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Protein deacetylation by sirtuins: delineating a post-translational regulatory program responsive to nutrient and redox stressors

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Abstract

Lysine acetylation/deacetylation is increasingly being recognized as common post-translational modification that appears to be broadly operational throughout the cell. The functional roles of these modifications, outside of the nucleus, have not been extensively studied. Moreover, as acetyl-CoA donates the acetyl group for acetylation, nutrient availability and energetic status may be pivotal in this modification. Similarly, nutrient limitation is associated with the deacetylation reaction. This modification is orchestrated by a novel family of sirtuin deacetylases that function in a nutrient and redox dependent manner and targets non-histone protein deacetylation. In compartment-specific locations, candidate target proteins undergoing lysine-residue deacetylation are being identified. Through these investigations, the functional role of this post-translational modification is being delineated. We review the sirtuin family proteins, discuss their functional effects on target proteins, and postulate on potential biological programs and disease processes that may be modified by sirtuin-mediated deacetylation of target proteins.

Keywords

Sirtuins; Lysine acetylation/deacetylation; Post-translational modifications; NAD⁺; Biological functions

Introduction

Deacetylation as a post-translational modification (PTM) was initially identified (and is to date being predominantly investigated) in the modification of histones to silence gene transcription. This PTM is orchestrated by a family of histone deacetylase enzymes (HDACs). It is now recognized that non-histone protein acetylation and deacetylation are common PTMs across multiple subcellular compartments including the nucleus, mitochondria, endoplasmic reticulum, and cytoplasm. Protein acetylation and deacetylation occur on numerous amino acid residues with the most common being on lysine residues. Acetylation has also been demonstrated on serine and threonine residues [1]. A major family of enzymes that function to deacetylate lysine residues of proteins are the sirtuin enzymes. These appear to be both nutrient and redox-stress responsive and modulate multiple substrates within the cell. This review article will focus on the biology of mammalian sirtuins, including their phylogeny, regulation, targets, and putative pathophysiological functions within mammalian systems.

Phylogeny and regulatory control

Identification of sirtuin proteins

The founding member of these enzymes is yeast Sir2, which silences chromatin via deacetylation of histones [2]. Sir2 enzymes have been shown to mediate lifespan extension through diverse programs including: suppression of rDNA recombination; telomeric silencing; attenuation of the consequences of oxidative damage; by the activation of DAF-16/Foxo and via the modulation of endoplasmic reticulum stress responses [2-5]. Mammals have seven sirtuin enzymes designated as SIRT1 through SIRT7 that exhibit distinct tissue distributions and subcellular localizations. These tissue and cellular location spatial differences may contribute to distinct biological functions of the different family members [6]. The mammalian sirtuins are phylogenetically divided into four subclasses based on the homology of their 250 amino acid core domain [7]. SIRT1, 2, and 3 are classified as the subclass I enzymes, and these show closest homology to yeast Sir2 and furthermore exhibit the most robust deacetylase activity. SIRT4 and SIRT5 are assigned to subclasses II and III, respectively, and SIRT6 and SIRT7 are classified as subclass IV enzymes [7]. Unlike the well-established deacetylase function of SIRT1-SIRT3, SIRT4 and SIRT6 have been reported to possess ADP-ribosyl transferase activity. However, recent biochemical analysis rather suggests that this ADP-ribosylation is more likely a consequence of sirtuins' deacetylase activity [8]. In this review we predominantly focus on the deacetylation function of these enzymes and do not discuss ADP-ribosyltransferase functioning in great detail.

Activation of sirtuins

The biochemical activation of sirtuin activity is dependent on NAD^+ . It has now been established that sirtuin activation is directly linked to the energetic and redox status of the cell as measured by the ratio of $\text{NAD}^+:\text{NADH}$, by the absolute levels of NAD^+ , NADH , and by the NAD^+ catabolite nicotinamide [9-11]. Interestingly, nicotinamide itself inhibits sirtuin activity and nicotinamide-depletion during NAD^+ biosynthesis inversely activates sirtuins [12].

NAD^+ biosynthetic pathways differ in prokaryotes and invertebrates compared to vertebrates (reviewed [13]), although we will only focus on vertebrate biochemistry here. De novo biosynthesis using tryptophan and nicotinic acid as precursors is the minor pathway for NAD^+ generation. This pathway is induced by exercise and following administration of the peroxisome proliferator activated receptor alpha ($\text{PPAR}\alpha$) agonists [14, 15]. The major pathway to generate NAD^+ involves salvage of NAD^+ with nicotinamide as the precursor. In mammals, there are two intermediary steps in NAD^+ generation, initiated by the conversion of nicotinamide to nicotinamide mononucleotide (NMN) via the nicotinamide phosphoribosyltransferase (NAMPT) enzyme. Nicotinamide/nicotinic acid mononucleotide adenylyltransferase (NMNAT) then converts NMN to NAD^+ . NAMPT has been identified as the rate-controlling step in NAD^+ biosynthesis in that overexpression of Nampt (but not Nmnat) increased cellular NAD^+ levels [16]. Whether distinct subcellular pathways control NAD^+ biology is being investigated with the identification of a mitochondrial-enriched NMNAT isoform supporting the concept of subcellular compartment-specific NAD^+ biosynthesis [17]. In a more recent study, AMPK has been found to enhance SIRT1 activity by increasing cellular NAD^+ levels [18].

Resveratrol is a polyphenol found in grapes, red wine, and many other plants, has been found to activate Sir2 family deacetylases and mimic the anti-age associated disease effects of SIRT1 [19]. Furthermore, small molecular activators of SIRT1, SRT2183, SRT1720, and SRT1460 (which are structurally unrelated to but more potent than resveratrol) have been

discovered by high-throughput screen analysis [20]. These compounds have been shown to bind to the SIRT1 enzyme-peptide substrate complex at an allosteric site amino-terminal to the catalytic domain and lower the Michaelis constant for acetylated substrates [20]. These observations have recently been questioned by three independent studies showing that neither resveratrol nor these small-molecule compounds are direct activators for SIRT1 and do not stimulate activities of native substrates of SIRT1 including p53 and acetyl-CoA synthetase 1 (AceCS1) [21-23].

Molecular regulation of sirtuin expression

SIRT1 has been the most extensively investigated mammalian sirtuin and hence our understanding of the regulatory control of the family of deacetylases pertains predominantly to the regulation of SIRT1. The regulatory control points have been identified at the level of transcription, RNA stability, and by post-translational modifications. At the transcriptional level TLX, FOXO3a, p53, the cell cycle regulator E2F1, and c-myc upregulate SIRT1 expression [24-27]. In contrast, HIC1 functions as a transcriptional repressor [28, 29]. At the post-transcriptional level miR34a, miR132 and miR199a bind to the 3' UTR region of SIRT1 mRNA inhibiting SIRT1 protein expression [30-32]. In contrast, the RNA-binding protein HuR stabilizes SIRT1 mRNA upon binding to its 3' UTR region [33]. Post-translational modifications modulating SIRT1 activity include activation by sumoylation and conversely desumoylation by SENP1 inhibits its activity [34]. Similarly, SIRT1 was found to be activated when phosphorylation by cell cycle-dependent kinase cyclinB/ Cdk1 and to be inactivated following dephosphorylation [35]. Finally, protein interactions with SIRT1 have been shown to modulate enzyme activity. DBC1 (Deleted in breast cancer) negatively regulates SIRT1 via direct binding and inactivation [36, 37]. In contrast, AROS binds to and activates SIRT1 deacetylase activity [38].

Biological function of sirtuins

In studies performed spanning yeast through mice, the overarching effects of sirtuin activation encompass adaptive reprogramming of the cell, organ, or animal, to promote longevity, improved DNA repair, control of reactive oxygen species biology, the modulation in cell tolerance to genotoxic stressors, and in the modulation of energy metabolism. The functions linked to the distinct mammalian sirtuins are described below and again illustrate that the majority of investigations have focused on SIRT1.

SIRT1

SIRT1 and genomic stability

Several lines of studies show SIRT1 involvement in maintaining genomic integrity. The most definitive data are that SIRT1 null mouse embryonic fibroblasts (MEFs) exhibit impaired DNA damage response and decreased ability to repair DNA damage, whether induced by γ -irradiation (induced double-strand DNA breaks) and/or UV radiation (single-strand breaks). The defect in DNA damage repair correlates with decreased formation of a DNA damage sensor, γ H2AX foci, caused by inhibition of γ H2AX phosphorylation in the absence of SIRT1 [39]. Additionally, SIRT1 targets Nijmegen Breakage syndrome protein NBS1, a component of DNA damage sensor MRN complex [40, 41] and is recruited to DNA double-strand breaks to promote homologous recombination-mediated repair [42]. Furthermore, SIRT1 directly binds to and deacetylates Apurinic/apyrimidinic endonuclease-1 (APE1) to activate APE1 mediated base excision repair (BER) [43]. In addition to activating DNA repair programs, SIRT1 also promotes the maintenance of genomic stability through the modulation of histone H3 K56 [40]. Taken together, these data show that SIRT1 functions at multiple levels in both the maintenance of and repair of

genomic DNA, which mirrors its homology to the yeast Sir2, and parallels its function of maintaining DNA fidelity.

SIRT1 and circadian clock

The role of the circadian clock in biological homeostasis is increasingly being recognized with an important role in controlling circadian metabolism. In this regard, the NAMPT-mediated NAD^+ salvage pathway as a novel circadian feedback loop and intracellular NAD^+ levels are implicated in the regulation of the core clock machinery [44, 45]. An additional finding that would support a role of the sirtuins in modulating circadian rhythm is that the central clock protein CLOCK has histone acetyl transferase (HAT) activities and acetylates its dimerization partner BMAL1 and histone H3. In turn, the CLOCK–BMAL1 complex activates Period (Per) and Cryptochrome (Cry) genes, leading to subsequent repression of CLOCK–BMAL1 by PER and CRY proteins. SIRT1 binds CLOCK and is recruited to the CLOCK:BMAL1 chromatin complex at circadian promoters where it deacetylates BMAL1 and H3 in a circadian manner. SIRT1 then deacetylates PER2 and promotes PER2 degradation [46, 47]. Inhibition of NAMPT releases the suppression of CLOCK:BMAL1 by SIRT1, in turn CLOCK binds to and up-regulates Nampt gene expression, thus completing a feedback loop involving NAMPT/ NAD^+ and SIRT1/CLOCK–BMAL1 [44].

SIRT1 and metabolism

As SIRT1 activation is nutrient-sensitive and as SIRT1 functions to control the circadian clock, a role for this deacetylase enzyme in broader metabolic control would be quite intuitive. This is indeed operational, as SIRT1 has been shown to modulate both lipid and glucose metabolism.

The role of SIRT1 in regulating lipid metabolism is quite extensive and the mechanisms delineated to date are described here. At the gene regulatory control level, SIRT1 regulates in vivo lipid metabolism through the deacetylation of the nuclear receptor LXR. Deacetylation of a lysine residue on LXR enables its subsequent ubiquitination, resulting in increased LXR transcription activity [48]. Murine SIRT1 depletion accordingly shows diminished expression of LXR target genes involved in lipid metabolism and impaired homeostasis of cholesterol and triglycerides [48]. Additionally, the conditional knockdown of SIRT1 in the liver promotes accumulation of hepatic free fatty acids and cholesterol under fasting conditions [49]. This appears to function in part by the inhibition of PGC-1 α , a master hepatic co-activator of metabolic pathways, via its hyperacetylation. PGC-1 α is a known substrate of SIRT1 where SIRT1 deacetylation of this transcriptional coactivator increases its activity [25, 50]. In skeletal muscle, SIRT1 increases fatty-acid oxidation and leads to a metabolic switch from glucose oxidation to fatty acid oxidation under low nutrient conditions [50]. In this same vein, in response to high-fat feeding, the absence of hepatic SIRT1 results in the development of hepatic steatosis, inflammation, and ER stress [51]. This study identified that SIRT1 mediates deacetylation and activation of another transcription factor, namely PPAR α to increase fatty-acid β -oxidation [51]. In contrast to the activation of PPAR α in the liver, SIRT1 represses PPAR γ to promote fat mobilization from white adipocytes [52]. The nuclear bile acid receptor FXR is additionally a target for SIRT1 deacetylation [53]. FXR acetylation is elevated in ob/ob mice and mice fed with a high-fat diet. Hepatic downregulation of SIRT1 increased FXR acetylation and impaired liver lipid metabolism [53]. SIRT1 can additionally modulate liver lipid metabolism via the modulation of signaling pathways as shown with the activation of AMPK kinase LKB1 [54].

In parallel with regulation of lipid metabolism, SIRT1 mediates gluconeogenesis through the modulation of similar and distinct regulatory proteins. This is evident as genes encoding for gluconeogenesis enzymes and hepatic glucose production are increased via SIRT1-mediated

deacetylation of PGC-1 α and FOXO1 [55, 56]. Additionally, deacetylation of STAT3 by SIRT1 inhibits its phosphorylation and releases STAT3-mediated suppression of gluconeogenesis [57]. The exquisite regulation of this system is further evident in that during the later stage of fasting when glucose output is diminished, the deacetylation of CRTC2 by SIRT1 leads to its degradation and the inhibition of subsequent hepatic glucose output [58]. Consistent with the role of SIRT1 in gluconeogenesis, the hepatic knockdown in SIRT1 exhibits lower fasting glucose levels and diminished hepatic gluconeogenesis in parallel to increased acetylation of STAT3, FOXO1, and PGC-1 α [49, 55]. However, global SIRT1 transgenic mouse models similarly show decreased hepatic glucose production and fasting glucose level [59, 60]. This discrepancy is probably due to extrahepatic perturbations and interaction of various tissues and organs in the maintenance of glucose homeostasis [61].

Consistent with the ameliorative effects of nutrient restriction-mediated induction of Sir2, SIRT1 is required for the ‘classical’ response to calorie restriction (CR) with the maintenance of energy homeostasis [62]. In this nutrient-limited environment, AMPK enhances SIRT1 activity by increasing the cellular NAD⁺ level, resulting in the deacetylation and modulation of cognate substrates including PGC-1 α , FOXO1, and FOXO3a to regulate energy metabolism in mouse skeletal muscle [18]. In an apparent perpetuating cycle, the deacetylation of AMPK kinase LKB1 by SIRT1 concordantly increase AMPK activity [54, 63]. Interestingly, and also illuminating, another level of regulation has been shown under energy-restrictive conditions where SIRT1 silences rRNA transcription to modulate cellular energy homeostasis [64].

In light of this spectrum of metabolic controls governed by SIRT1, the role of this deacetylase would be expected to be operational in metabolic disease models. In cell culture studies, SIRT1 levels are downregulated during the induction of insulin resistance and are similarly upregulated by insulin sensitization therapy [65]. In vivo, the global SIRT1 transgenic mice exhibit resistance to high-fat-diet-induced insulin resistance and diabetes and can rescue the metabolic profile in db/db mice [59]. Similarly, SIRT1 overexpression protects against lipid-induced inflammation, and against high-fat-diet-induced hepatic steatosis [66]. The latter beneficial effects are due to activation of the candidate regulatory proteins already described in addition to the dampening of inflammatory cytokines production via the inhibition of NF- κ B [66]. These anti-inflammatory effects are similarly evident in adipocytes and macrophages [67, 68]. The administration of questionable SIRT1 activator SRT1720 evokes a similar profile with the enhancement of murine physical endurance and protection against high-fat-diet-induced obesity and insulin resistance [69]. Furthermore, in obesity and insulin-resistant mice, SRT1720 treatment reduces liver lipid accumulation via the reduction in hepatic lipogenic gene expression including SREBP-1c, ACC, and FAS, as well as via decreasing oxidative stress and inflammation [70]. A recent study, however, disputes this observation by being unable to demonstrate that SRT1720 can reduce plasma glucose level or improve mitochondrial capacity in mice fed with a high-fat diet [71]. An additional mechanism of SIRT1-mediated enhancement in insulin sensitivity is evident via the transcriptional repression of a member of the protein tyrosine phosphatase (PTP) family PTP1B [72]. In adipocytes, SIRT1 inhibits anti-inflammatory effects and improves insulin sensitivity [67, 68].

SIRT1 and neuronal function

The role of SIRT1 in modulating genomic stability and metabolism would implicate that this regulatory protein may be operational in the pathophysiology of degenerative diseases and under pathologic conditions that require adaptive metabolic remodeling. As degenerative diseases in the brain have robust phenotypic consequences, the role of SIRT1 in modulating degenerative processes has predominantly been investigated in this context. In established

neurodegenerative diseases, SIRT1 expression is dramatically decreased in a Huntington's diseases model [73] and the depletion of SIRT1 closely associates with the accumulation of amyloid-beta and tau in the cerebral cortex of Alzheimer's disease (AD) patients [74]. In a mouse model for AD, 'activation' of SIRT1 by resveratrol reduces neurodegeneration, prevents learning impairment, and decreases acetylation of SIRT1 targets PGC1a and p53 [75]. Furthermore, introduction of SIRT1 into AD mice confers protection against neurodegeneration. SIRT1 overexpression and resveratrol replicate these ameliorative effects in cell-based models of AD and amyotrophic lateral sclerosis [75]. SIRT1 additionally inhibits NF- κ B signaling pathway to protect amyloid- β -induced toxicity in microglia cells [76] and the activation of (or overexpression of) SIRT1 similarly protect through ROCK1 signaling pathway both in vitro and in vivo [77]. The potential protective role of SIRT1 against additional neurodegenerative conditions is evident where further suggested where the putative activators SRT647 and SRT501 show protective effects against optic neuritis [78].

Despite the beneficial effects of SIRT1 in neurodegenerative diseases, its role in neuronal oxidative stress pathology appears to have neutral or adverse effects. In neuron-specific SIRT1 transgenic mice, exposure to ischemia and MPTP-induced neuronal damage was not improved [79]. Interestingly, in these mice, the excess SIRT1 levels were associated with reference memory deficits [79]. In contrast, inhibition of SIRT1 increases acetylation and decreases phosphorylation of IRS-2 and reduced Ras/ERK1/2 pathway. This inhibition of SIRT1 and Ras/ERK1/2 pathway shows resistance to oxidative damage [80]. Additionally, under oxidative stress and inflammatory conditions, SIRT1 promotes differentiation of neuron progenitor cells towards the astrocytes lineage through its inhibition of Mash1 transcription [81].

SIRT1 and cancer

The effects of SIRT1 on tumorigenesis is not uniform but is actively being investigated as multiple mediators of cell survival and apoptosis are known substrates of SIRT1 deacetylation. SIRT1 mediated pro-survival programs including the inactivation of p53 [28, 82, 83], via Ku70-mediated sequestration or the mitochondrial proapoptotic factor Bax in the cytosol [84], by inhibiting the transcriptional activity and apoptotic functioning of the cell cycle apoptosis regulators [26] and by Smad7 deacetylation with subsequent degradation with the resulting inhibition of TGF- β -induced apoptosis [85]. SIRT1 increases cellular stress resistance through its interaction with the FOXO family to inhibit FOXO-dependent apoptosis [86, 87]. Consistent with these mechanisms of action, SIRT1 is highly expressed in several tumors including prostate and gastric carcinoma and lymphoma [88-91] and is implicated in the migration of ovarian and breast cancer cells [92]. In contrast, in several cell lines, SIRT1 was shown to suppress cell growth [93, 94] and many human cancers such as breast cancer and hepatic carcinoma display reduced levels of SIRT1 [95]. SIRT1 is also shown to augment TNF- α -induced apoptosis through its interaction with RelA/p65 subunit of NF- κ B [96]. In mice, overexpression of SIRT1 significantly reduces colon cancer formation, proliferation, and animal morbidity by interaction with β -catenin [97]. Furthermore, SIRT1^{+/-} p53^{+/-} mice develop tumors in multiple tissues and the activation of SIRT1 by resveratrol reduces tumorigenesis [95]. SIRT1 has also shown an inhibitory effect on BRCA1-associated breast cancer, which is caused by mutations of tumor suppressor genes BRCA1 [39, 95]. Taken together, these data divergently implicate SIRT1 as an oncogene and/or as a tumor suppressor. These paradoxical observations suggest that a more detailed understanding of SIRT1 is required prior to the therapeutic modulation of SIRT1 for cancer therapy.

SIRT1 and cardiovascular function

As SIRT1 is expressed in the heart, its role in modulating cardiac function has begun to be explored in the context of the cardiac response to biomechanical and redox stressors. SIRT1 deacetylates a histone variant H2A.z leading to its degradation thereby opposing H2A.z-mediated cardiac hypertrophy [98]. In a similar vein, the protective effect of fructose feeding against pressure overload-mediated cardiac hypertrophy in mice is associated with the induction of SIRT1 expression in the heart [99]. In parallel with its anti-apoptotic effects in the brain, inhibition of endogenous SIRT1 in cardiomyocytes enables p53-dependent apoptosis and SIRT1 overexpression protects cardiomyocytes from serum starvation induced cell death [100]. A role of SIRT1 in redox stress tolerance is also evident in that the modest induction of SIRT1 in the heart in transgenic mice prevents aging-associated cardiac dysfunction and protects against paraquat-induced oxidative stress [101]. This ameliorative effect is 'gene-dose dependent' as a very high copy number of the SIRT1 transgene induces cardiomyopathy and increases oxidative stress [101].

The role of SIRT1 in the cardiovascular system extends beyond the heart as it has been found to modulate vascular homeostasis [102]. Knockdown of SIRT1 in endothelial cells blocks sprout formation, migration, and the assembly of primitive vascular networks, in part via the activation of FOXO1, a negative regulator of angiogenic activity [102-104]. SIRT1 additionally modulates vascular reactivity via the deacetylation and stimulation of eNOS activity [105]. The beneficial effects on endothelial function are also shown by resistance to high-fat-diet-induced endothelial dysfunction and decreased atherosclerosis in apolipoprotein E-deficient mice in mice with enrichment of endothelial SIRT1 expression [106]. Treatment with SIRT1 activator resveratrol or overexpression of SIRT1 in vascular smooth-muscle cells decreases angiotensin II type I receptor (ATIR) expression and resveratrol administration attenuates angiotensin II-induced hypertension [107]. Additional potential adaptive effects of SIRT1 on the vasculature include the diminution of oxidative stress and the induction of mitochondrial biogenesis [108, 109].

The diversity of functions of SIRT1 in cellular function and in organ distinct pathways are schematized and summarized in Fig. 1.

SIRT2

Among the seven mammalian sirtuins, SIRT2 is the only isoform predominantly localized in cytoplasm, although it does transiently reside in the nucleus during the phases of mitosis [110]. In the cytosol, SIRT2 co-localizes with microtubules and specifically deacetylates lysine 40 of α -tubulin. Interestingly, HDAC6 is also found in this complex with SIRT2 and microtubules. However, our understanding of the mechanisms of the interplay between these different deacetylases on tubulin function is not fully elucidated [111, 112].

In contrast to the cytoprotective effects of SIRT1, the overexpression of SIRT2 promotes neurodegeneration [113] and diminishes resilience to oxidative stress in cardiac-derived cells [114]. Interestingly, a potent SIRT2 inhibitor rescues α -synuclein-mediated toxicity in a cellular model of Parkinson's disease [115] and SIRT2 knockdown is protective in neuronal and cardiac cells [114, 115]. One important mechanism for promotion of neuron degeneration by SIRT2 is attributed to its deacetylation of tubulin to impair microtubule stability in neurons [113, 115]. In addition, SIRT2 is also found to inhibit the myelin-producing glial cells oligodendrocytes differentiation through its deacetylation of α -tubulin [116]. In the cardiac-derived cells, SIRT2 downregulated the cytosolic chaperone 14-3-3Z thereby diminishing resilience to redox stress [114].

Additionally, SIRT2 is an important cell-cycle regulator by controlling mitotic exit and checkpoint transitions by blocking the entry to chromosome condensation and subsequent hyperploid cell formation in response to microtubule inhibitors [117, 118]. The mechanisms whereby SIRT2 regulates the cell cycle may include its control of tubulin deacetylation and/or via the deacetylation of lysine16 on histone 4 (H4K16) during G2/M transition and mitosis [119].

In addition, SIRT2 is also shown to interact with several transcription factors. In 3T3-L1 adipocytes SIRT2 deacetylates FOXO1 to reduce insulin-stimulated phosphorylation of FOXO1 [120] and increase FOXO1's binding to PPAR γ [121], resulting in the inhibition of adipocytes differentiation due to nuclear localization of FOXO1 and repression on PPAR γ transcriptional activity [120, 121]. SIRT2 similarly deacetylates FOXO3 with the subsequent induction of its target genes including p27kip1, MnSOD, and Bim [122]. Additional regulation of transcription factors includes interaction with HOXA10, a sequence-specific DNA-binding transcription factor important for developmental regulation [123], and the regulation of p53 activity at the posttranslational level by deacetylation and at the transcriptional level through its interaction with 14-3-3 β/α [124].

In recent years, the regulation of SIRT2 itself has begun to be explored and shows that SIRT2 activity is modulated by phosphorylation and acetylation. SIRT2 is phosphorylated on serine residues 368 and 372 and their substitution with alanine's diminishes SIRT2 activity [125]. Cyclin-dependent kinase 1 (CDK1) phosphorylation of serine 368 is required for the SIRT2-mediated delay in cell cycle progression [110]. In contrast, the phosphorylation of SIRT2 at serine 331 by cyclin E-Cdk2, cyclin A-Cdk2, and p35-Cdk5 inhibit its catalytic activity and this modification inhibits the cell adhesion and migration functions of SIRT2 [126]. Finally, interaction between SIRT2 and the acetyl-transferase p300 acetylates and inactivates SIRT2 [127]. In a counter-regulatory fashion, SIRT2 deacetylates lysine residues in the catalytic domain of p300 resulting in increased p300 recruitment to an integrated VP16-responsive gene to enhance transcription [128].

Mitochondrial sirtuins-SIRT3, 4, and 5

Proteomic analysis recently demonstrated that approximately 20% of mitochondrial proteins exhibit nutrient-dependent lysine residue acetylation [129]. The spectrum of proteins that undergo this post-translational modification include proteins modulating mitochondrial oxidative phosphorylation, the citric acid cycle, the electron transfer chain and proteins involved in amino acid metabolism and antioxidant defenses [129]. As three sirtuins, i.e., SIRT3, 4, and 5, reside in the mitochondria [130], they would be ideal candidates to facilitate this deacetylation of proteins and their respective cognate targets are being actively being explored.

Despite SIRT4 being structurally aligned with the other sirtuin family members, this sirtuin is a mitochondrial ADP-ribosyltransferase with no detectable deacetylation activity [131]. SIRT4 uses NAD⁺ to ADP-ribosylate glutamate dehydrogenase (GDH) and consequently decrease its enzyme activity at least 50%. SIRT4-mediated inactivation of GDH leads to inhibition of insulin secretion in pancreatic β -cells [131]. As will be described below, SIRT3 counter-regulates GDH via deacetylation, and it is necessary to further investigate how this NAD⁺-dependent deacetylation and ADP-ribosylation concomitantly regulate GDH enzyme activity and biological functions. SIRT4 was additionally shown to associate with the metalloprotease IDE, which is known to degrade insulin and with the mitochondrial translocase ANT2 [132]. A role of this sirtuin in pancreatic β -cell function is being explored as SIRT4 is expressed in β -cells and its genetic depletion in insulin producing cells results in increased glucose-dependent insulin secretion [132].

SIRT3 has been the most extensively studied mitochondrial sirtuin and is the most robust mitochondrial deacetylase when compared to SIRT4 and SIRT5 [133]. Mitochondrial localization of the human SIRT3 has been more comprehensively demonstrated [134-136], though one study does show that human SIRT3 is originally localized in nuclei and is imported into mitochondria during stress conditions [137]. However, the exclusive mitochondrial localization of mouse SIRT3 has been keenly debated. This debate was initiated as the proposed initial sequence of mouse Sirt3 lacked a classic amino-terminal mitochondrial localization sequence in contrast to the comparable human gene [134, 138]. Two recent studies have now identified two longer isoforms of the murine SIRT3 and furthermore questioned whether the initial short isoform has biological significance [139, 140]. However, using knockout SIRT3 mouse embryonic fibroblasts, we have recently demonstrated that reconstitution of the mouse short SIRT3 isoform does have similar mitochondrial deacetylase activity compared to the longer isoform [141]. The studies in this arena are, however, still not completely resolved, as the overexpression of the short mouse isoform has nuclear and cytosolic regulatory effects [142-144], but whether this represents endogenous functioning or results from overexpression artifact requires further investigation [141].

Several mitochondrial proteins have been identified as SIRT3 targets which link SIRT3 to various metabolic pathways. Acetyl-CoA synthetase 2 (AceCS2) was the first mitochondrial protein found to be deacetylated and activated by SIRT3 [145, 146]. AceCS2 converts free acetate, which is generated from endogenous cellular reactions or absorbed from the gut, into an active metabolite acetyl-CoA for energy production through the TCA cycle. Regulation of AceCS2 activity by sirtuins is conserved from bacteria to mammals. Though unlike bacteria, which requires acetate metabolism for growth, AceCS2 activation in mammals plays an important role in acetate conversion under ketogenic conditions such as diabetes. In addition, two other mitochondrial matrix proteins, GDH, an enzyme important for amino acid metabolism, and isocitrate dehydrogenase 2 (IDH2), a key regulation enzyme for TCA cycle, are also deacetylated and activated by SIRT3 [133, 147]. Of note, unlike SIRT4, deacetylation of GDH by SIRT3 only increases its activity very modestly (<20%), implying that SIRT4 might play a major role for GDH activity. Interestingly, several components of the electron transfer chain (ETC) have been found to be regulated by SIRT3. For example, NDUFA9, a subunit for ETC complex I [148], succinate dehydrogenase flavoprotein (SdhA), the complex II subunit [149], and ATP synthase alpha and beta subunits [150] were shown to interact with SIRT3. Interaction with SIRT3 increases both complex I and complex II activities [148, 149], implying that SIRT3 is a central player for energy metabolism. Consistent with this, SIRT3 is found to regulate and maintain tissue basal ATP levels in vivo [148]. Moreover, one study shows that SIRT3 interacts with FOXO3a in mitochondria and increases FOXO3a-dependent gene expression [151]. However, as SIRT3 knockout mice show no obvious metabolic abnormalities [133], the regulatory role of SIRT3 is most likely operational under certain nutrient stress conditions, such as during caloric restriction and in response to nutrient overload. Indeed, CR activates SIRT3 expression in both brown and white adipose tissues, and cold exposure upregulates SIRT3 expression in brown fat [142] and SIRT3 knockout mice only show that adaptive thermogenesis under fasting conditions but not under the fed state [133, 152]. In the same study, SIRT3 was reported to promote mitochondrial fatty-acid oxidation through its interaction with long-chain acyl co-enzyme A dehydrogenase (LCAD) [152]. Interestingly, a recent study indicates that SIRT3 is a tumor suppressor in the mammary gland and SOD2 might play a potential role in this defect [153].

Besides functioning as a metabolic regulator, several lines of evidence suggest that SIRT3 may be involved in longevity. Here, the Ang II type 1 receptor (AT1A) knockout mouse, which display increased life span, have an associated upregulation of SIRT3 expression in

the kidney [154]. Furthermore, polymorphisms in SIRT3 gene are linked to survival in the elderly [155, 156] and muscle SIRT3 expression declines in sedentary individuals with aging [157]. However, and in contrast, the regulation of cell survival by SIRT3 is controversial. The conflicting data show that both SIRT3 and SIRT4 are required for cell survival under genotoxic stress conditions in a NAD⁺-dependent manner [158] but in contrast, SIRT3 has been found to participate in Bcl-2 and JNK2-mediated apoptosis in several human cancer cell lines [159], and Kaempferol, a natural flavonoid, induces apoptosis in K562 and U937 cell lines via activation of SIRT3 [160]. Recently, SIRT3 has also been shown to directly modulate the functioning of the mitochondrial ribosome, where SIRT3 activation inhibits mitochondrial ribosomal functioning and protein synthesis [161].

Several studies using the gain-of-function approach have discovered profound functions of SIRT3. In brown adipocytes, overexpression of the short isoform of mouse SIRT3 increases PGC1 α and UCP-1 expression and the mitochondrial biogenesis [142]. In a transgenic mouse model, overexpressing this same construct in the heart attenuates cardiac hypertrophy [144]. The innate biological validity of these observations will be continued to be debated, however, until the understanding of the biological activity of the different murine isoforms are definitively characterized.

In contrast to SIRT3, SIRT5 has weak mitochondrial deacetylase activity when histones were employed as the substrate to determine activity [128, 137]. SIRT5 appears to be localized to both the mitochondrial matrix and to the intermembrane space [147, 162, 163]. A recent study using SIRT5 knockout mice identified carbamoyl phosphate synthetase 1 (CPS1), an enzyme regulating urea cycle for excess ammonia detoxification and disposal, as a functional target SIRT5 in an NAD⁺-dependent manner [162]. SIRT5-mediated CPS1 regulation is important for the adaptation to conditions linked to increased amino acid catabolism such as prolonged fasting and CR. However, in contrast to this finding, another recent study reported that CPS1 acetylation is increased during CR [164]. SIRT5 is also found to interact with cytochrome c in mitochondrial intermembrane space [147, 163]. The biochemical activity and biological functional changes following deacetylation of cytochrome c have not been delineated. However, since cytochrome c is a pivotal component of the mitochondrial ETC and a key regulator of apoptosis, it will be interesting to interrogate this post-translational modification in the regulation of energy metabolism and cell survival.

The role of mitochondrial sirtuins in the control of the diverse array of mitochondrial functions is probably quite extensive and we are at the early stages in understanding this biology. Figure 2 shows the known mitochondrial programs shown to exhibit changes in protein acetylation status [129] and illustrates the functional substrates to date ascribed to the individual sirtuins. In addition to the identification of other functional targets of the mitochondrial sirtuins in this pathway, this figure illustrates areas of study that are required to enable our comprehensive understanding of the role of acetylation/deacetylation in mitochondrial functioning.

SIRT6

SIRT6 was initially found to function as a nuclear ADP-ribosyltransferase enzyme [165], although subsequent studies have expanded its portfolio to include deacetylase functioning with histone H3 lysine 9 (H3K9) and lysine 56 (H3K56) as substrates [166, 167]. As this enzyme mediates nuclear post-translational regulation, the biological effects of this SIRT6 regulation are probably substantial. To date, it has been demonstrated that deacetylation of H3K9 by SIRT6 plays an important role in maintaining telomere integrity and its depletion leads to telomere dysfunction with end-to-end chromosomal fusion and premature

senescence [166]. Furthermore, the depletion of SIRT6 results in cellular hypersensitivity to DNA damage with increased genomic instability with defects in BER [168]. Consistent with these functions, SIRT6 knockout mice show dramatically shortened life span with the development of degenerative abnormalities, lymphopenia, loss of subcutaneous fat, lordokyphosis, and severe metabolic defects with precipitous drops in serum glucose and IGF-1 levels, within 2 weeks of birth, succumbing to these deficits within 4 weeks [168].

Additionally, SIRT6 modulates the NF- κ B-dependent signaling pathway by interacting with NF- κ B subunit RELA with subsequent recruitment to NF- κ B target gene promoters. Here, deacetylation of H3K9 destabilizes NF- κ B binding, with the inhibition of cognate target gene expression [169]. The functional consequences of this interaction in SIRT6-deficient cells is the hyperacetylation of H3K9 with increased apoptotic resistance and induction of senescence via NF- κ B-dependent pathway. In parallel, decreasing NF- κ B signaling by crossing SIRT6^{-/-} mice with RelA^{+/-} mice extends the life span of SIRT6^{-/-} mice [169].

SIRT6 is implicated in the modulation of metabolic programs as steady-state SIRT6 levels are shown to be increased upon nutrient deprivation in cultured cells, following fasting and with calorie-restricted diets in mice [170]. The role of SIRT6 in metabolic control is further supported in that the overexpression of SIRT6 attenuates nutrient excess-induced insulin resistance and fat accumulation, in part via the downregulation of lipogenic genes [171]. Furthermore, SIRT6 has been shown to function as a corepressor of HIF-1 α in the control of glucose metabolism [172].

SIRT7

SIRT7 is the least investigated mammalian sirtuin isoform although its depletion has a profound phenotype, with the mice exhibiting a reduced mean and maximal life span by approximately 50% with the development of degenerative cardiac hypertrophy and inflammatory cardiomyopathy [173]. This profound phenotype is postulated to occur in part due to p53 hyperacetylation and diminished resistance to genotoxic and oxidative stress [173]. Additional SIRT7 substrates that modulate genomic integrity include an association of SIRT7 with RNA polymerase I to activate rDNA transcription [174] and its interaction with the rDNA transcription factor UBF to regulate the onset of rDNA transcription at the exit from mitosis [175].

SIRT7 is also implicated in the regulation of cell growth, although these effects are probably cell-specific. Depletion of SIRT7 in U2OS cells led to inhibition of cell proliferation and triggers apoptosis [174], which is compatible with the apoptotic profile in primary cardiomyocytes [173]. In contrast, SIRT7 inhibits basal cell growth and under oxidative stress in MEF and 10T1/2 fibroblasts [176]. Moreover, SIRT7 mRNA expression inversely correlates with the tumorigenic potential in several murine cell lines [176] and SIRT7 gene expression levels are similarly increased in breast cancer biopsies [177].

Conclusions and future directions

The sirtuins are a distinct group of protein deacetylases that share overlapping function with the HDACs in nuclear regulation. However, they have expanded the portfolio of protein residue deacetylation to include lysine residues on non-histone proteins within and outside of the nucleus. The requirement of SIRT1, 6, and 7 in normal development and in tissue homeostasis is evident in the knockout mouse models of these isoforms that exhibit defective development, survival, or disease onset without the addition of added biological stressors. In contrast, the study of SIRT2, 3, 4, and 5 knockout mice only exhibit deficits in response to an array of biological stressors. SIRT2 is distinct in that not only is it the predominant cytosolic isoform, but its induction appears to be a negative regulator of

adaption to stress and its depletion may enhance stress resilience. The mitochondrial-enriched sirtuins appear to be more important under stress-mediated alternations in mitochondrial metabolic and redox demands. Their integral roles in this biology may be important in disease processes linked to metabolic stressors such as obesity, diabetes, and cancer, and further studies into these arenas are being actively explored. In conclusion, the nutrient- and redox-dependent modulation through the post-translational modification of lysine residues by deacetylation is an exciting and potentially pivotal program in the control of diverse homeostatic programs. Furthermore, the identification of pharmacologic modulators of this family of proteins additionally suggests that the modulation of these regulatory programs in disease processes is an exciting potential future avenue for exploration.

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Fig. 1. Schematic to show the cellular and organ-specific targets for SIRT1-mediated deacetylation with examples of biological consequences of this posttranslational modification of lysine residues

Fig. 2.
Illustrates the known mitochondrial programs shown to exhibit changes in protein acetylation coupled with functional substrates to date ascribed to the individual sirtuins. In addition, the *box* suggests areas of study that would be required to enable our comprehensive understanding of the role of acetylation/deacetylation in mitochondrial functioning