammals possess seven members of the sirtuin family, from the well-studied SIRT1 to SIRT7. Three sirtuin enzymes, SIRT3, SIRT4 and SIRT5, are known to be located in the mitochondria and regulate post-translational modifications in this organelle.

In this review we will discuss the emerging role of lysine acylation in the mitochondrion and summarise the evidence suggesting that mitochondrial sirtuins are important players in the modulation of mitochondrial energy metabolism via their effects on protein post-translational modifications. We also highlight some of the inconsistencies and deficiencies in the literature regarding mitochondrial sirtuin biology and propose some key areas for future research.

2. Reversible lysine acetylation

The most well studied form of acyl modification is lysine acetylation. Acetylation was first described in histones in the nucleus approximately fifty years ago [15], and has since been shown to be a major regulator of gene expression and chromatin structure [16]. Acetylation is the covalent addition of an acetyl group from acetyl-CoA to the ε-amino group of lysine residues, which neutralises the positively charged lysine, changing the way it interacts with other nearby proteins and molecules [17]. As a result of this change, reversible lysine acetylation is known to affect enzymatic activity, protein stability, protein interactions and subcellular localisation of target proteins [17].

With advances in mass spectrometry, proteomic studies in the last decade have shown that acetylation is a post-translational modification that extends well beyond the nucleus, being common to many non-histone proteins across the cell [9–11,18–20]. In combination, these studies have shown that more than 4000 mammalian proteins are acetylated, pointing to reversible lysine acetylation as a major post-translational modification, that has a regulatory scope comparable to that of other major protein modifications such as phosphorylation or ubiquitination, as originally predicted [21]. Consistent with this wide spectrum of target proteins, acetylation has been shown to influence a multitude of cellular processes, including apoptosis and the cell cycle, ageing, antioxidant defences, cancer, circadian rhythms, gene expression, and metabolism [10,22–25].

3. Acetylation is highly prevalent in the mitochondrion

While it is wide-spread across the cell, acetylation is particularly prominent in the mitochondria. In 2006, Kim et al. used a combination of studies in HeLa cells and mouse liver to show that acetylation was abundant in the mitochondria [11]. Subsequent proteomic studies examining the global acetylome of whole cells and tissues revealed thousands of acetylation sites, and mitochondrial proteins were highly represented in these studies [9,10]. In addition to identifying individual proteins that may be acetylated, these reports also highlighted that proteins may be acetylated at multiple lysine sites. Integrated analysis of several recent mammalian proteomic screens estimates that approximately 35% of all mitochondrial proteins have at least one lysine that is able to be acetylated [8], while another recent report puts this number as high as 65% [26]. The majority of these proteins have only one or two acetylation sites, however, just over 10% of identified proteins have greater than 10 unique acetylation sites [8].

Additional proteomic studies have been published recently expanding the scope of specific tissues, species and conditions under which acetylation has been assessed. Some of these recent reports investigating the acetylome include examination of tissue specific changes across multiple rat tissues [19], liver acetylation changes in mice during calorie restriction [26], an alcoholic liver disease model in mice [27], and SIRT3 dependent changes in murine embryonic fibroblasts and mouse liver [12,28]. Collectively these studies have further highlighted the diversity and potential scope of acetylation for impacting upon mitochondrial metabolism.

Acetylated residues are observed in all major metabolic pathways in the mitochondria, including enzymes of the tricarboxylic acid (TCA) cycle, the urea cycle and fatty acid β-oxidation [9]. With respect to the functional impact of acetylation, Zhao et al. showed that the enzyme malate dehydrogenase (MDH) in the TCA cycle was able to be acetylated at four lysine sites, and that this acetylation was dependent on the glucose concentration in the cells and caused an increase in the activity of the enzyme [9]. Increasing the complexity of these systems, acetylation appears to both inhibit and activate different metabolic enzymes, such that while MDH is activated by acetylation, other mitochondrial enzymes, such as long chain acyl-CoA dehydrogenase (LCAD) and ornithine carbamylase (OTC) are inhibited by acetylation [29,30]. In addition, because some acetylated proteins have multiple lysine acetylation sites, validation studies are necessary to delineate which of the modified lysines are responsible for changes in activity of the target protein. In the case of LCAD, an enzyme involved in fatty acid oxidation, while there are 8 acetyllysine sites on the protein, only one has been shown to be associated with modified enzyme activity in the models studied thus far [30].

4. Sirtuin deacylase enzymes

Reversible acetylation is controlled by the actions of acetyltransferase and deacetylase enzymes, which catalyse the addition and the removal of acetyl groups on lysine residues of target proteins respectively. In contrast to the many hundreds of enzymes that control protein phosphorylation and ubiquitination, there are only a limited number of regulatory enzymes for acetylation, with approximately 30 acetyltransferases and 18 deacetylases identified in humans [24]. Amongst the enzymes that regulate deacetylation is the sirtuin family of NAD^+-dependent deacetylase enzymes, which play an important role in regulating lysine acetylation in different cellular compartments.

Sirtuins are categorised as Class III deacetylases and unlike classic deacetylases, which hydrolyse the acetyl group, sirtuins deacetylate lysine residues in an unusual chemical reaction that consumes NAD^+, releases nicotinamide, *O*-acetyl ADP ribose, and the deacetylated substrate. The name sirtuin is derived from its founding member, yeast Sir2 (silent information regulator 2). The sirtuins are highly conserved across species from bacteria to humans [31] and are associated with increased lifespan in yeast, nematodes and fruit fly [32–34] and improved healthspan in mammals [35–37]. In mammals there are seven sirtuin proteins (SIRT1–7), which display diverse subcellular localisations. SIRT1, SIRT6 and SIRT7 are predominantly nuclear, SIRT2 is cytoplasmic and SIRT3, SIRT4 and SIRT5 reside in the mitochondria. In addition to being present in disparate parts of the cell, it has come to light that mammalian sirtuins also catalyse a range of different enzymatic reactions other than deacetylation. These additional enzymatic roles include desuccinylation, demalonylation, demyristolation and ADP-ribosylation [6,38,39], and consistent with phylogenetic analyses of mammalian sirtuins [31], it appears that different mammalian sirtuins have evolved to have distinct roles within the cell. From a metabolic perspective, the NAD^+ dependency of the sirtuin deacylation reaction indicates that sirtuins are perfectly positioned at the crossroads of metabolic flux,

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