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Mitochondrial sirtuins and metabolic homeostasis

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Abstract

The maintenance of metabolic homeostasis requires the well-orchestrated network of several pathways of glucose, lipid and amino acid metabolism. Mitochondria integrate these pathways and serve not only as the prime site of cellular energy harvesting but also as the producer of many key metabolic intermediates. The sirtuins are a family of NAD⁺-dependent enzymes, which have a crucial role in the cellular adaptation to metabolic stress. The mitochondrial sirtuins SIRT3, SIRT4 and SIRT5 together with the nuclear SIRT1 regulate several aspects of mitochondrial physiology by controlling posttranslational modifications of mitochondrial protein and transcription of mitochondrial genes. Here we discuss current knowledge how mitochondrial sirtuins and SIRT1 govern mitochondrial processes involved in different metabolic pathways.

Keywords

Deacetylase; Energy homeostasis; Metabolism; Mitochondria; Sirtuins

1. Introduction

Mitochondria are organelles composed of a matrix enclosed by a double (inner and outer) membrane (1). Major cellular functions, such as nutrient oxidation, nitrogen metabolism, and especially ATP production, take place in the mitochondria. ATP production occurs in a process referred to as oxidative phosphorylation (OXPHOS), which involves electron transport through a chain of protein complexes (I-IV), located in the inner mitochondrial membrane. These complexes carry electrons from electron donors (e.g. NADH) to electron acceptors (e.g. oxygen), generating a chemiosmotic gradient between the mitochondrial intermembrane space and matrix. The energy stored in this gradient is then used by ATP synthase to produce ATP (1). One well-known side effect of the OXPHOS process is the production of reactive oxygen species (ROS) that can generate oxidative damage in biological macromolecules (1). However, to neutralize the harmful effects of ROS, cells have several antioxidant enzymes, including superoxide dismutase, catalase, and peroxidases (1). The sirtuin silent information regulator 2 (Sir2), the founding member of the sirtuin protein family, was identified in 1984 (2). Sir2 was subsequently characterized as important in yeast replicative aging (3) and shown to possess NAD⁺-dependent histone deacetylase activity (4), suggesting it could play a role as an energy sensor. A family of

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conserved Sir2-related proteins was subsequently identified. Given their involvement in basic cellular processes and their potential contribution to the pathogenesis of several diseases (5), the sirtuins became a widely studied protein family.

In mammals the sirtuin family consists of seven proteins (SIRT1-SIRT7), which show different functions, structure, and localization. SIRT1 is mostly localized in the nucleus but, under specific physiological conditions, it shuttles to the cytosol (6). Similar to SIRT1, also SIRT6 (7) and SIRT7 (8) are localized in the nucleus. On the contrary, SIRT2 is mainly present in the cytosol and shuttles into the nucleus during G2/M cell cycle transition (9). Finally, SIRT3, SIRT4, and SIRT5, are mitochondrial proteins (10).

The main enzymatic activity catalyzed by the sirtuins is NAD⁺-dependent deacetylation, as known for the progenitor Sir2 (4,11). Along with histones also many transcription factors and enzymes were identified as targets for deacetylation by the sirtuins. Remarkably, mammalian sirtuins show additional interesting enzymatic activities. SIRT4 has an important ADP-ribosyltransferase activity (12), while SIRT6 can both deacetylate and ADP-ribosylate proteins (13,14). Moreover, SIRT5 was recently shown to demalonylate and desuccinylate proteins (15,16), in particular the urea cycle enzyme carbamoyl phosphate synthetase 1 (CPS1) (16). The (patho-)physiological context in which the seven mammalian sirtuins exert their functions, as well as their biochemical characteristics, are extensively discussed in the literature (17,18) and will not be addressed in this review; here we will focus on the emerging roles of the mitochondrial sirtuins, and their involvement in metabolism. Moreover, SIRT1 will be discussed as an important enzyme that indirectly affects mitochondrial physiology.

Sirtuins are regulated at different levels. Their subcellular localization, but also transcriptional regulation, post-translational modifications, and substrate availability, all impact on sirtuin activity. Moreover, nutrients and other molecules could affect directly or indirectly sirtuin activity. As sirtuins are NAD⁺-dependent enzymes, the availability of NAD⁺ is perhaps one of the most important mechanisms to regulate their activity. Changes in NAD⁺ levels occur as the result of modification in both its synthesis or consumption (19). Increase in NAD⁺ amounts during metabolic stress, as prolonged fasting or caloric restriction (CR) (20-22), is well documented and tightly connected with sirtuin activation (4,19). Furthermore, the depletion and or inhibition of poly-ADP-ribose polymerase (PARP) 1 (23) or cADP-ribose synthase 38 (24), two NAD⁺ consuming enzymes, increase SIRT1 action.

Analysis of the SIRT1 promoter region identified several transcription factors involved in up- or down-regulation of SIRT1 expression. FOXO1 (25), peroxisome proliferator-activated receptors (PPAR) α/β (26,27), and cAMP response element-binding (28) induce SIRT1 transcription, while PPAR γ (29), hypermethylated in cancer 1 (30), PARP2 (31), and carbohydrate response element-binding protein (28) repress SIRT1 transcription. Of note, SIRT1 is also under the negative control of miRNAs, like miR34a (32) and miR199a (33). Furthermore, the SIRT1 protein contains several phosphorylation sites that are targeted by several kinases (34,35), which may tag the SIRT1 protein so that it only exerts activity towards specific targets (36,37). The beneficial effects driven by the SIRT1 activation - discussed below- led the development of small molecules modulators of SIRT1. Of note, resveratrol, a natural plant polyphenol, was shown to increase SIRT1 activity (38), most likely indirectly (22,39,40), inducing lifespan in a range of species ranging from yeast (38) to high-fat diet fed mice (41). The beneficial effect of SIRT1 activation by resveratrol on lifespan, may involve enhanced mitochondrial function and metabolic control documented both in mice (42) and humans (43). Subsequently, several powerful synthetic SIRT1 agonists have been identified (e.g. SRT1720 (44)), which, analogously to resveratrol,

improve mitochondrial function and metabolic diseases (45). The precise mechanism of action of these compounds is still under debate; in fact, it may well be that part of their action is mediated by AMP-activated protein kinase (AMPK) activation (21,22,46), as resveratrol was shown to inhibit ATP synthesis by directly inhibiting ATP synthase in the mitochondrial respiratory chain (47), leading to an energy stress with subsequent activation of AMPK. However, at least in β -cells, resveratrol-mediated SIRT1 activation and AMPK activation seem to regulate glucose response in the opposite direction, pointing to the existence of alternative molecular targets (48).

Another hypothesis to explain the pleiotropic effects of resveratrol suggests it inhibits cAMP-degrading phosphodiesterase 4 (PDE4), resulting in the cAMP-dependent activation of exchange proteins activated by cyclic AMP (Epac1) (40). The consequent Epac1-mediated increase of intracellular Ca^{2+} levels may then activate of CamKK β -AMPK pathway (40), which ultimately will result in an increase in NAD^+ levels and SIRT1 activation (21). Interestingly, also PDE4 inhibitors reproduce some of the metabolic benefits of resveratrol representing yet another putative way to activate SIRT1.

The regulation of the activity of the mitochondrial sirtuins is at present poorly understood. SIRT3 expression is induced in white adipose (WAT) and brown adipose tissues upon CR (49), while it is down-regulated in the liver of high-fat fed mice (50). SIRT3 activity changes also in the muscle after fasting (51) and chronic contraction (52). All these processes are associated with increase (20,53) or decrease (50) in NAD^+ levels. From a transcriptional point of view, SIRT3 gene expression in brown adipocytes seems under the control of peroxisome proliferator-activated receptor gamma coactivator-1 α (PGC-1 α) - estrogen-related receptor α (ERR α) axis, and this effect is crucial for full brown adipocyte differentiation (54,55). SIRT4 expression is reported to be reduced during CR (12), while the impact of resveratrol on SIRT4 is still under debate (56). Finally, upon ethanol exposure, SIRT5 gene expression was shown to be decreased together with the NAD^+ levels (57), probably explaining the protein hyperacetylation caused by alcohol exposure (58).

2. Metabolic homeostasis

The maintenance of metabolic homeostasis is critical for the survival of all species to sustain body structure and function. Metabolic homeostasis is achieved through complicated interactions between metabolic pathways that govern glucose, lipid and amino acid metabolism. Mitochondria are organelles, which integrate these metabolic pathways by serving a physical site for the production and recycling of metabolic intermediates.

2.1 Glucose metabolism

2.1.1. Overview—Glucose homeostasis is regulated through various complex processes including hepatic glucose output, glucose uptake, glucose utilization and storage. The main hormones regulating glucose homeostasis are insulin and glucagon, and the balance between these hormones determines glucose homeostasis. Insulin promotes glucose uptake in peripheral tissues (muscle and WAT), glycolysis and storage of glucose as glycogen in the fed state, while glucagon stimulates hepatic glucose production during fasting. Sirtuins influence many aspects of glucose homeostasis in several tissues such as muscle, WAT, liver and pancreas.

2.1.2 Gluconeogenesis—The body's ability to synthesise glucose is vital in order to provide an uninterrupted supply of glucose to the brain and survive during starvation. Gluconeogenesis is a cytosolic process, in which glucose is formed from non-carbohydrate sources, such as amino acids, lactate, the glycerol portion of fats and tricarboxylic acid (59) cycle intermediates, during energy demand. This process, which occurs mainly in liver and

kidney, shares some enzymes with glycolysis but it employs phosphoenolpyruvate carboxykinase, fructose-1,6-bisphosphatase and glucose-6-phosphatase to control the flow of metabolites towards glucose production. These three enzymes are stimulated by glucagon, epinephrine and glucocorticoids, whereas their activity is suppressed by insulin.

The role of mitochondrial sirtuins in the control of gluconeogenesis is not well established. SIRT3 is suggested to induce fasting-dependent hepatic glucose production from amino acids by deacetylating and activating the mitochondrial conversion of glutamate into the TCA cycle intermediate α -ketoglutarate, via the enzyme glutamate dehydrogenase (GDH) (Fig. 1A) (60,61). As *SIRT3*^{-/-} mice do not display changes in GDH activity (62), the mechanism requires further clarification. In contrast to SIRT3, SIRT4 inhibits GDH via ADP-ribosylation under basal dietary conditions (Fig. 1A-B) (12). Conversely, SIRT4 activity is suppressed during CR resulting in activation of GDH, which fuels the TCA cycle and possibly also gluconeogenesis (12). Therefore, mitochondrial sirtuins may function to support gluconeogenesis during energy limitation, but further research is required to understand the exact roles of mitochondrial sirtuins in gluconeogenesis.

2.1.3 Glucose uptake and insulin signaling—The entry of glucose into the cell is mediated by a set of glucose transporters. Insulin reduces blood glucose levels by increasing the translocation of the insulin-sensitive glucose transporter (GLUT4) from intracellular storage sites to the plasma membrane through the activation of a pathway of insulin signalling molecules. The binding of insulin to its receptor results in the phosphorylation of insulin receptor substrate proteins (IRS) tyrosine residues. In contrast, serine phosphorylation of IRS proteins by kinases such as JNK blunts insulin signalling. The down-stream targets of IRS proteins are phosphatidylinositol 3-kinase/Akt/Akt substrate of 160 kDa pathway, which regulates GLUT4 translocation, and the Ras-mitogen activated protein kinase (MAPK/ERK) pathway, which mediates the effect of insulin on cell growth and differentiation cooperatively with phosphatidylinositol 3-kinase and Akt.

Of the mitochondrial sirtuins, SIRT3 is known to influence insulin sensitivity as *SIRT3*^{-/-} mice have impaired glucose clearance (63). The loss of *SIRT3* increased oxidative stress, enhanced JNK activity, decreased tyrosine, but increased serine phosphorylation of IRS-1 and reduced activation of Akt and ERK in myoblasts (63). Interestingly, a functional single nucleotide polymorphism of *SIRT3* has been shown to associate with metabolic disease in humans (50). The impact of overexpression or activation of SIRT3 on insulin signaling has, however, not been investigated. Therefore, SIRT3 may have a role in the regulation of insulin sensitivity but clearly more studies are needed to address this issue.

2.1.3. Glucose utilization—Glycolysis is the first step of glucose utilization in the fed state. It is a cytosolic process leading to the conversion of glucose to pyruvate. Three irreversible reactions catalyzed by hexokinase (or glucokinase), phosphofructokinase and pyruvate kinase are the major sites for the regulation of glycolysis and are under allosteric and hormonal (insulin and glucagon) control. During anaerobic conditions (e.g. in exercising muscle), pyruvate is converted to lactate in a reaction catalyzed by lactate dehydrogenase. In aerobic conditions, pyruvate is converted to acetyl-CoA by pyruvate dehydrogenase complex, the rate of which is the major indicator of glucose oxidation. Acetyl-CoA, generated via glycolysis or after the degradation of fatty acids (FFAs), is used in the mitochondrial OXPHOS to form ATP (see Introduction). However, not all energy liberated in the respiratory chain is coupled to ATP formation. Uncoupling proteins are mitochondrial inner membrane proteins that can dissipate the proton gradient before it can be used to provide the energy for OXPHOS.

SIRT3 plays, together with SIRT1, an important role in the control of glucose utilization. First, SIRT1 represses glycolysis by inducing PGC-1 α , which turns down the transcription of glycolytic genes (64). Second, SIRT1 deacetylates and reduces the activity of hypoxia-inducible factor α (HIF1 α) (65) that stimulates expression of several genes governing glycolysis (66) and indirectly inactivates pyruvate dehydrogenase (67). SIRT3 inhibits the ROS-mediated stabilization of HIF1 α by activating the antioxidant enzyme manganese superoxide dismutase (68). Moreover, SIRT3 activates GDH and isocitrate dehydrogenase 2 (IDH2) boosting NADH production and consequently, the antioxidant enzyme glutathione reductase (Fig. 1A) (69,70). In addition, SIRT3 deacetylates and diminishes the activity of cyclophilin D, thereby, promoting the dissociation of hexokinase II from the mitochondrial membrane and slowing down the entry of glucose into glycolysis (71).

Sirtuins are also critically involved in the regulation of the OXPHOS machinery. SIRT1 stimulates OXPHOS by activating PGC-1 α , which increases mitochondrial biogenesis and transcription of OXPHOS genes (reviewed in (72)). SIRT3 can physically interact with subunits of complex I (Ndufa9), (73) complex II (SDHA) (74,75), and complex III (Fig. 1A) (63). SIRT3 also regulates mitochondrial protein translation by deacetylating the mitochondrial ribosomal protein L10 (76). SIRT3 and SIRT5 also bind to ATP synthase (77) and cytochrome c (78), respectively, but the physiological relevance of these interactions is currently unclear.

Collectively, it appears that SIRT1 and SIRT3 support proper mitochondrial oxidation and ATP production under basal conditions. This notion is bolstered by the finding that SIRT3^{-/-} mice exhibit significant reduction in basal ATP levels in several tissues (79). As tumor cells have a high reliance on glycolysis, known as the Warburg effect, the development of a cancer treatment, which shifts metabolism from glycolysis to oxidative metabolism is of interest.

2.1.4 Insulin secretion—Glucose is transported into the pancreatic β -cell via the GLUT2 transporter, which after glycolysis and OXPHOS leads to the generation of ATP. The subsequent increase in ATP/ADP ratio increases intracellular free calcium, which subsequently triggers the release of insulin.

SIRT4 blunts amino-acid induced insulin secretion during normal glucose conditions by repressing the activity of GDH (Fig. 1B), as discussed in the section 2.1.2 (12,80). However, GDH is released from the SIRT4-mediated inhibition via an undefined mechanism during CR, thereby enhancing amino acid-induced insulin secretion (12,80). Accordingly, in SIRT4 deficient mice GDH activity is enhanced in islets, leading to the enhancement of glucose and amino acid-stimulated insulin secretion (12). Moreover, SIRT4 inhibits glucose-stimulated insulin secretion possibly by modifying cytoplasmic ATP concentrations as SIRT4 interacts with adenine nucleotide translocator 2, which facilitates the transfer of mitochondrially produced ATP into the cytosol and ADP into the mitochondrial matrix (Fig. 1B) (80). Finally, SIRT3 may activate GDH by promoting its deacetylation (Fig. 1A) (see section 2.2.1) (78), but there is no evidence currently supporting the role of SIRT3 in insulin secretion via the modification of GDH activity (12). Taken together, SIRT4 may modulate amino-acid induced insulin secretion according to the actual metabolic demands of the organism, whereas evidence for a role of SIRT3 in insulin secretion is currently lacking.

2.2 Lipid metabolism

2.2.1 Overview—Lipid metabolism is a complex process involving lipid synthesis, uptake, storage and utilization. Lipid metabolism is controlled by many nutrients, intermediary metabolites and hormones. Several studies have also highlighted the role of sirtuins in the metabolism of different lipid types such as bile acids, cholesterol, and FFAs. As fatty acid

synthesis and storage are mainly under the control of SIRT1 and they do not involve mitochondrial events, we focus here on lipid utilization through β -oxidation.

2.2.2 Lipid utilization—In response to energy demand such as during fasting and CR, FFAs are released from adipose tissue stores and transported to peripheral tissues, where they are taken up into cells via different fatty acid transporter proteins (81). After entering the cell, FFAs are converted to long chain acylcarnitines by carnitine palmitoyltransferase 1 and transported across the inner mitochondrial membrane where they are converted to long chain acyl-CoAs that are used for β -oxidation, ultimately yielding acetyl-CoA, which then fuels OXPHOS. Hormones, nutrients, metabolic intermediates, and various signal transduction pathways can control FFA transport and β -oxidation.

Skeletal muscle utilizes FFAs as fuel during prolonged exercise. The switch to oxidative metabolism, i.e. the induction of mitochondrial biogenesis, fatty acid oxidation (FAO), and cellular respiration, is controlled by the opposing actions of corepressors, such as nuclear receptor co-repressor 1 (NCoR1) (82), and coactivators, such as PGC-1 α (reviewed in (72,83)), which fine-tune the transcriptional activity of downstream targets controlling oxidative metabolism (84). A key event triggering oxidative metabolism is the activation of a cellular energy sensor, AMPK, by an increase in the AMP/ATP ratio during exercise or energy demand (85). Activated AMPK phosphorylates PGC-1 α directly (86) and induces SIRT1 indirectly by elevating cellular NAD⁺ levels (21). Nonetheless, the activation of SIRT1 may be independent of AMPK in skeletal muscle during caloric restriction (87,88), although this is not always the case (89). Once SIRT1 is activated by NAD⁺, it deacetylates and locks PGC-1 α in an active state (64), which together with diminished activity of NCoR1 (82) favors oxidative metabolism. Recent data have suggested that the nuclear abundance of the key PGC-1 α acetyltransferase, GCN5 (general control of amino-acid synthesis 5) is also reduced in response to exercise, which also attenuates PGC-1 α acetylation (90). However, further investigation is clearly needed to clarify the relationship between coactivators, such as GCN5 and PGC-1 α , and corepressors, such as NCoR1, in the control of oxidative metabolism.

The mitochondrial sirtuins, SIRT3 and SIRT4, have been suggested to modulate FAO in skeletal muscle. The knock-down of SIRT4 enhances FAO rate and oxygen consumption in myotubes (Fig. 1B) (91). SIRT3-dependent deacetylation and activation of long-chain acyl CoA dehydrogenase (LCAD), the key enzyme involved in the oxidation of long-chain substrates, alters FAO in muscle (Fig. 1B) (92). Furthermore, SIRT3 may enhance fatty acid utilization in response to exercise and lack of nutrients through AMPK and PGC-1 α , as phosphorylated AMPK and PGC-1 α mRNA levels are significantly reduced in SIRT3^{-/-} skeletal muscle (93). Moreover, SIRT3 and PGC-1 α protein expression are increased in rat muscles in response to chronic exercise (52,94). Thus, concurrent changes of SIRT3 and PGC-1 α expression in the muscle could indicate that SIRT3 may participate to the regulation of PGC-1 α or vice versa, as PGC-1 α regulates SIRT3 expression via ERR α in brown adipocytes (54); the mechanism(s) underlying these observations warrant future investigation.

FFAs are an important energy source in the liver during fasting, caloric restriction and high-fat feeding. Notably SIRT1 promotes mitochondrial FAO through activating PPAR α and its coactivator PGC-1 α (95), curbing the onset of diet-induced obesity (42,45). Accordingly, hepatic SIRT1^{-/-} mice develop hepatic steatosis (95-97). Treatment with resveratrol or SIRT1 agonists ameliorates steatosis in diet-induced and genetically obese mouse models (98-101), and reduces liver lipid content in obese humans (43), thereby supporting the notion that SIRT1 activation enhances lipid utilization.

Of the mitochondrial sirtuins, SIRT3 is closely involved in the regulation of hepatic FAO as SIRT3 deacetylates and activates LCAD (Fig. 1A) (92). Like *SIRT1*^{-/-} mice, *SIRT3*^{-/-} mice are susceptible to hepatic steatosis (92). In contrast, SIRT4 seems to be a negative regulator of FAO since *SIRT4* knock-down induces expression of genes involved in FAO and cellular respiration (Fig. 1B) (91). Collectively, these results argue that SIRT1 and SIRT3 activation and SIRT4 inhibition could stimulate fat oxidation and point to a potential role of these targets in the management of hepatic steatosis and steato-hepatitis.

2.2.3 Ketogenesis—Ketogenesis supports energy supply to extrahepatic tissues under fasting conditions, when liver mitochondria are overwhelmed by FAO. Acetoacetate, β -hydroxybutyrate, and acetone, referred to as ketone bodies, are produced in mitochondria from acetyl-CoA that is either generated from acetate, through the enzymes Acetyl-CoA Synthase1 (AceCS1) and AceCS2 (102,103), or from FAO. Acetyl-CoA is further converted into 3-hydroxy-3-methylglutaryl-CoA, by hydroxy-3-methylglutaryl-CoA synthase (HMGCS2), which is finally converted into the above-mentioned ketone bodies. SIRT3 deacetylates and activates both mitochondrial enzymes AceCS2 (104) and HMGCS2 (105) increasing the rate of ketogenesis upon fasting (Fig. 1A). Thus, the role of SIRT3 as a regulator of ketone bodies highlights again its important role in the adaptive response to fasting.

2.3. Urea metabolism

The urea cycle is a metabolic pathway by which excess nitrogen is incorporated into urea. In mammals the urea cycle takes primarily place in the liver and much less in the kidney (106), that is the main urea excretory organ. Ammonia production, and consequently urea cycle activity, increases during conditions that boost amino acid breakdown, such as CR and fasting (107). Since the urea cycle consists of five reactions two of which occur in mitochondria (107), is noteworthy that the three mitochondrial sirtuins may affect directly or indirectly urea cycle.

SIRT4 for example inhibits, through ADP-ribosylation, the mitochondrial enzyme GDH (Fig. 1B) (12), which converts glutamate to α -ketoglutarate and ammonia, thus attenuating ammonia production. Therefore, SIRT4 inhibition under CR (12) might contribute to the increase of ammonia production.

Also SIRT5, localized in the mitochondrial matrix, seems also to be involved in the control of urea cycle. Indeed, SIRT5 was reported to deacetylate -although the exact residues deacetylated were not established- and activate CPS1 (62), the enzyme catalyzing the first step of the urea cycle (Fig. 1B) (107). Consistently, *SIRT5*^{-/-} mice CPS1 activity is reduced compared to wild type mice under food starvation with a consequent accumulation of ammonia into the blood (62). Despite this report suggesting that SIRT5 may be a CPS1 deacetylase, SIRT5 was recently shown to primarily demalonylate and desuccinylate proteins, including CPS1 (16). Further studies are hence required to elucidate how SIRT5 affects two distinct post-translational modifications in a single protein. Finally, ornithine transcarbamoylase (OTC) is deacetylated and activated by SIRT3 (Fig. 1A) (108). OTC is the second enzyme involved in the mitochondrial steps of the urea cycle (107). Thus, in *SIRT3*^{-/-} mice the absence of SIRT3 hampers the stimulation of OTC in response to caloric restriction, causing alteration in the urea cycle metabolites levels. These observations reinforce the idea that sirtuins promote metabolic adaptations during stress, and suggest that sirtuin modulators could be valuable agents to improve these stress-inflicted abnormalities.

Summary

The recent discoveries in the biology of mitochondria have shed light on the metabolic regulatory roles of the sirtuin family. To maintain proper metabolic homeostasis, sirtuins sense cellular NAD⁺ levels, which reflect the nutritional status of the cells, and translate this information to adapt the activity of mitochondrial processes via posttranslational modifications and transcriptional regulation. SIRT1 and SIRT3 function to stimulate proper energy production via FAO and SIRT3 also protects from oxidative stress and ammonia accumulation during nutrient deprivation. SIRT4 seems to play role in the regulation of gluconeogenesis, insulin secretion and fatty acid utilization during times of energy limitation, while SIRT5 detoxifies excess ammonia that can accumulate during fasting. However, we are only at the beginning of our understanding of the roles of the mitochondrial sirtuins, SIRT3, SIRT4 and SIRT5 in complex metabolic processes. In the coming years, further research should identify and verify novel sirtuin targets in vivo and in vitro. We need also to elucidate the regulation and tissue-specific functions of these mitochondrial sirtuins, as well as to understand the potential crosstalk and synchrony between the different sirtuins in different subcellular compartments. Ultimately, the understanding of mitochondrial sirtuin functions may open new possibilities, not only for treatment of cancer and metabolic diseases characterized by mitochondrial dysfunction, but also for disease prevention and health maintenance.

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Research agenda

- Studies are needed to identify and validate more targets of mitochondrial sirtuins.
- The regulation of the activity of the mitochondrial sirtuin activity requires clarification.
- Tissue-specific functions of the mitochondrial sirtuins needs to be determined using somatic knock-out mice.
- Loss-of function approaches, should be complemented by gain-of-function studies.
- The synchronization of the action of mitochondrial and nuclear sirtuins needs attention.

Figure 1. Summary of mitochondrial sirtuins' role in mitochondrial pathways

Mitochondrial sirtuins influence glucose, lipid and amino acid metabolism through affecting several aspects of mitochondrial physiology. Ovals indicate mitochondrial sirtuins SIRT3 (green), SIRT4 (red), and SIRT5 (yellow), whereas cyan rectangles represent the sirtuin targets. Overall, mitochondrial sirtuin functions highlight their importance in promoting metabolic adaptations during stress. For details see in the text.