Review

Special Issue: The evolving role of mitochondria in metabolism

Mitochondrial sirtuins: regulators of protein acylation and metabolism

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Sirtuins are NAD$^+$-dependent protein deacetylases and have been implicated in the regulation of metabolism, stress responses, and aging. Three sirtuins are located in mitochondria: SIRT3, 4, and 5. SIRT3 deacetylates and regulates the enzymatic activity of many metabolic enzymes in mitochondria, whereas SIRT5 removes two novel post-translational modifications, lysine malonylation and succinylation. Here, we review the current knowledge of how mitochondrial sirtuins function in metabolism and metabolic diseases, and offer a conceptual model how they may regulate mitochondrial function through distinct deacylation activities (deacetylation, demalonylation, or desuccinylation).

The mitochondrial sirtuins

Mitochondria are crucial intracellular organelles involved in energy production, metabolism, and intracellular signaling [1]. Mitochondrial number and/or activity change in response to a variety of physiological conditions such as nutrients, exercise, and change in temperature or oxygen levels, as well as during aging. Because they represent the main provider of cellular energy, altered mitochondria function can have a great impact upon the health of an organism [2]. Indeed, mitochondrial dysfunction has been linked to diseases including obesity, type 2 diabetes, and cancer, as well as normal aging [3,4]. However, how mitochondrial dysfunction contributes to disease pathogenesis is not fully understood.

Sirtuins, a family of NAD$^+$-dependent protein deacetylases, are molecular sensors of cellular energy balance, and regulators of metabolic responses to changes in nutritional availability in multiple tissues [5,6]. The canonical catalytic reaction removes an acetyl group from the lysine side chain of protein substrates, consumes NAD$^+$ as a cosubstrate, and generates nicotinamide (NAM) and 2′-*O*-acetyl-ADP-ribose [7]. Intracellular levels of NAD$^+$ and the ratio of NAD$^+$ to its reduced form NADH are sensitive to cellular oxygen metabolism and redox state, and this is probably one of the mechanisms by which the activity of sirtuins responds to cellular energy status [5]. Seven sirtuin members (SIRT1–7) are present in mammals. They have distinct subcellular localizations: SIRT1, 6, and 7 are found primarily in the nucleus, whereas SIRT2 is cytosolic, and SIRT3, 4 and 5 are mitochondrial [8].

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SIRT3 is the major deacetylase in mitochondria, and in its absence mitochondrial proteins become hyperacetylated [9,10]. By contrast, SIRT4 and SIRT5 only show weak deacetylase activity [11]. SIRT4 ADP-ribosylates glutamate dehydrogenase (GLUD1) [12], but it is not yet clear if this represents its general activity. SIRT5 has robust demalonylase and desuccinylase activities instead of deacetylase activity [13,14], and SIRT5 null mice show no difference in lysine acetylation levels but dramatic increases in lysine malonylation and succinylation [14]. Despite these biochemical differences, germline knockout of SIRT3, 4, or 5 does not produce gross phenotypes in young mice, under normal conditions [15]. Nonetheless, SIRT3 null mice have constitutive deficits in ATP production [9,16] and, when challenged with fasting or a high-fat diet (HFD), demonstrate phenotypes including cold-intolerance, reduced ketone-body production, and a propensity to develop the metabolic syndrome [17–19]. Similar constitutive and conditional phenotypes probably await discovery for SIRT4 and SIRT5 null mice.

The divergence of enzymatic activity among sirtuin family proteins may be explained by their phylogeny (Figure 1). Sirtuins can be categorized into five classes, I–IV and U, based on the conservation of a 250 amino acid core domain. SIRT1, 2, 3 are class I sirtuins, show high homology to yeast homologs (Sir2, Hst1, Hst2), and exhibit robust deacetylase activity, whereas weak deacetylases SIRT4 and SIRT5 belong to classes II and III, respectively [20]. The discovery of SIRT5's novel deacylase activities opens up the possibility that sirtuins in other classes with weak deacetylase activity, such as SIRT4, might possess other kinds of deacylase activity.

SIRT3: the *bona fide* mitochondrial deacetylase

At least 20% of mitochondrial proteins are acetylated in proteomic surveys [21,22], and the importance of acetylation is suggested by the high conservation of many sites, from *Drosophila* to humans [23]. Acetylation and deacetylation of target proteins has complex effects on activity, stability, or complex formation. In both prokaryotes and eukaryotes, reversible acetylation of metabolic enzymes appears to be crucial for the regulation of metabolic flux in response to different sources of metabolic fuel or diverse metabolic states [24–26].

SIRT3 is the major mitochondrial deacetylase. In mice lacking SIRT3, global levels of protein acetylation increase dramatically in a variety of tissues [10]. Hyperacetylation

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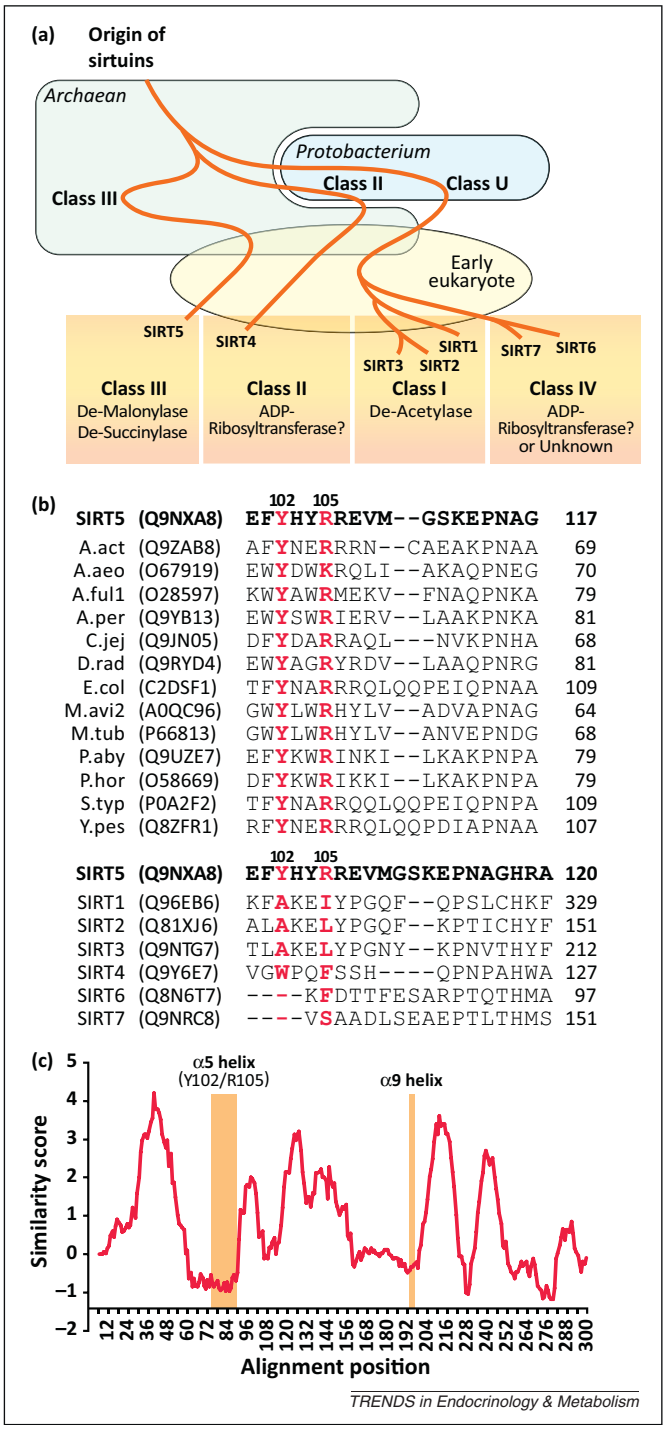

Figure 1. Evolution of sirtuins dictates their enzymatic activities. **(a)** Phylogenetic analysis divides sirtuins into five classes, class I, II, III, IV, and U. SIRT3, 4, and 5 belong to classes I, II, and III, respectively. Sirtuins within a specific class seem to possess unique deacylase activities, although the activity for some classes is currently unknown (adapted from Frye in *Histone Deacetylases*, edited by Eric Verdin [91]). In addition, it is postulated that α-proteobacterium became engulfed by an archaean cell to form the first eukaryotic cell. The engulfed α-proteobacterium contributed a class II sirtuin and a class U sirtuin and the archaean parent contributed the class III sirtuin. **(b)** Protein sequence alignments within class III sirtuins across species (top alignment) and between mammalian sirtuins of all classes (bottom alignment) reveal two highly conserved amino acids, Y102 and R105 that are present in all class III sirtuins but not in other classes (adapted from Figure S15 of Du *et al.* [13], and Figure 3 in Schuetz *et al.* [92]). Organism names are abbreviated and the UniProtKB entry numbers are indicated in parentheses. **(c)** Regions determining SIRT5 substrate specificity are poorly conserved among the three mitochondrial sirtuins. We aligned the core catalytic domains of human SIRT3, 4 and 5 using Clustal 2.1, then plotted a moving average of sequence similarity scores (BLOSUM62) in an 11 amino acid window. The SIRT5 α5 helix, containing residues Y102 and R105, is among the least conserved regions. The SIRT5 α9 helix, which interacts with the α5 helix, also lies in a poorly conserved region.

at specific lysine sites of metabolic enzymes such as long chain acyl-CoA dehydrogenase (ACADL) and isocitrate dehydrogenase 2 (IDH2) have been linked to various metabolic phenotypes in SIRT3 null mice [26]; SIRT3-mediated deacetylation of metabolic enzymes most often leads to their enzymatic activation [17,27,28]. In a few cases, such as cyclophilin D, SIRT3 deacetylation instead impedes function [29,30]. In addition, specific lysine sites may be targeted under different conditions. For example, four distinct sites on Mn superoxide dismutase (SOD2) are deacetylated by SIRT3 under specific conditions: K53 and K89 during calorie restriction [31], K122 in response to ionizing radiation stress [32], and K68 in response to increased ROS levels [33].

Acetylation levels are sensitive to metabolic states and dietary conditions. A HFD, fasting, and calorie restriction (CR) have all been linked to hyperacetylation of liver mitochondrial proteins [19,34,35]. The increase in global protein acetylation may be due to increased fatty acid (FA) oxidation and acetyl-CoA production under these conditions. However, although global acetylation increases under these conditions, the acetylation of specific, functionally important lysines may show a relative decrease due to SIRT3 deacetylation [17]. For instance, although global acetylation increases in the liver during CR [35], acetylation at K53/89 of SOD2 is decreased [31]. This deacetylation is absent in SIRT3 knockout mice, leading to deficient SOD2 activation [31], and suggesting that SIRT3 activity is crucial for the deacetylation of these two key sites and the activation of SOD2.

Regulation of SIRT3 expression and activity

Basal SIRT3 expression varies widely, and is highest in the most metabolically active tissues including liver, kidney, and heart [9,36]. Diet and nutrient availability dramatically affect SIRT3 levels in different tissues. SIRT3 expression in liver and adipose tissue is increased in glucose-poor, fasting states including CR [17,37–40]. Expression in skeletal muscle also increases in CR [16,39], but has been reported to both increase [36,37] and decrease [16] with fasting. Exercise increases SIRT3 expression in skeletal muscle in both mice [36,41,42] and humans [43,44]. Interestingly, although HFD initially induces SIRT3 expression in liver and skeletal muscle, chronic HFD feeding leads to a decrease [16,19,36,39,45]. SIRT3 expression also decreases in mouse models of type 1 or 2 diabetes mellitus [16,36].

Regulation of SIRT3 has been best studied at the level of transcription, where the peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) coactivates the transcription factor estrogen receptor-related (ERR) α bound to an ERR response element within the SIRT3 promoter [46,47]. Chronic HFD is associated with decreased PGC-1α and SIRT3 expression, and SIRT3 expression can be rescued in the liver after administration of an adenovirus overexpressing PGC-1α [19]. Other factors that influence SIRT3 gene expression include the liver X receptor (LXR), peroxisome proliferation-activated receptor α (PPARα),

angiotensin II, and the Jun and Fos oncogenes; however, the underlying mechanisms are poorly defined [37,48–50]. There are no reports yet of regulation of SIRT3 protein levels via post-transcriptional mechanisms.

Post-translational modifications are crucial regulators of SIRT1, and these include phosphorylation, sumoylation, methylation, and nitrosylation [51]. Several phosphoserine sites were identified on SIRT3 by mass spectrometry, but their significance remains unknown [52]. Surprisingly, two mass spectrometry surveys did not identify any acetylation sites [21,22]. Modification of cysteine by 4-HNE is observed in ethanol-treated mice [53] and a model of Friedreich's ataxia [54], and impairs SIRT3 activity.

SIRT3 regulates intermediary metabolism

Since acetyl-coenzyme A synthetase (ACSS2) was identified as a reversibly acetylated mitochondrial protein and the first SIRT3 target [27,55], more than a dozen SIRT3 deacetylation targets have been reported (Table 1). Many SIRT3 targets appear to play a role in mediating the switch to fasting metabolism, moving away from glucose as a source of energy and metabolic intermediates and towards increased use of lipids and amino acids (Figure 2).

SIRT3 and lipid metabolism

In liver and peripheral tissues, SIRT3 activity promotes the efficient utilization of lipids as a primary source of acetyl-CoA (Figure 2). SIRT3 deacetylates and activates ACADL, a key enzyme in the β-oxidation of FAs [17]. Mice lacking SIRT3 accumulate β-oxidation precursors and intermediates, including triglycerides and long-chain FAs. These mice also share other characteristics observed in human disorders of FA oxidation, including cold-intolerance and reduced basal ATP levels [17]. During fasting, many tissues rely on ketone bodies produced in the liver as an alternative source of energy to glucose. SIRT3 regulates ketone-body production by deacetylating and activating 3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2), the rate-limiting enzyme in ketone-body biosynthesis. Accordingly, mice lacking SIRT3 show reduced fasting serum levels of ketone bodies [18]. In addition to ketone bodies, acetate produced in the liver from acetyl-CoA is another soluble form of lipid-derived energy that can be distributed to extrahepatic tissues [56]. SIRT3 deacetylates and activates acetyl-CoA synthetase 2 (ACSS2), an enzyme present in extrahepatic tissues that is used to activate acetate into acetyl-CoA [27,55]. Therefore, SIRT3 facilitates the catabolism of FAs in the liver and the peripheral use of lipid-derived acetate and ketone bodies during fasting.

SIRT3 and nitrogen metabolism

Glycolysis yields tricarboxylic acid (TCA) cycle intermediates via the conversion of pyruvate into oxaloacetate. Under fasting conditions, amino acids provide an alternative source of carbon backbones for the TCA cycle [57]. SIRT3 accelerates amino acid catabolism and nitrogen waste disposal by deacetylating and activating the mitochondrial matrix enzyme glutamate dehydrogenase (GLUD1) [58]. Catabolism of most amino acids requires transfer of the α-amino moiety to α-ketoglutarate by an aminotransferase, forming glutamate. GLUD1 regenerates α-ketoglutarate from glutamate, and releases nitrogen to the urea cycle as ammonia [57]. SIRT3 accelerates the urea cycle by deacetylating and activating ornithine transcarbamoylase (OTC), the key mitochondrial enzyme in the urea cycle.

Table 1. Known substrates of mitochondrial sirtuins

Mitochondrial Sirtuins	Gene Symbol	Gene Name	Confirmed by *in vitro* deacetylase assay?	Function	Reference
SIRT3	PPID	Peptidylprolyl Isomerase D (Cyclophilin D)	No	Glycolysis	[30]
	ACADL	Acyl-CoA Dehydrogenase, Long Chain (LCAD)	Yes	Fatty Acid Oxidation	[17]
	HMGCS2	3-Hydroxy-3-Methylglutaryl-CoA Synthase 2, Mitochondrial	Yes	Ketone Body Synthesis	[18]
	ACSS2	Acyl-CoA Synthetase Short-Chain Family Member 2	Yes	Acetate Metabolism	[27,55]
	OTC	Ornithine Transcarbamoyltransferase	Yes	Urea Cycle	[38]
	GLUD1	Glutamate dehydrogenase 1 (GDH)	Yes	Amino Acid Catabolism	[58]
	NDUFA9	NADH Dehydrogenase (Ubiquinone) 1 α Subcomplex, 9, 39-kDa	No	Oxidative Phosphorylation	[9]
	SDHA	Succinate Dehydrogenase Complex, Subunit A, Flavoprotein	No		[65,66]
	ATP5E	F_1F_0-ATPase Subunit α	No		[45]
	IDH2	Isocitrate Dehydrogenase 2, Mitochondrial	Yes	TCA Cycle	[28]
	SOD2	Superoxide Dismutase 2, Mitochondrial (MnSOD)	Yes	ROS	[31–33]
	ALDH2	Aldehyde Dehydrogenase 2 Family, Mitochondrial	No	Ethanol Metabolism	[93]
	MRPL10	Mitochondrial Ribosomal Protein L10	Yes	Mitochondrial Protein Synthesis	[94]
	FOXO3	Forkhead Box O3	Yes	Transcriptional Activation	[95]
	STK11	Serine/Threonine Kinase 11 (LBK1)	Yes	Tumor Suppressor, AMPK Signaling	[48]
	HISTH3	Histone Cluster 3, H3 (Specific to H3K56-Ac)	No	DNA Damage Response	[96]
	XRCC6	X-ray Repair Complementing Defective Repair in Chinese Hamster Cells 6 (Ku70)	Yes	Apoptosis	[97]
SIRT4	GLUD1	Glutamate Dehydrogenase 1 (GDH)	Yes	Amino Acid Catabolism	[12,69]
SIRT5	CPS1	Carbamoyl Phosphate Synthetase 1	Yes	Urea Cycle	[13,72]

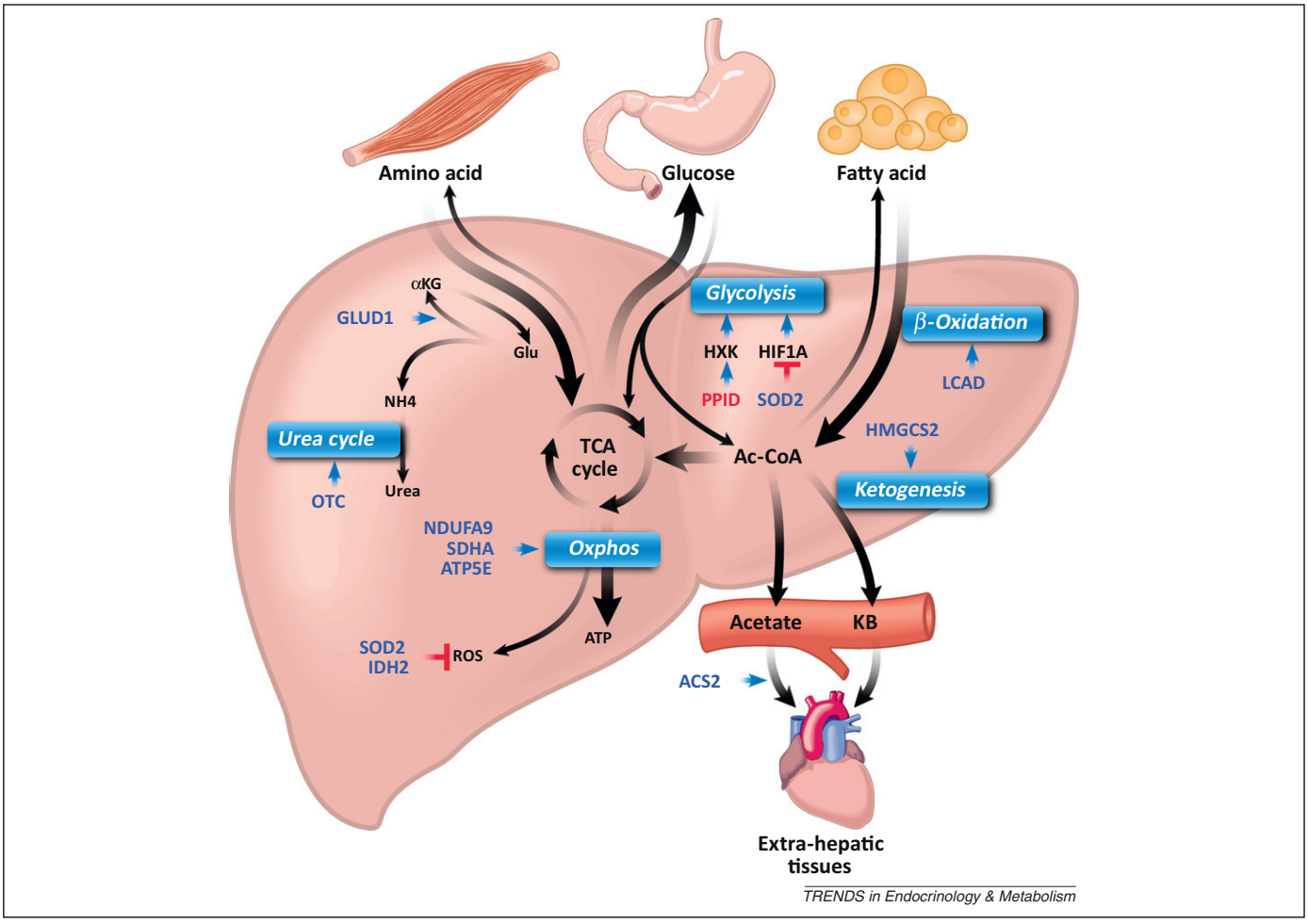

Figure 2. SIRT3 tunes the body to fasting metabolism. The SIRT3-mediated regulation of fasting metabolism is illustrated. In times of abundance, glucose supplies both acetyl-CoA to generate ATP, and carbon backbones to synthesize metabolic intermediates. In glucose-poor states such as fasting, ATP is instead generated predominantly from the β-oxidation of FAs derived from adipose tissue, and carbon backbones from the catabolism of amino acids from muscle. Acetyl-CoA is also converted to ketone bodies or, to a lesser extent acetate, which are then distributed to extrahepatic tissues through the bloodstream. SIRT3 regulates many of the enzymes involved in this switch to fasting metabolism. Proteins in blue, activated by SIRT3. In red, deactivated by SIRT3. More detailed descriptions of SIRT3 target genes are given in Table 1.

Mice lacking SIRT3 exhibit a metabolic profile similar to human disorders of the urea cycle, including increased serum ornithine and reduced citrulline levels – the substrate and product, respectively, of OTC [38].

SIRT3 and carbohydrate metabolism

SIRT3 regulates carbohydrate metabolism in cancer cells, one aspect of its emerging role in cancer biology [59]. The Warburg effect refers to the preference of cancer cells for glucose utilization as a source of energy [60]. Downregulation of SIRT3 can enhance the Warburg effect by two mechanisms [30,61,62]. First, SIRT3 regulates hypoxia-inducible factor 1 α (HIF1α), a transcription factor driving the expression of glycolytic genes, via cellular levels of reactive oxygen species (ROS). In the absence of SIRT3, ROS production increases (below) leading to deactivation of prolyl hydroxylases [63] and stabilization of HIF1α [62]. Second, hyperacetylation of the peptidyl-prolyl isomerase cyclophilin D in the absence of SIRT3 helps maintain hexokinase II (HK2) in an active state on the outer mitochondrial membrane, facilitating the rapid production of glucose-6-phosphate [30,64].

SIRT3 and mitochondrial respiration

SIRT3 facilitates mitochondrial oxidative phosphorylation (OXPHOS) by deacetylating and activating several components of the respiratory transport chain, including NDUFA9 (complex I) [9] and SDHA (complex II) [65,66]. Accordingly, mice lacking SIRT3 show reduced complex I and II activity in the liver and brown adipose tissue [65,66]. SIRT3 also regulates complex IV/V activity, probably via the deacetylation of ATP synthase [45]. Together, these effects on OXPHOS are probably responsible for the 10% reduced O_2 consumption and up to 50% reduced ATP production observed in mice lacking SIRT3 [9,16].

SIRT3 and ROS

SIRT3 also enhances the ability of mitochondria to cope with ROS that are generated as a by-product of OXPHOS. First, SIRT3 deacetylates and activates isocitrate dehydrogenase 2 (IDH2), an enzyme in the TCA cycle that helps to replenish the mitochondrial pool of NADPH [28]. NADPH is used by glutathione reductase to maintain glutathione in its reduced antioxidant form [57]. Normal mice develop age-related hearing loss due to ROS-induced

cochlear cell damage, a phenotype that is ameliorated by CR via SIRT3-dependent deacetylation of IDH2 leading to reduced ROS levels [28]. Second, SIRT3 deacetylates and activates the ROS-scavenging enzyme Mn superoxide dismutase (SOD2), thereby reducing oxidative damage in the liver [31–33].

SIRT3 in metabolic diseases and aging?

Several lines of evidence support a role for SIRT3 in metabolic disorders. Mice lacking SIRT3 fed a HFD show accelerated obesity, glucose intolerance, insulin resistance, hyperlipidemia, and steatohepatitis [19]. These effects are partly due to increased stearoryl-CoA desaturase 1 (SCD1) activity in the liver caused by increased saturated lipid levels. Deletion of SCD1 rescues both wild-type and SIRT3-knockout mice from HFD-induced metabolic disorders [19]. Furthermore, SIRT3-knockout mice show impaired insulin signaling in skeletal muscle due to increased oxidative stress; this leads to activated JNK and decreased insulin receptor substrate-1 (IRS-1) signaling following insulin receptor activation [16]. Although there are no known human monogenic diseases associated with loss of SIRT3 function, a SIRT3 single nucleotide polymorphism (SNP) that reduces SIRT3 catalytic activity by 20% is associated with increased risk of developing the metabolic syndrome in two independent human cohorts [19].

A major goal for future research is deciphering the tissue-specific roles of SIRT3 in regulating these complex metabolic phenotypes. For example, hyperglycemia can be caused by defects in pancreatic insulin secretion, regulation of gluconeogenesis in the liver, or uptake and utilization of glucose in muscle [67]. A key limitation of the current knockout models of SIRT3 is that their phenotypes represent the integration of SIRT3 function over space and time: they mask tissue-specific effects that could be additive or compensatory, and adult metabolic studies may be confounded by developmental changes or the emergence of secondary effects. Further, because increased SIRT3 expression is observed under CR, it will also be important to study the possible protective role of increased SIRT3 expression/activity. Tissue-specific or inducible SIRT3 mouse models can overcome these limitations, but the first such study illustrates the challenges that await: neither liver- nor muscle-specific SIRT3 knockout recapitulates the phenotypes of germline global knockout [68]. Despite the daunting complexity, understanding the tissue- and temporal-specific effects of SIRT3 could prove a boon for understanding the complex pathogenesis of metabolic disease in humans.

SIRT4: still a mysterious enzyme

SIRT4 is abundantly expressed in pancreatic β cells and is involved in the regulation of insulin secretion [12,69]; however, its precise enzymatic functions remain unclear. It displays no detectable NAD-dependent deacetylase activity [10,12,69] and may possess weak ADP-ribosyltransferase activity [12,69]; however, this activity is more than 1000-fold slower than that of a bacterial ADP-ribosyltransferase, casting doubt on its physiological significance [13]. SIRT4 suppresses GLUD1 activity via ADP-ribosylation [12], in contrast to SIRT3, which activates GLUD1 via deacetylation. SIRT4-mediated inhibition of GLUD1 reduces the generation of ATP from the catabolism of glutamate and glutamine, which is essential for the ability of β cells to secrete insulin in response to amino acids. Mice lacking SIRT4 show correspondingly increased amino acid-stimulated insulin secretion [12]. In addition, SIRT4 interacts with insulin degrading enzyme (IDE) and the ADP/ATP carrier proteins ANT2 and ANT3 [69], although the implications of these interactions remain unclear.

Little is known of the functions of SIRT4 outside pancreatic β cells, but there are more hints of an interesting contrast with SIRT3. In contrast to SIRT3, SIRT4 expression is reduced during CR and increased in mouse models of diabetes [35,70]. SIRT4 is a negative regulator of FA oxidation in liver and muscle. Knockdown of SIRT4 expression both *in vitro* and *in vivo* increases the expression of genes involved in FA oxidation and oxidative phosphorylation, including SIRT1, medium chain acyl-CoA dehydrogenase (MCAD), carnitine palmitoyltransferase 1 (CPT1), PGC-1α, cytochrome *c*, ATP synthase, and IDH2, thereby enhancing FA oxidation and mitochondrial respiration [70]. Interestingly, increased expression of SIRT1 in response to SIRT4 knockdown is required for the observed increase in FA oxidation [70]. Nevertheless, how SIRT4 enzymatic activity in the mitochondrion affects gene transcription in the nucleus is unknown. Identifying the true enzymatic activity of SIRT4 and its mitochondrial target will undoubtedly shed light on its function.

SIRT5: the new frontier of protein deacylation

SIRT5 was an enzymatic enigma until the recent finding that it possesses unique, potent demalonylase and desuccinylase activities [13,14]. Malonyl-lysine and succinyl-lysine modifications have been identified in a variety of organisms ranging from yeast, worms, flies, and mice to humans [14,71]. Mice lacking SIRT5 exhibit global protein hypermalonylation and hypersuccinylation, suggesting that SIRT5 is the major protein demalonylase and desuccinylase in mammals [14]. Interestingly, two amino acids – tyrosine (Y102) and arginine (R105), located within the catalytic pocket of SIRT5 – are required for the demalonylase and desuccinylase activities [13]. The positive charge of this arginine may explain the preference of SIRT5 for negatively charged acyl groups such as malonyl-lysine and succinyl-lysine. Strikingly, these two amino acids are specifically conserved across class III sirtuins from lower organisms to mammals [13], but are not conserved between different classes (Figure 1b,c), suggesting a structural basis for distinct enzymatic activities between sirtuin classes.

The biological significance of lysine malonylation and succinylation is currently unknown. Many of the malonylated or succinylated proteins identified so far are important metabolic enzymes, including IDH2, serine hydroxylmethyltransferase, glyceraldehyde 3-phosphate dehydrogenase, GLUD1, malate dehydrogenase 2, citrate synthase, carbamoyl phosphate synthetase 1 (CPS1), HMGCS2, thiosulfate sulfurtransferase, and aspartate aminotransferase [13,14,71]. How lysine malonylation and succinylation modulate the function of these enzymes has yet to be investigated. However, based on what we

have learned while studying protein acetylation, and given the larger size and negative charge associated with malonylation or succinylation, one can reasonably expect that these modifications and their regulation by SIRT5 will play a significant role in metabolic regulation.

In addition to these novel enzymatic activities, SIRT5 may also function as a protein deacetylase on the urea cycle enzyme CPS1, and thereby increase its activity [72]. SIRT5 has also been shown to regulate CPS1 activity through desuccinylation [13].

An orchestra of three

Of the seven mammalian sirtuins, SIRT3, 4 and 5 are located in mitochondria where they are not functionally redundant; instead, as described above, they exhibit distinct deacylase activities and their expression levels respond differently to changing metabolic states. As an orchestra of three, mitochondrial sirtuins may coordinate mitochondrial function through multiple regulatory layers of post-translational protein modifications in response to dynamic changes of nutrient availability and metabolic states.

Acetyl-CoA, malonyl-CoA and succinyl-CoA are important intracellular metabolites. They are present in both the mitochondrion and cytosol, and are variously derived from the catabolism of carbohydrates, FAs, or proteins (Figure 3). Acetyl-CoA is produced during the aerobic catabolism of carbohydrates and during β-oxidation of long-chain FAs [57]. Catabolism of some amino acids or decarboxylation of malonyl-CoA also produces acetyl-CoA. Acetyl-CoA can then feed into the TCA cycle for energy production or used for biosynthesis, such as in ketogenesis, during glucose-poor conditions [57]. Intra-mitochondrial concentrations of acetyl-CoA are in the millimolar range [73], a level that can initiate non-enzymatic acetylation reactions [74]. Importantly, global protein acetylation in mitochondria correlates with elevated production of acetyl-CoA in such varied states as fasting, CR, HFD, and ethanol intoxication [17,19,35,75,76].

Malonyl-CoA pools in the mitochondrion and cytosol are tightly regulated. Cytosolic malonyl-CoA is synthesized by carboxylation of acetyl-CoA by the enzyme acetyl-CoA carboxylase (ACC); the decarboxylation of malonyl-CoA by malonyl-CoA decarboxylase (MCD) regenerates acetyl-CoA [77]. The mitochondrial pool of malonyl-CoA, however, is generated by the activity of propionyl-CoA carboxylase (PCC) on acetyl-CoA, with the reverse reaction again catalyzed by MCD [77]. The activity of both ACC and MCD are regulated by many physiological factors including glucose, insulin, or AMP-activated protein kinase (AMPK), through allosteric or phosphorylation mechanisms [77]. Whole-cell malonyl-CoA levels decrease dramatically in fasting and diabetic conditions, but increase to twice the normal levels after feeding [77]. Malonyl-CoA is not only the precursor for *de novo* FA synthesis but is also an inhibitor of fatty acid oxidation. It binds to and inhibits CPT1 on the mitochondrial outer membrane, thereby inhibiting the transport of FAs into mitochondria for β-oxidation [77]. Consistently, pharmacological inhibition or genetic disruption of MCD activity leads to increased intracellular malonyl-CoA levels, decreased FA oxidation, and a switch to glucose oxidation [78,79]. In mammals, two isoforms of ACC are expressed, ACC1 and ACC2, with different tissue distribution and function: ACC1 is

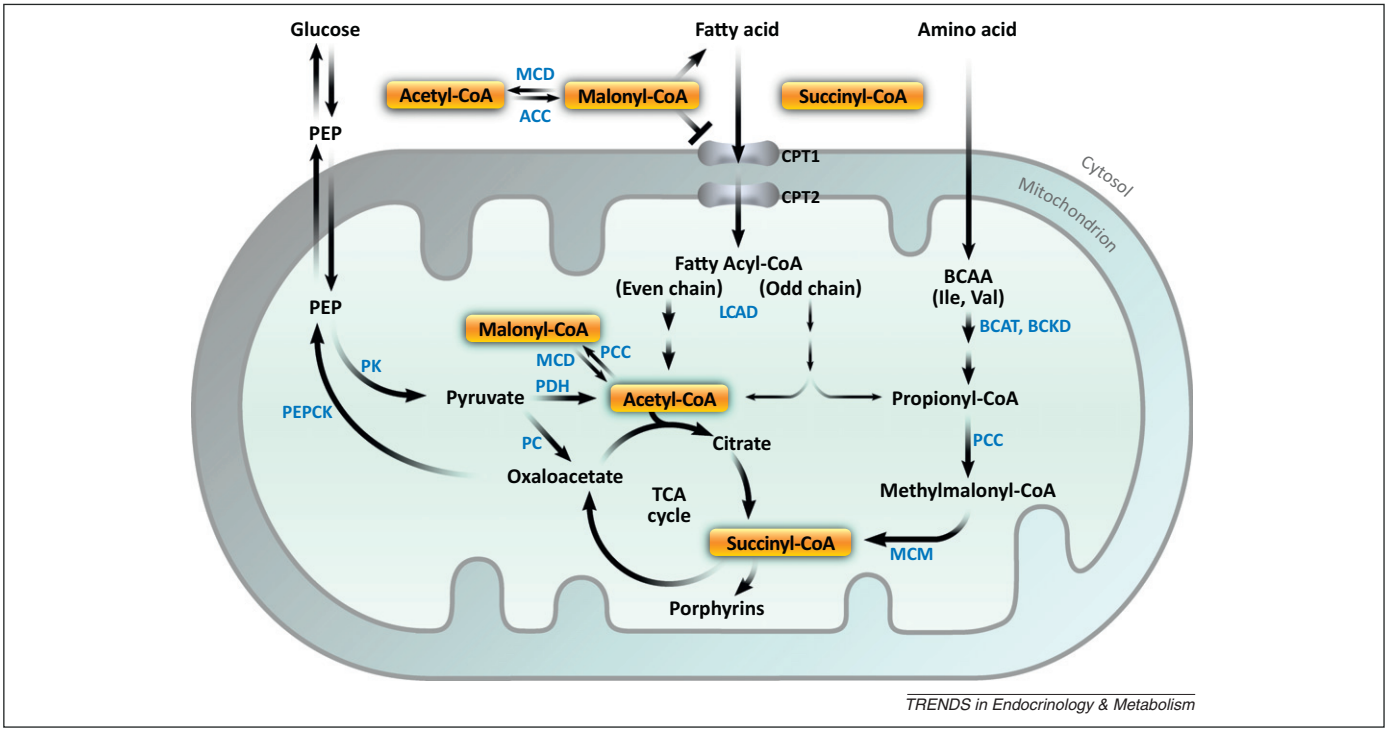

Figure 3. Simplified view of acetyl-CoA, malonyl-CoA, and succinyl-CoA metabolism. Pathways for the synthesis and catabolism of acetyl-CoA, malonyl-CoA, and succinyl-CoA suggest possible targets of feedback and feedforward regulation by mitochondrial sirtuins. For example, SIRT3 regulates both the synthesis of acetyl-CoA from FAs and its conversion to ketone bodies. ACC, acetyl-CoA carboxylase; BCAT, branched-chain aminotransferase; BCKD, branched-chain α-keto acid dehydrogenase; CPT1 and 2, carnitine palmitoyltransferases 1 and 2; LCAD, long-chain acyl-CoA dehydrogenase; MCD, malonyl-CoA decarboxylase; MCM, methylmalonyl-CoA mutase; PC, pyruvate carboxylase; PCC, propionyl-CoA carboxylase; PDH, pyruvate dehydrogenase; PEP, phosphoenolpyruvate; PEPCK, phosphoenolpyruvate carboxykinase.

enriched in lipogenic tissues where it produces malonyl-CoA in the cytosol for lipogenesis; ACC2 is preferentially expressed in oxidative tissues, where it negatively regulates FA oxidation [80]. ACC1 knockout in mice is embryonic lethal [81], whereas deletion of ACC2 leads to increased β-oxidation in both liver and muscle [82]. ACC2 knockout mice are lean, hyperphagic, and resistant to obesity and diet-induced diabetes [82,83]. ACC2 is suggested to inhibit CPT1 by creating a local high concentration of malonyl-CoA at the mitochondrial outer membrane [84]. However, whether ACC2 is located on the outer membrane of mitochondria or inside mitochondria has been difficult to demonstrate. Malonyl-CoA also regulates, directly or indirectly, physiological or pathological conditions such as muscle contraction, cardiac ischemia, β-cell secretion of insulin, and the hypothalamic control of appetite [77]. These findings notwithstanding, our understanding of the role of malonyl-CoA as a metabolic regulator is still very incomplete. With the discovery of malonylation as a post-translational protein modification, one intriguing question is whether protein malonylation represents one of the mechanisms by which malonyl-CoA levels regulate intermediary metabolism.

Succinyl-CoA is an intermediate in the TCA cycle and also a precursor for porphyrin synthesis [57]. Catabolism of odd-chain FAs, and of some branched-chain amino acids (BCAAs) such as isoleucine or valine, generates propionyl-CoA, which is first carboxylated to methylmalonyl-CoA and then converted to succinyl-CoA [57]. Odd-chain FAs are rare in the human diet [85], but BCAAs such as leucine, isoleucine and valine are the most abundant essential amino acids [86]. Catabolism of three BCAAs is initiated by a common enzyme, BCAA aminotransferase (BCAT), and followed by the rate-limiting, irreversible processing by branched-chain α-keto acid dehydrogenase complex (BCKD). Both enzymes are highly regulated by nutritional, hormonal, and pathological factors through allosteric- or phosphorylation-based mechanisms [86]. Strikingly, in humans >50% of the capacity of the tissues to catabolize BCAAs resides in skeletal muscle [87], a reflection of the role of skeletal muscle as a fuel reserve in starvation or other glucose-poor states [88,89]. We do not know yet whether succinyl-CoA levels and global protein succinylation correlate, as has been observed for acetyl-CoA levels and mitochondrial protein acetylation. In addition, BCAAs also play a number of regulatory roles in protein synthesis, insulin secretion, and amino acid uptake in the brain, although the underlying mechanisms are incompletely understood [87–89]. Whether protein succinylation is involved is currently unknown.

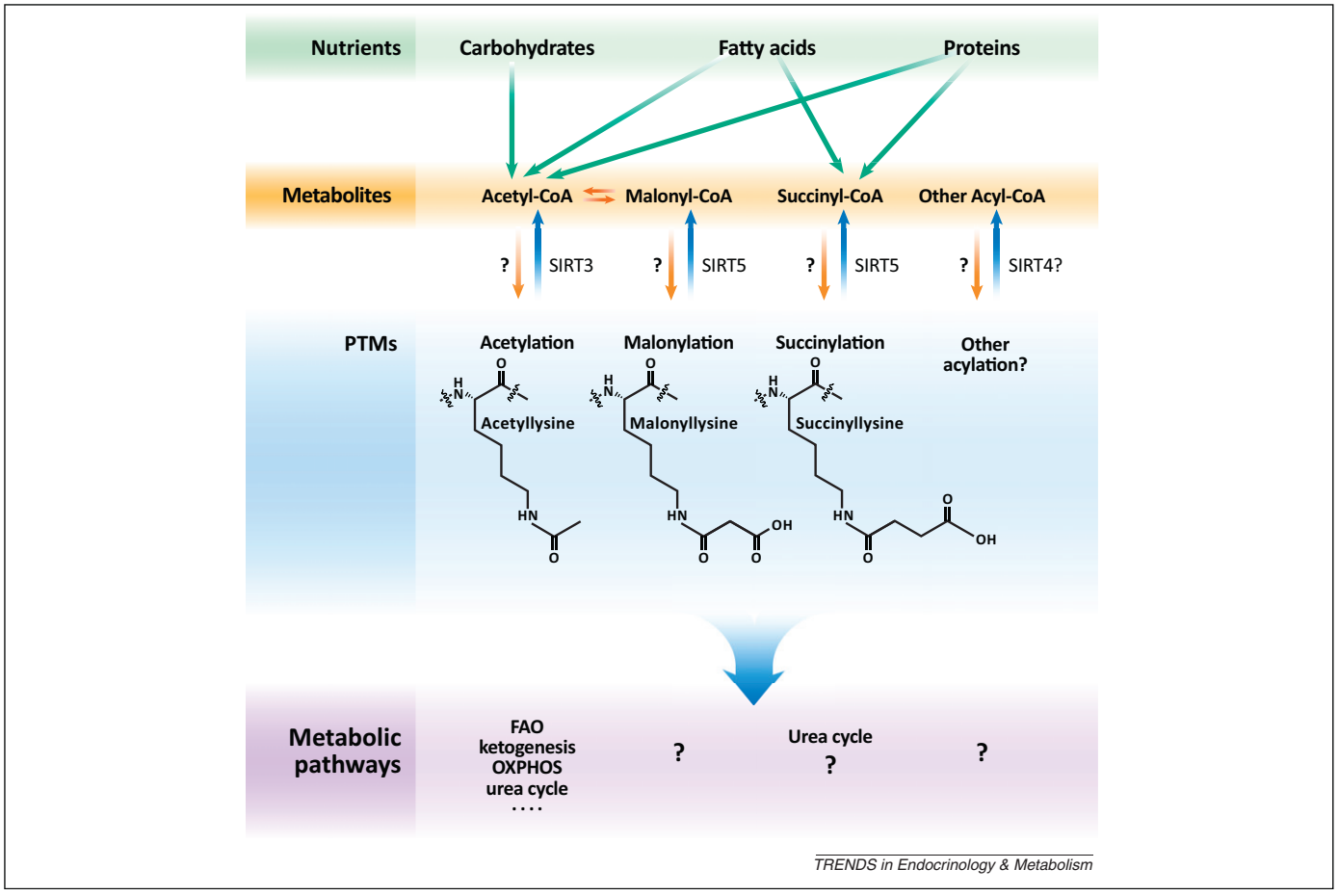

Figure 4. Protein acylation and removal by sirtuins are important regulators of metabolism. A model for reversible protein acylation in the control of metabolic flux is shown. A variety of acyl-CoAs can be generated from the catabolism of carbohydrates, FAs, or amino acids. The addition of acyl groups from acyl-CoA to lysine residues in mitochondrial proteins may be non-catalytic or proceed by as yet unknown acyltransferases. SIRT3 removes acetylated moieties, whereas SIRT5 removes malonylated and succinylated moieties. The extent of SIRT4 deacylation activity is currently unknown. The acetylation and deacetylation of many metabolic enzymes by SIRT3 has a significant impact on a broad range of metabolic pathways. So far, SIRT5 is only known to desuccinylate CPS1 and regulate the urea cycle, leaving much to be discovered. BCAAs, branched-chain amino acids; OCFAs, odd-chain fatty acids.

Acetylation of mitochondrial proteins has significant consequences. In the absence of SIRT3, hyperacetylation of ACADL and HMGCS2 during fasting disrupts the normal metabolic switch towards FA utilization [17,19]. Because many metabolic enzymes are also malonylated or succinylated [14,71], SIRT5-mediated demalonylation or desuccinylation of metabolic enzymes may modulate metabolic pathways in a similar fashion, under conditions of high malonyl-CoA or succinyl-CoA levels. SIRT5 is likely to emerge in the near future as an important regulator of intermediary metabolism.

Altogether, we propose a conceptual model for the coordinated regulation of metabolism by an orchestra of three mitochondrial sirtuins (Figure 4). When energy homeostasis or nutrient availability changes, levels of metabolites including acyl-CoAs will change correspondingly. Acyl-CoAs derived from different nutrient resources influence mitochondrial function by causing the acylation of metabolic enzymes. Through evolution, mitochondrial sirtuins could have evolved first as 'detoxifying enzymes' necessary to remove acetyl, malonyl and succinyl groups from mitochondrial proteins. Such a detoxifying mechanism might have further evolved into complex sensing and regulatory mechanism at a later point in evolution, as we have recently demonstrated for SIRT3. Protein acylation and its reversal by SIRT3, 4, or 5 then function as a crucial switch at the nodes of multiple metabolic pathways, to coordinate the network of metabolic fluxes in response to dynamic changes of metabolic states.

Concluding remarks

Our knowledge of the dynamic interactions between protein acetylation and energy metabolism has been revolutionized in the past decade. Accumulating evidence suggests that protein acetylation is sensitive to nutrient stimuli, and that SIRT3 plays important roles in modulating the enzymatic activity of crucial enzymes in metabolic pathways. Other acylation reactions, including the newly identified malonylation and succinylation reactions, may also occur during distinct metabolic states. Future studies will test the hypothesis that SIRT5 plays a similar but distinct role to SIRT3 in metabolic regulation. Other acylations derived from acyl-CoAs may also be regulatory, such as propionylation and butyrylation, and their corresponding propionyl-CoA and butyryl-CoA [90]. Whether diverse acyl-CoAs can serve as cell signaling molecules, and whether other deacylases exist, remain challenging and exciting questions to be explored.

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