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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 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 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 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 mitochondria. In 2006, Kim et al. used a combination of studies in HeLa cells and mouse liver to show that acetylation was abundant in 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,

with the ability to sense and respond to both the redox and metabolic state of the cell (Fig. 1). Furthermore, the fact that acyl donor molecules for acylation such as acetyl-CoA, malonyl CoA and succinyl CoA are themselves important metabolites that fluctuate with changes in cellular energy flux, further highlights the potential role of sirtuins in the metabolic control system.

As noted above, SIRT3, SIRT4 and SIRT5 are all present in the mitochondria, and these enzymes have been reported to catalyse different reactions, with SIRT3 being the predominant mitochondrial deacetylase, SIRT4 displaying ADP-ribosylase activity and some deacetylase activity towards specific targets and SIRT5 primarily being a desuccinylase and demalonylase enzyme. Interestingly, despite the mitochondrial sirtuins being quite well studied, so far no mitochondrial acyltransferases have been described, although GCN5L1 has been reported in the literature as a possible mitochondrial acetyltransferase [40]. Since the majority of the mitochondrial proteins are nuclear encoded, it is plausible that nuclear encoded mitochondrial proteins may be acylated prior to their trafficking to the mitochondria, however, since proteins encoded by the mitochondrial genome are found to be acetylated, it is likely that unidentified protein acetyltransferases are present [11]. One alternate possibility is that mitochondrial proteins may in some cases be directly acylated due to high intramitochondrial concentrations of succinyl-CoA, malonyl-CoA or acetyl-CoA, and with respect to acetylation, non-protein mediated addition of an acetyl group has been demonstrated in vitro for histones [41].

5. The mitochondrial deacetylase SIRT3

SIRT3 is the most well characterised member of the mitochondrial sirtuins and is a soluble protein found in the mitochondrial matrix [42]. SIRT3 is nuclear encoded and expressed as a 45 kDa protein containing an N-terminal mitochondrial targeting sequence, that is cleaved off after import into the mitochondria, leaving an enzymatically active 28 kDa protein [42]. Reports persist that SIRT3 may also be found in the nucleus [43,44], however most publications now agree that SIRT3 is mitochondrial in localisation [45–47]. SIRT3 has the most robust deacetylase activity of the three mitochondrial sirtuins and SIRT3 deletion in mice leads to marked upregulation of global acetylation in mitochondria [5,48]. The levels of SIRT3 are highly responsive to the prevailing nutrient availability of the cell. Calorie restriction, fasting and exercise training have all been reported to increase SIRT3 levels in different tissues [29,49–51], although a recent study showed a reduction in SIRT3 in skeletal muscle during fasting [52]. In contrast the expression and/or activity of SIRT3 has been shown to be lower in high-fat fed rodents [50,53,54], in mouse models of type 2 diabetes [55] and in human subjects with the metabolic syndrome [50].

5.1. Protein targets of SIRT3

The first reported target of SIRT3 described in the literature was acetyl-CoA synthetase 2 (AceCS2) [56,57]. AceCS2 was shown to be activated upon the deacetylation of lysine residue Lys^{642} by SIRT3. Since then, SIRT3 has been reported to directly affect the function of diverse mitochondrial enzymes and components of mitochondrial respiration complexes which are discussed in detail below. A comprehensive list of reported SIRT3 substrates is presented in Table 1, highlighting the tissues or cell model in which these substrates were validated. Interestingly, most SIRT3 targets were confirmed in vitro in immortalised cell lines, or in vivo in mouse liver, with most studies taking advantage of liver tissues from the SIRT3 knockout (SIRT3 KO) mouse model. Because the

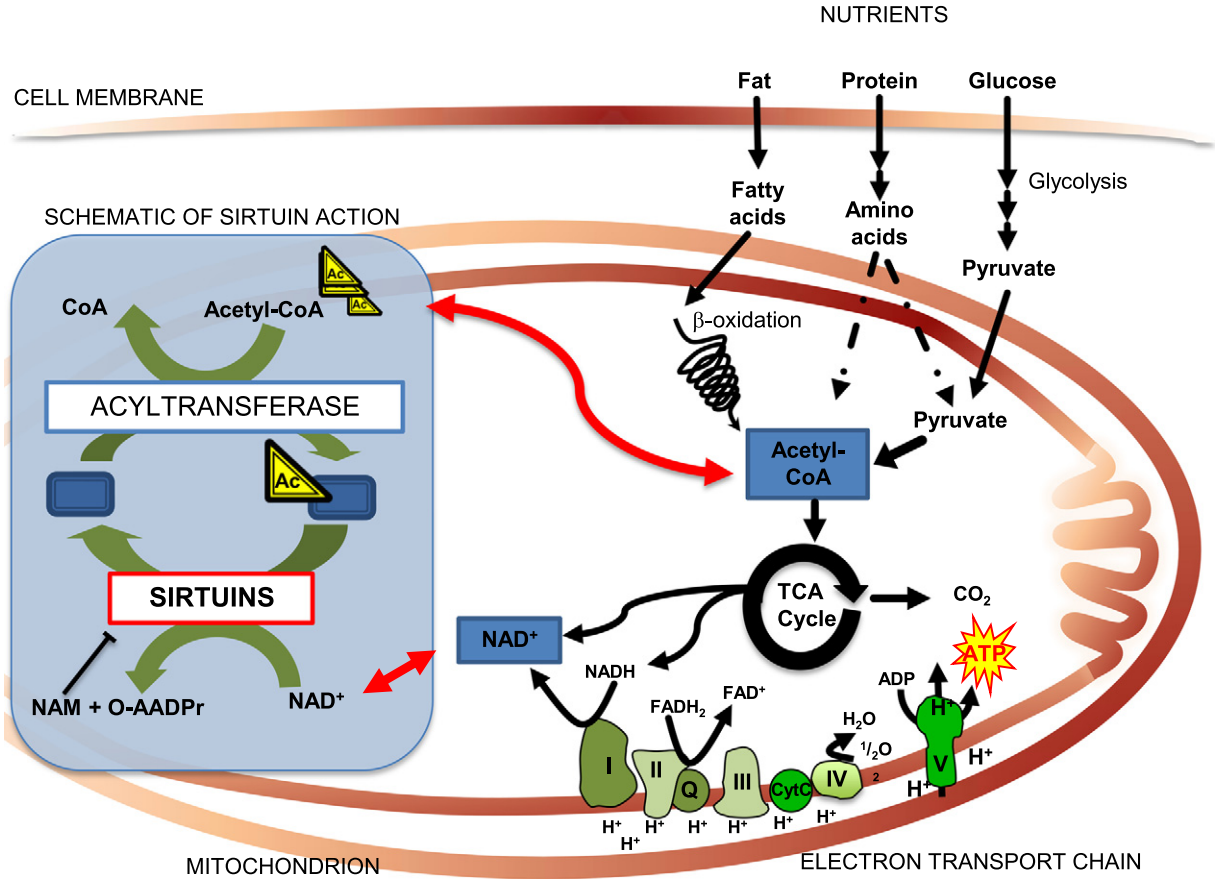

Fig. 1. Mitochondrial sirtuins are highly responsive to changes in nutrient flux. Mitochondrial sirtuins form part of a cycle that regulates the activity of specific enzymes and metabolic pathways in response to changes in the levels of key nutrient intermediates, including NAD^+ and acetyl-CoA. Other intermediates such as succinyl-CoA and malonyl-CoA can also participate in acylation reactions. NAD^+, nicotinamide adenine dinucleotide; NAM, nicotinamide; H^+, proton; Ac, acetyl modification.

Table 1
Reported substrates of SIRT3.

Substrate	Tissue type/model studied	Validation		Ref
		Deacetylase assay	MS	
AceCS2	In vitro & cell culture models; *E. coli*	✓		[55,56]
ATPase subunit α	HepG2 cells; mouse MEFs; mouse primary hepatocytes			[53]
Cyclophilin D	Mouse heart and heart mitochondria from SIRT3 KO mice; human cell lines		✓	[65,70]
FOXO3a	Mouse cardiomyocytes overexpressing SIRT3	✓		[84]
GDH	SIRT3 KO mouse liver mitochondria	✓		[48,85]
HMGCS2	SIRT3 KO mouse liver mitochondria	✓	✓	[26,58]
IDH2	CR model in the brain, inner ear & liver of SIRT3 KO mice; human cell lines	✓	✓	[61,85]
Ku70[a]	Mouse cardiomyocytes and cell lines overexpressing SIRT3	✓		[64]
LCAD	SIRT3 KO mouse liver: high fat fed/fasted	✓	✓	[30]
MRPL10	Bovine liver mitochondrial ribosomes; human cell lines; SIRT3 KO mouse liver; C2C12 cells	✓		[86]
NDUFA9/CI	SIRT3 KO mouse liver		✓	[12,57]
OTC	Mouse liver & blood; SIRT3 KO mice and CR	✓	✓	[19,29]
P300/histone H3[a]	HeLa cells			[87]
PDH E1α	Muscle SIRT3 KO mice; C2C12 cells		✓	[52]
SDHa	BAT, liver and liver mitochondria of SIRT3 KO mice; brown preadipocyte cell line	✓	✓	[59,60]
SOD2	Cell lines and MEFs overexpressing SIRT3; CR model in the liver of SIRT3 KO mice; SIRT3 KO MEFS	✓		[62,63]

Abbreviations: CR = calorie restriction, KO = knockout mouse model, MEFs = mouse embryonic fibroblasts, BAT = brown adipose tissue, MS = mass spectrometric validation.
[a] Nuclear localisation requires further validation.

interaction of SIRT3 with its substrates may be tissue and condition specific, more studies on other tissues and in conditions other than global SIRT3 deletion are required.

More recently, detailed proteomic studies have sought to investigate the full acetylome regulated by SIRT3. Quantitative proteomics of SIRT3 KO murine embryonic fibroblast (MEF) cells found that of all the acetylation sites identified in the screen, SIRT3 knockdown was found to modulate the acetylation of more than a quarter of them [28]. Further investigation of the effect of calorie restriction [26] or fasting [12] in combination with SIRT3 deletion using SIRT3 KO liver cells and high resolution mass spectrometry has further established SIRT3 as a major regulator of mitochondrial acetylation state [12,26]. Collectively, these studies have identified a number of new putative SIRT3 targets that require validation, and as this information accumulates further understanding of the dynamic regulatory roles this enzyme plays in mitochondrial metabolism will emerge.

5.2. Metabolic pathways regulated by SIRT3

SIRT3 KO mice have been utilised extensively in the field to elucidate the role of SIRT3 in metabolism. Lombard et al. first published that a global SIRT3 KO mouse model, despite exhibiting marked hyperacetylation of mitochondrial proteins, showed no detectable metabolic phenotype in response to fasting or cold exposure, and exhibited normal weight, food intake and oxygen consumption compared to wildtype controls [48]. An independent SIRT3 KO mouse model also showed marked hyperacetylation of mitochondrial extracts, and although accompanied by functional changes including a 50% reduction in ATP levels in the liver, kidney and heart tissues, and a reduction in complex I activity, there was no major pathology observed in these knockout animals [58].

Since the initial investigation of these knockout mice, SIRT3 has been ascribed a number of metabolic functions. Hirschey et al. reported an important role for SIRT3 in regulating hepatic fatty acid oxidation [30]. In this study SIRT3 KO mice displayed reduced markers of fatty acid oxidation compared to control mice under conditions of fasting. This was linked with regulation of the enzyme LCAD, which is deacetylated by SIRT3 in the liver of normal mice, activating LCAD and stimulating fatty acid oxidation when dietary energy intake is low [30]. Hallows et al. also employed metabolomics screening and peptide spot arrays in tissues and plasma from SIRT3 KO mice to show that SIRT3 is involved in regulating fatty acid oxidation, likely by affecting multiple β-oxidation enzymes in addition to LCAD [29]. Furthermore, as well as directly influencing fatty acid oxidation, SIRT3 also regulates other aspects of lipid metabolism. The activity of 3-hydroxy-3-methylglutaryl CoA synthase 2 (HMGCS2), the rate-limiting step in ketone body formation, is regulated by SIRT3-mediated deacetylation, with SIRT3 KO mice displaying an impaired production of ketone bodies during prolonged fasting [59]. Additionally, as mentioned above, SIRT3 also deacetylates and activates AceCS2, which is an enzyme that converts acetate (largely derived from lipid in the liver) to acetyl-CoA in extrahepatic tissues [56,57]. These latter two functions of SIRT3 have been proposed to promote the coordinated use of lipid-derived carbons under conditions when lipid catabolism is high, such as during fasting.

Interestingly, while studies in SIRT3 KO mice have typically indicated reduced lipid utilisation in the liver of these animals, a recent study examining the muscle of SIRT3 KO mice suggests this may not be the case for all tissues. Deletion of SIRT3 in skeletal muscle in vivo and knockdown of SIRT3 in myoblasts led to hyperacetylation of the E1α subunit of pyruvate dehydrogenase (PDH), altering phosphorylation of this enzyme and decreasing PDH activity [52]. Due to the critical role of PDH for channelling carbohydrate-derived carbons into oxidative pathways, the decrease in PDH activity in SIRT3-deficient muscle resulted in a switch towards fatty acid utilisation rather than glucose metabolism, even under fed conditions when nutrient levels were high. These findings suggest a critical role for SIRT3 in regulating the ability of muscle to appropriately switch between fuel substrates (i.e. metabolic flexibility) [52].

On top of influencing carbohydrate and lipid metabolism, another important pathway regulated by SIRT3 involves the acceleration of amino acid utilisation and subsequent disposal of nitrogen waste via the urea cycle. SIRT3 deacetylates and activates glutamate dehydrogenase (GDH), a key enzyme involved in the catabolic processing of most amino acids [48]. The reaction catalysed by GDH releases nitrogen to the urea cycle as ammonia, and SIRT3 enhances the urea cycle by activating the key mitochondrial enzyme involved, OTC [29].

Several protein subunits of the mitochondrial respiratory chain have been reported to be regulated by SIRT3. These include NDUFA9 (complex I), succinate dehydrogenase subunit a (SDHa) (a subunit of complex II) and ATP synthase α subunit (complex V) [54,58,60,61]. Accordingly, tissues and cells with deletion or knockdown of SIRT3 show reduced activity of specific mitochondrial complexes, as well as reduced oxygen consumption. These changes in oxidative phosphorylation may, in part, explain up to 50% reduced ATP levels observed in mice lacking SIRT3 [58].

In addition to regulating enzymes involved in the metabolism of specific nutrients, SIRT3 also modulates a number of stress-related pathways in mitochondria. Firstly, SIRT3 enhances the capacity of

mitochondria to detoxify reactive oxygen species (ROS), which are a by-product of mitochondrial substrate metabolism. SIRT3 deacetylates and activates isocitrate dehydrogenase 2 (IDH2) [62], which is a TCA cycle enzyme that plays a critical role in maintaining the mitochondrial pool of NADPH, which is in turn used by glutathione reductase to maintain glutathione in its reduced antioxidant form. Secondly, SIRT3-mediated deacetylation of the superoxide scavenger Mn superoxide dismutase (SOD2) activates this enzyme and reduces mitochondrial ROS production [63,64]. Thirdly, SIRT3 has also been shown to act via interaction with FOXO3a to increase the transcription of antioxidants in cardiomyocytes [65]. As well as its role in regulating oxidative stress levels, SIRT3 also modifies the activity of another key stress-sensitive pathway in mitochondria, the mitochondrial permeability transition pore (mPTP). SIRT3 deacetylates one of the core regulatory components of the mPTP, cyclophilin D, leading to reduced opening of this mitochondrial pore [66].

Collectively many of the metabolic pathways regulated by SIRT3 indicate that this enzyme may function as a master switch that mediates the change from glucose-mediated metabolism towards a fasting metabolism, promoting the utilisation of lipids and amino acids under conditions where nutrient availability is low. Furthermore, in addition to its effects on intermediary metabolism, SIRT3 also appears to play a key role in the resistance to various mitochondrial stresses.

5.3. SIRT3 and disease

Due to their ability to regulate longevity in lower organisms [32,33], mammalian sirtuin proteins have received much attention for their role in modifying age-related diseases. SIRT3 has been reported to mediate the benefits of calorie restriction (CR) on hearing loss triggered by increased oxidative damage in the cochlear in ageing mice [62] and ameliorated by CR in a SIRT3-dependent manner. SIRT3 also protects against palmitate induced lipotoxicity and its attendant increase in ROS in kidney cells and hepatocytes [54,67]. Via its interaction with the mPTP, SIRT3 has also been shown to suppress age-related cardiac hypertrophy [66].

Cancer can be described as a prime example of aberrant metabolism, where the metabolism of a cell is largely shifted towards a state of increased proliferation and anabolic processes. It has been reported that loss of SIRT3 can trigger metabolic reprogramming, supporting tumorigenesis, and providing a selective advantage that drives tumour growth via increased glycolytic flux [23,68,69]. Reduced SIRT3 activity has also been proposed to drive tumourigenesis through excess ROS production and stabilisation of HIF-1α [70]. Many transformed cell lines and human tumours have reduced SIRT3 expression [71] and there is a growing body of evidence supporting a role for SIRT3 as a tumour suppressor [23,68], including the observation that SIRT3 KO mice have a high incidence of spontaneous tumours [69]. However, it should be noted that SIRT3 levels have also been shown to be increased in certain types of cancers (e.g. oral cancer) [72], and a recent report suggests that SIRT3 favours carcinogenesis, by providing resistance to stress and apoptotic stimuli through its interaction with cyclophilin D [73]. Thus the precise role of SIRT3 in regulating tumourigenesis may be cell-type and condition-specific, and additional studies in this area are required.

With regard to metabolic disease, SIRT3 expression and/or activity has been consistently shown to be reduced in rodent models of obesity and type 2 diabetes [50,55]. SIRT3 knockout mice display mild glucose intolerance and knockdown of SIRT3 in muscle cells reduces insulin action, likely as a result of oxidative stress [55]. Verdin and colleagues have also shown that SIRT3 KO mice display accelerated development of the metabolic syndrome, but only when exposed to a very long-term (8–12 months) high fat diet [50]. With respect to humans, a polymorphism in the human SIRT3 gene encoding a protein with reduced enzymatic efficiency is correlated with the development of the metabolic syndrome [50].

5.4. Timing and site of action may be important for SIRT3 function

One caveat of the abovementioned studies is that they have relied largely on global SIRT3 knockout mice to define the physiological roles of SIRT3. Deletion of SIRT3 in all tissues from birth is likely to elicit a number of developmental adaptations that may have secondary effects on metabolism. Furthermore, a major deficiency in this area is that there has been little investigation of the effect of SIRT3 gain-of-function. It is also unresolved if particular tissues play a more dominant role in mediating the whole body effects of SIRT3. In this regard, Auwerx and colleagues recently generated liver- and muscle-specific SIRT3 knockouts and reported that these animals have no detectable changes in metabolic phenotype in response to high fat feeding [74], throwing some doubt on the idea that liver changes in fatty acid oxidation are responsible for the detrimental phenotype observed in the global SIRT3 KO mice [50]. Of note is that in the tissue specific model, mice were only maintained on the high fat diet for 8 to 16 weeks, which is shorter than studies in the global SIRT3 KO. Also, as the authors point out, the genetic background of the animals and the developmental onset of the SIRT3 deletion differ between models which may additionally have a bearing on these disparate findings [74]. Overall these studies highlight that there are still many areas that require investigation to fully elucidate the metabolic role of SIRT3.

6. Metabolic role of SIRT4

Relatively little is known about the substrate specificity and physiological relevance of SIRT4, or its precise role in metabolism. SIRT4 deletion does not cause any major phenotype in chow-fed mice under standard laboratory conditions [39]. In contrast to SIRT3 null mice, SIRT4 KO mice do not display hyperacetylation of mitochondrial proteins, suggesting that SIRT4 is not involved in the global deacetylation of lysine residues in mitochondrial proteins, at least not under the conditions investigated [48]. Concomitant with this, SIRT4 was initially reported to exhibit little deacetylase activity, but some ADP-ribosylase activity [39].

The ADP-ribosyltransferase activity of all mitochondrial sirtuins is low, and has even been described as an inefficient side-reaction [75], however it remains likely that this activity of SIRT4 is physiologically relevant, especially in the case of its regulation of GDH [39]. Several groups have shown that SIRT4 is expressed abundantly in pancreatic beta cells and has the ability to regulate GDH and impact insulin secretion [76,77]. More specifically SIRT4 has been shown to ADP-ribosylate GDH, reducing its activity and decreasing amino acid-stimulated insulin secretion. Indeed SIRT4 null mice have increased GDH activity and insulin secretion [39].

The regulation of GDH by SIRT4 has been shown to also be involved in the modulation of the mPTP, and hence in the pathways of cell survival and apoptosis [78]. The role of SIRT4 in glutamine metabolism and glutamine flux through the TCA cycle has also been studied in the context of DNA damage responses, cell cycle and tumorigenesis. SIRT4 has been proposed to be a potent tumour suppressor in human cancer and mouse models, acting via its effect on glutamine metabolism, and like SIRT3, its decrease is associated with fuel switching to support proliferation and tumour progression [79]. SIRT4 null mice spontaneously develop lung tumours, and many human tumour lines show downregulation of SIRT4 expression [79]. DNA damage in healthy cells blocks glutamine metabolism, inducing cell cycle arrest to limit the effects of this cellular stress. Loss of SIRT4 allows cells to overcome this limitation, utilising glutamine metabolism to drive proliferation and create a tumorigenic environment [79].

In addition to the regulation of glutamine metabolism, SIRT4 knockdown was also reported to enhance pathways of fatty acid oxidation, increasing various targets including SIRT1, MCAD, CPT1, and PGC1α [77]. A recent paper has also reported a novel role for SIRT4 in controlling lipid metabolism, via the regulation of malonyl-CoA decarboxylase

(MCD) activity. Despite the initial reports of little deacetylase activity for SIRT4 [39], Haigis and colleagues have shown that SIRT4 deacetylates MCD, reducing its enzymatic activity and thus causing a rise in malonyl-CoA levels. The result of this increased malonyl-CoA is an increase in lipogenesis and reduction in fatty acid oxidation. Consistent with this newly described function, SIRT4 deficient mice display enhanced lipid oxidation and reduced lipogenesis in vivo and are partially protected from the deleterious effects of high fat feeding. Interestingly, unlike levels of SIRT1 and SIRT3, which generally increase during calorie restriction (CR), SIRT4 levels have been shown to decrease in response to fasting and CR [51,80]. This decrease of SIRT4 under conditions of nutrient scarcity appears to promote glutamine oxidation via the TCA cycle and also enhance fatty acid oxidation, allowing efficient use of available fuel sources when dietary intake is low [39,80].

7. Novel deacylase functions of SIRT5

The enzymatic role of SIRT5 in the mitochondria has until recently been largely mysterious. Like SIRT4 null mice, SIRT5 null mouse strains were shown to have unremarkable acetylation profiles, and SIRT5 was described as a weak deacetylase enzyme [48]. One of the first substrates reported to be deacetylated (and activated) by SIRT5 was carbamoyl phosphate synthetase (CPS1), an important enzyme of the urea cycle [81,82]. SIRT5 null mice display decreased activity of CPS1 and have reduced capacity to detoxify ammonia via the urea cycle and as such are less able to cope with situations of high amino acid catabolism such as CR or high-protein diets [81].

An exciting development in 2011 was the discovery of novel enzymatic activities for SIRT5, namely, lysine demalonylation and desuccinylation [5,6]. Using a combination of in vitro studies and experiments in SIRT5 KO tissues, it was shown that amongst all protein deacetylases, SIRT5 has the unique ability to remove malonyl and succinyl groups from lysine residues. The significance of these post-translational modifications is still unclear, however many metabolic enzymes, including IDH2, GDH, citrate synthase, CPS1 and HMGCS2 have been identified to be malonylated or succinylated [5,6,83]. Interestingly, many of these show overlap with deacetylase targets of SIRT3, and hence there may be a coordinated web of post-translational modifications of lysine residues at play in the mitochondria, tightly regulating the metabolism of nutrients and reflecting specific changes in the availability of succinyl-CoA, malonyl-CoA, and acetyl-CoA in the cell [14]. A detailed validation of the lysine modifications removed by SIRT5 will be necessary to fully appreciate the importance of this enzyme in mitochondrial metabolism and more broadly in other cellular processes.

8. Conclusions and future directions

It is now clear that dynamic changes in post-translational modifications of mitochondrial proteins can influence energy metabolism. Due to their NAD^+ dependency, the activity of mitochondrial sirtuins is intimately tuned to the metabolic and redox state inside mitochondria and numerous studies have reported that sirtuin-mediated alterations in specific acyl modifications allow for adjustments in mitochondrial metabolism and signalling in response to changes in nutrient flux. However, there are still many unresolved questions on the exact metabolic role of the mitochondrial sirtuins. In future studies it will be critical to define the tissue- and condition-specific roles of mitochondrial sirtuins, as most studies to date have relied on global knockout mouse models, which only manifest a significant metabolic phenotype under specific conditions. Furthermore, the large number of putative target proteins that have been identified in proteomic studies requires validation to determine the functional impact of sirtuin-regulated acyl modifications in these targets and their subsequent effect on substrate metabolism. Additional work is also required to define if the activity and abundance of mitochondrial sirtuins is regulated by diverse inputs and post-translational modifications, as is the case for SIRT1 [84,85]. Finally, it remains to be determined if enhancing mitochondrial sirtuin activity can have beneficial metabolic effects that are therapeutically relevant for treating metabolic diseases such as obesity and type 2 diabetes. These exciting areas of future research will no doubt provide new insight into the role of mitochondrial sirtuins in modulating energy metabolism.

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