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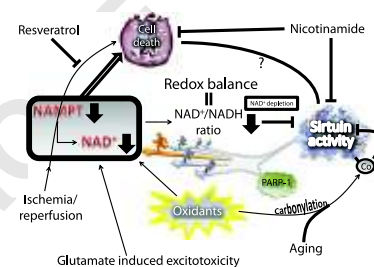
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Graphical Abstract

Redox-regulating sirtuins in aging, caloric restriction, and exercise

Zsolt Radak^{a,b}, Erika Koltai^a, Albert W. Taylor^a, Mitsuru Higuchi^{a,b}, Shuzo Kumagai^c, Hideki Ohno^d, Sataro Goto^a, Istvan Boldogh^e^a Research Institute of Sport Science, Semmelweis University, H-1085 Budapest, Hungary^b Graduate School of Sport Sciences, Waseda University, Saitama, Japan^c Institute of Health and Sports Science & Medicine and Graduate School of Human-Environment Studies, Kyushu University, Kasuga, Fukuoka 816-8580, Japan^d Department of Molecular Predictive Medicine and Sport Science, School of Medicine, Kyorin University, Mitaka, Tokyo, Japan^e University of Texas Medical Branch at Galveston, Galveston, TX 77555, USA

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Highlights

► Sirtuins deacetylate important transcription factors that adjust the redox balance. ► Sirtuin-mediated protein stabilization was readily related to housekeeping and redox regulation. ► Sirtuins are able to alter the progress of aging. ► Adaptation to caloric restriction and physical exercise involves sirtuin-regulated pathways.



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Review Article

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ABSTRACT

The consequence of decreased nicotinamide adenine dinucleotide (NAD⁺) levels as a result of oxidative challenge is altered activity of sirtuins, which, in turn, brings about a wide range of modifications in mammalian cellular metabolism. Sirtuins, especially SIRT1, deacetylate important transcription factors such as p53, forkhead homeobox type O proteins, nuclear factor κB, or peroxisome proliferator-activated receptor γ coactivator 1α (which controls the transcription of pro- and antioxidant enzymes, by which the cellular redox state is affected). The role of SIRT1 in DNA repair is enigmatic, because it activates Ku70 to cope with double-strand breaks, but deacetylation of apurinic/aprimidinic endonuclease 1 and probably of 8-oxoguanine-DNA glycosylase 1 decreases the activity of these DNA repair enzymes. The protein-stabilizing effects of the NAD⁺-dependent lysine deacetylases are readily related to housekeeping and redox regulation. The role of sirtuins in caloric restriction (CR)-related longevity in yeast is currently under debate. However, in mammals, it seems certain that sirtuins are involved in many cellular processes that mediate longevity and disease prevention via the effects of CR through the vascular, neuronal, and muscular systems. Regular physical exercise-mediated health promotion also involves sirtuin-regulated pathways including the antioxidant-, macromolecular damage repair-, energy-, mitochondrial function-, and neuronal plasticity-associated pathways. This review critically evaluates these findings and points out the age-associated role of sirtuins.

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Abbreviations: 8-oxoG, 8-oxo-7, 8-dihydroguanine; APE1, apurinic/aprimidinic endonuclease 1; BER, DNA base excision repair; CR, caloric restriction; DSB, DNA double-strand break; FOXO, forkhead homeobox type O protein; eIF2α, eukaryotic initiation factor 2α; eNOS, endothelial nitric oxide synthase; IL-1β, interleukin 1β; OGG1, 8-oxoguanine-DNA glycosylase 1; PIG3, p53-induced gene 3; PARP1, poly(ADP-ribose) polymerase 1; PGC-1α, peroxisome proliferator-activated receptor γ coactivator 1α; MST1, mammalian sterile 20-like kinase 1; NAD⁺, nicotinamide adenine dinucleotide; NAMPT, nicotinamide phosphoribosyltransferase; NOXA, NADPH activator A; SIRT1, sirtuin (silent mating type information regulation 2 homolog) 1 or NAD⁺-dependent deacetylase sirtuin 1; Nrf2, nuclear factor (erythroid-derived 2)-like 2; Sir2, silent information regulator 2; TNF-α, tumor necrosis factor α; Trx, thioredoxin; UCP2, uncoupling protein 2

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Introduction

Sirtuins (silent information regulator 2 (Sir2)1 proteins) belong to an ancient family of evolutionarily conserved NAD⁺-dependent enzymes with deacetylase and/or mono-ADP-ribosyltransferase activity and are implicated in diverse cellular processes. The sirtuin family is ubiquitously distributed in mammals, with seven homologs (SIRT1–7), and their expression/activity shows organ and organelle specificity. Powerful protein deacetylase activity of SIRT1, SIRT2, SIRT3, and SIRT5 has been reported toward histones, whereas SIRT4, SIRT6, and SIRT7 have no such detectable enzymatic activity on histone peptide substrate [1,2]. SIRT3–5 are predominantly localized to the mitochondria. Mammalian sirtuins are closely involved in metabolism [2–4], which is linked to the mitochondrial generation of reactive oxygen species (ROS) [5,6]. SIRT1 is downstream in ROS signaling because of a dependence on the availability of NAD, but it can be important upstream in cellular regulators, including forkhead homeobox type O factor 3 (FOXO3) [7], muscle-specific RING finger protein 1 [8], and the v-Akt murine thymoma viral oncogene homolog 1 (Akt1) [9].

The crystal structure of human SIRT1 (a homolog of yeast Sir2) reveals a large groove intersected by a pocket lined with hydrophobic residues, conserved with class-specific protein-binding sites of each Sir2 class [10]. Activity of most of the sirtuins is controlled by posttranslational modifications, as well as the availability of NAD⁺. It has been shown that they are phosphorylated at N- and C-terminals, which play a role in substrate binding [11]. Moreover, in addition to phosphorylation, it appears that S-nitrosylation of SIRT1 impairs the catalytic activity of enzymes via a nitrosylated glyceraldehyde-3-phosphate dehydrogenase-mediated process [12]. Additionally, thioredoxin (Trx) regulates cellular redox balance through reversible oxidization of its redox-active cysteine residues (-Cys-Gly-Pro-Cys-), which can mediate protein S-denitrosylation [13–15] and hence the activity of

sirtuins. The deacetylase domain of sirtuins consists of approximately 250 amino acids, differentiated by divergent N- and C-terminal extensions [16]. In the budding yeast *Saccharomyces cerevisiae*, the Sir proteins are involved in a wide array of cellular processes, including the nonhomologous end-joining repair of DNA [17], the stabilization of the replication forks in the ribosomal (r) DNA to prevent DNA breaks, recombination, and the generation of extrachromosomal rDNA circles [18], which leads to aging of this organism [19].

One of the first studies that linked sirtuins to aging was based on the observation that proteins encoded by SIR genes are responsible for silencing the rDNA of *S. cerevisiae* [20]. The same group of investigators also demonstrated that Sir2 is redox sensitive because of its NAD⁺ dependence. Moreover, they showed that sirtuins have deacetylase activity from eubacteria to humans [21]. Guarente and his group then demonstrated that redistribution of the Sir2 complex from a telomere to the nucleolus is associated with aging in yeast [19,22], and overexpression of this gene extends their life span [23]. Sinclair and co-workers showed that life extension in yeast could be done via the salvage pathway of NAD⁺ [24], and life-span extension during caloric restriction (CR) is associated with activation of Sir2 genes in yeast and the mammalian homolog SIRT1 of human cells [25,26]. Now, mounting data suggest an active role for sirtuins in aging and age-associated diseases. However, as with most phenomena in science, the convincing effects of sirtuins in mammalian aging are not without debate [27].

This paper reviews the redox sensitivity of sirtuins and the role of these lysine deacetylases, especially SIRT1, in the aging process. The authors also crucially review the data of knockout and overexpression models, as well as the effects of CR and physical exercise. The effects of resveratrol, which is a potent stimulator of SIRT1, have been reviewed elsewhere [28–31].

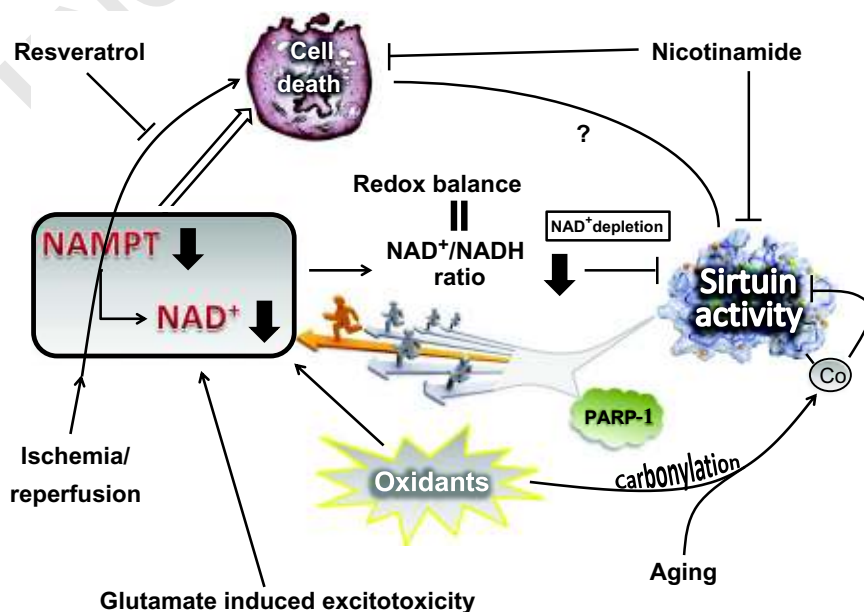


Fig. 1. The suggested mechanisms between the availability of NAD and the activities of sirtuins and PARP is shown. Metabolic challenges modulate NAD levels, NAD:NADH ratio, and cell fate by shifting to either pro- or antiapoptotic pathways.

1 Role of sirtuins in cellular redox balance

2 Redox balance and sirtuins: up- and downstream regulators

3 The activity of sirtuins can be readily modified by the availability
4 of NAD⁺, expressed as a function of NAD:NADH ratio, which is a
5 marker of cellular redox balance. Metabolic challenges, such as
6 hypoxemia–ischemia and reoxygenation, result in decreased NAD
7 and nicotinamide phosphoribosyltransferase (NAMPT) levels and
8 decreased activity of sirtuins [32–35]. It was recently shown that
9 treatment with glycated low-density lipoprotein resulted in increased
10 ROS production in vascular endothelial cells and in lowered mito-
11 chondrial membrane potential, the NAD:NADH ratio [36]. It has been
12 shown that glutamate-induced excitotoxicity in neurons and ische-
13 mia in mouse brains result in marked decreases in NAD⁺ levels,
14 which is a precursative step before cell death [32]. ROS can readily
15 induce DNA damage, and in response to DNA strand breaks poly
16 (ADP-ribose) polymerase 1 (PARP1) consumes significant amounts of
17 NAD⁺ to produce poly(ADP-ribose) polymers on target proteins in a
18 process called poly-ADP-ribosylation [37]. Significant DNA damage-
19 associated PARP activation can lead to NAD⁺ depletion resulting in
20 energy crisis [38].

21 Hence, administration of nicotinamide, which is a NAD⁺
22 precursor and a noncompetitive inhibitor of SIRT1, has rescued
23 cells from apoptosis and necrosis [32]. On the other hand
24 resveratrol supplementation did not increase cell survival.
25 In contrast, in ischemic-reperfused cardiomyocytes resveratrol
26 supplementation mediated cell survival via mitogen-activated
27 protein kinase pathways [39].

28 The concentration of NAD⁺ seems to be crucial, because when
29 PARP1 was knocked out the phenotype of mice resembled the
30 SIRT1 of overexpressed phenotypes, and similar phenomena have
31 occurred when PARP1 was inhibited by pharmacological agents
32 [40]. These data strongly point to a competition between PARP1
33 and SIRT1 for the common cofactor NAD⁺ (Fig. 1). On the other
34 hand, when the NAD⁺ level was depleted first by oxidants, such
35 as hydrogen peroxide or cigarette smoke, then the PARP1 inhibi-
36 tion was not effective in gaining back SIRT1 activity. Nonetheless,
37 the NAD⁺ content was restored [35]. One of the causative factors
38 could be that oxidants resulted in carbonylation of amino acid
39 residues of SIRT1, which, in turn, could curb the activity of the
40 enzyme [35,41]. This could happen with aging also. Koltai and
41 colleagues [42] observed that with aging there is an increase
42 in the level of SIRT1, which is associated with decreased activity
43 of the enzyme and appears to be due to increased carbonylation.
44 In this study, carbonylation of SIRT1 was not studied specifically;
45 however, the overall carbonylation of the cytosolic proteins has
46 been found to significantly increase in older animals. In a recent
47 study in which pelvic human skin samples were used, DNA
48 damage was positively correlated with aging and negatively
49 correlated with NAD⁺ levels [43]. Therefore, it seems that
50 oxidants could affect sirtuins via depletion of NAD⁺ and oxida-
51 tive modification of amino acid residues, leading to decreased
52 activity or level of the enzyme [40,44]. On the other hand, it has
53 also been shown that the overexpression of SIRT1 has beneficial
54 effects in resistance to oxidative stress [39,44,45].

55 The effects of sirtuin-regulated transcription factors on redox balance

56 Sirtuin-mediated deacetylation of key transcription factors results
57 in altered gene expression of key antioxidant enzymes or those
58 producing ROS. Here we focus on redox regulation by sirtuins via
59 p53, FOXOs, nuclear factor κ B (NF- κ B), and peroxisome proliferator-
60 activated receptor γ coactivator 1 α (PGC-1 α) as examples.

61 SIRT1 is involved in stress responses, cellular metabolism, and
62 aging through deacetylation of a variety of substrates including

63 p53. p53 is acetylated by CBP/p300 acetyltransferases at lysine
64 residues, including Lys 370, 372, 382, and 386 at the carboxy-
65 terminal region [46,47]. Activated p53 then enhance ROS produc-
66 tion through mitochondrial dysfunction and/or increased expres-
67 sion of genes that are involved in redox modulation, such as the
68 p53-upregulated modulator of apoptosis (PUMA), NADPH activa-
69 tor A (NOXA), and p53-induced gene 3 (PIG3) [48,49]. In addition,
70 it seems that NADPH oxidase 1-generated ROS inhibit the acet-
71 ylation of p53 at Lys 382 via SIRT1. Deacetylation at Lys 382 is
72 important in the control of gene expression from PUMA, NOXA,
73 and PIG3, i.e., the proapoptotic function of p53 [50]. Interestingly,
74 p53 via p21 indirectly activates nuclear factor (erythroid-derived
75 2)-like 2 (Nrf2), a central transcription factor in the antioxidant
76 response. Nrf2 binds to the antioxidant-response element 2, a cis-
77 acting enhancer found in promoters, and, via increased antiox-
78 idant gene expression, counteracts oxidative challenge [51].

79 In mammals there are four FOXO transcription factors, and,
80 among them, FOXO1, FOXO3a, and FOXO4 are involved in redox
81 regulation [52,53]. FOXOs are negatively regulated by the phos-
82 phatidylinositol 3-kinase–Akt signaling pathway. When cells
83 were treated with hydrogen peroxide, the FOXO3 acetylation
84 was increased by CBP/p300 and, as a result, the resistance against
85 oxidative stress was enhanced [54]. Moreover, oxidants caused
86 translocation of FOXO3 into the nucleus, where it can be deace-
87 tylated by SIRT1, which would enhance the activation of GADD45.
88 This is important for stress resistance by the induction of DNA
89 repair, because GADD45 is required for efficient DNA base exci-
90 sion repair (BER) and nucleotide excision repair (NER) [54,55].
91 In addition, FOXO3 directly activates the transcription of Mn-
92 superoxide dismutase (SOD) and catalase [56] to attenuate the
93 toxicity of mitochondrial-derived superoxide anion and hydrogen
94 peroxide, thereby preventing damage to genomic and mitochon-
95 drial (mt) DNA, lipids, and proteins. In addition to FOXO3, FOXO1
96 can also alter redox states. For instance, Akt-mediated phosphor-
97 ylation of PGC-1 α at serine 570 results in inactivation, and this
98 conformational change leads to reversible disruption of the
99 FOXO1 response element of the catalase promoter, therefore
100 curbing the expression of catalase [57]. In addition, it appears
101 that FOXO4 inhibits NF- κ B and thereby the proinflammatory
102 chemokine/cytokine expression and ROS production associated
103 with the activation of inflammatory cells [58].

104 NF- κ B-dependent signaling plays a role in the aging process,
105 with a wide range of effects in addition to inflammation [59]. NF-
106 κ B is a family of inducible transcription factors that plays a
107 central role in controlling expression of networks responsible for
108 proinflammatory gene expression, cell proliferation, differentia-
109 tion, or apoptosis [60]. NF- κ B release from cytoplasmic inhibition
110 is necessary, but not sufficient, for target gene induction, e.g., that
111 induced by ROS RelA/p65 phosphorylated at serine 276, which is
112 coincident with its release from I κ B α in the canonical pathway.
113 Upon phosphorylation, RelA/p65 translocates into the nucleus
114 and mediates the promoter-specific recruitment of p300/CBP,
115 which acetylates it at lysine 510 to enhance transcription. It has
116 also been shown that SIRT1 deacetylates the RelA/p65 on lysine
117 310 and thus blocks the transcriptional activity of NF- κ B [61],
118 resulting in active silencing of the chromatin (Fig. 2). SIRT1
119 has been shown to be capable of decreasing NF- κ B-induced
120 inflammation by deacetylation and prevents the tumor necrosis
121 factor α (TNF- α)-mediated activation of matrix metalloproteinase
122 9, interleukin 1 β (IL-1 β), IL-6, and inducible nitric oxide synthase
123 [62]. In addition, it has been suggested that SIRT1 binds to the
124 promoter regions of IL-1 β and TNF- α in a cell model of sepsis
125 and plays a role in epigenetic regulation during endotoxin tolerance [63].
126 However, it is clear that suppression of NF- κ B activity by SIRT1
127 modulates redox signaling, not just by regulating inflammation but
128 also through the expression of Mn-SOD [64,65].

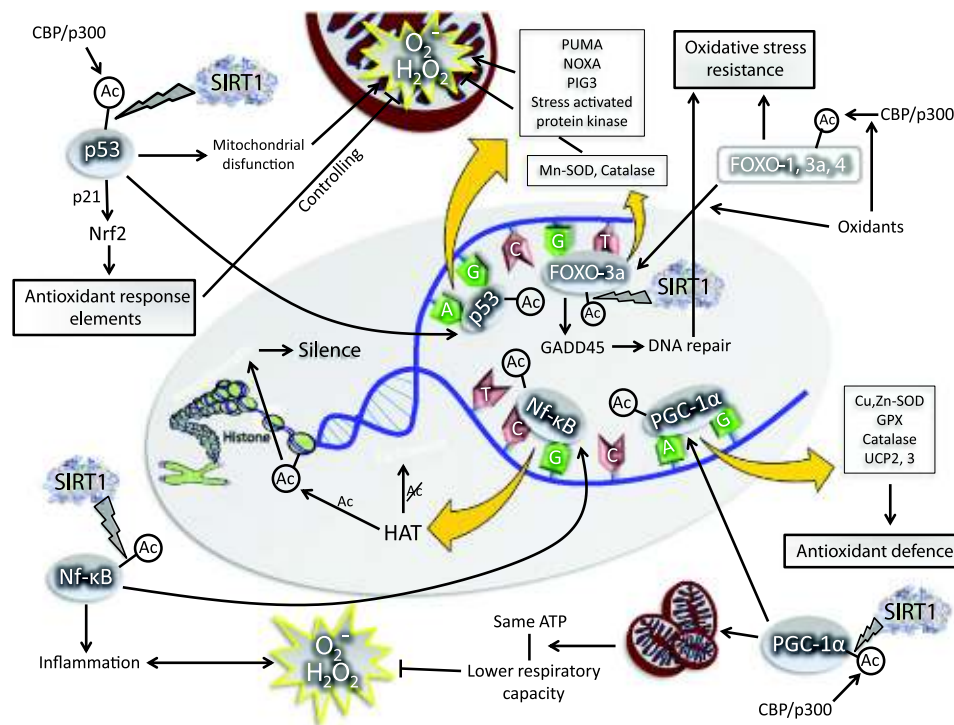


Fig. 2. Sirtuins are involved in a complex way in redox regulation. By the deacetylation of transcription factors, such as p53, FOXOs, NF- κ B, and PGC-1 α , they modulate the transcription of pro- and antioxidant genes, mitochondrial biogenesis, activity of oxidative damage-repairing enzymes, and chromatin structure.

PGC-1-related proteins are expressed ubiquitously, but PGC-1 α and -1 β are enriched in mitochondria-rich tissues such as cardiac and skeletal muscles. PGC-1 α is a master transcriptional coactivator that modulates the gene expression involved in energy metabolism and energy expenditure. PGC-1 α is a direct link between external physiological stimuli and the regulation of mitochondrial biogenesis. PGC-1 α regulates the activity of a large number of transcription factors, including peroxisome proliferator-activated receptors α and γ , estrogen receptor-related α , FOXO1, hepatocyte nuclear factor 4 α , and nuclear respiratory factor 1. Moreover, PGC-1 α also regulates gene transcription encoding mitochondrial electron transport chain proteins, uncoupling protein 2 (UCP2) and UCP3, and key antioxidant enzymes, such as Cu,Zn-SOD, glutathione peroxidase, and catalase [66,67]. Its transcriptional coactivator function is primarily regulated by acetylation via p300/CBP and deacetylation by SIRT1 [68]. Mitochondrial biogenesis itself can be an important cog in redox homeostasis, because for the same ATP production more mitochondria can work at a lower respiratory capacity, and then fewer ROS are produced. Contrarily, decreased mitochondrial capacity at metabolic stress, such as exercise or ischemia, would result in enhanced ROS production. These observations support PGC-1 α 's active involvement in antioxidant defense [69]. Inflammation is associated with increased ROS production and oxidative damage, and the antioxidant role of PGC-1 α is further supported by the anti-inflammatory effects of this coactivator [70].

Sirtuins in regulation of genome integrity

One of the first studies on sirtuins and DNA repair as well as telomere silencing was done in yeast cells (*S. cerevisiae*). These studies revealed that Ku protein interacts with sirtuins during DNA double-strand break (DSB) repair of telomere regions [71,72]. Studies have also shown that SIRT1 deacetylates Ku70 in mammalian cells, revealing antiapoptotic effects [26,73]. Another sirtuin family member, SIRT6, interacts with PARP1 to

enhance the efficiency of repair of DSBs [74]. SIRT6 is also important for other DNA repair pathways, because in SIRT6-ablated cells, overexpression of a single-strand break gap-filling protein, DNA polymerase β , rescued cells, implying that SIRT6-mediated deacetylation has a crucial role in BER processes [75]. Consequently, a lack of SIRT6 in knockout mice decreased the life span [76]. Moreover, many human cancers show decreased levels of SIRT1, and SIRT1 mutant mice exhibit an increased incidence of cancer [77].

The apurinic/apyrimidinic endonuclease 1 (APE1) plays a central role in the repair of oxidized and alkylated bases in mammalian genomes via the BER pathway. APE1 also functions as redox effector factor 1 for several transcription factors, including activator protein-1 (AP-1), HIF1 α , and p53 [78]. Importantly, both repair and transcriptional function (coactivator and corepressor) of APE1 are modulated by acetylation in redox-dependent and redox-independent mechanisms [79]. It has also been shown that APE1 is a target of SIRT1-mediated deacetylation, and the administration of resveratrol increases the activity of this important BER enzyme [80]. Along with APE1, human 8-oxoguanine-DNA glycosylase 1 (OGG1) is the major DNA glycosylase for excision of 7,8-dihydro-8-oxoguanine (8-oxoG) and ring-opened fapyguanine. OGG1 has been shown to be acetylated by p300/CBP at lysines 338 and 341, which results in a significant increase in its activity [81]. We have recently demonstrated that Ac-OGG1 is present in human skeletal muscle and rat tissues, and the level of acetylation decreases with aging, whereas a parallel pattern was found for SIRT1 activity [82,83]. Moreover, we have shown that resveratrol administration decreases the acetylation level of OGG1 and ablation of SIRT1 by siRNA-increased levels of Ac-OGG1. This finding suggests that SIRT1 is counteracted by OGG1 (Sarga, Z. Radak, and I. Boldogh, unpublished observation). It has been recently shown that the OGG1-8-oxoG complex interacts with and catalyzes GDP-GTP exchange in the canonical Ras family GTPases H-, K-, and N-Ras. These data imply that in the presence of 8-oxoG base, OGG1 acts as a guanine nucleotide

exchange factor [84]. In turn, Ras-GTP via Raf1 (v-raf-1 murine leukemia viral oncogene homolog 1) phosphorylates the mitogen-activated kinases MEK1,2/ERK1, which may lead to ROS production. In addition to BER, NER is also modulated by sirtuins, because the xeroderma pigmentosum group A is deacetylated and suppressed by SIRT1 [85,86]. Moreover, it was also shown that human cancers have decreased levels of SIRT1, and SIRT1 mutant mice exhibited an increased incidence of cancer [77].

Sirtuins in protein stability and cellular stress response

Housekeeping of oxidative stress and damage is crucial in redox balance as it affects the fate of the cells. A group of genes involved in preserving cellular homeostasis during stress conditions, called vitagenes, and sirtuins, among others, are involved in a wide spectrum of cellular defense [14,87,88]. Indeed, one of the ways by which sirtuins regulate the stress response is through protein stability, which can readily affect the levels of oxidatively modified/damaged proteins [89]. One of the ways by which SIRT1 protects cells from oxidative stress is its involvement in the integrated stress response pathway via eukaryotic initiation factor 2 α (eIF2 α). During stress, cells suppress the levels of protein synthesis and mobilize most of the available sources to cope with stress [90], and the reduction in eIF2-GTP levels leads to a general reduction in global protein synthesis. It turns out that SIRT1 interacts with mediators of eIF2 α dephosphorylation, suggesting a role for SIRT1 in the eIF2 α -related early stress response [90], which partly depends on the NAD:NADH ratio.

Cellular stress response is also mediated by heat shock transcription factor 1 (HSF1), which when acetylated negatively regulates DNA binding. On the other hand, SIRT1-mediated deacetylation of HSF1 enhances its binding to the heat shock protein 70 promoter by maintaining HSF1 in a deacetylated, DNA-binding-competent state [91]. This finding established the role of

SIRT1 in the heat shock response and longevity, because HSF1 has been linked to longer life spans [92], as well as in protein stability, because heat shock proteins are important modulators of protein degradation. Indeed, the effect of acetylation on protein stability is another acting point in the cellular stress response by which redox balance is supervised by sirtuins [82,93–96].

The role of acetylation and deacetylation of protein stability has been reviewed by others [97,98]. One of the first proteins for which acetylation was linked to stability was p53 [47,99]. Acetylation of lysine residues prevents ubiquitination of the given residues, thus hampering signaling for proteolytic degradation [98]. Indeed, inhibition of SIRT1 by nicotinamide decreased the polyubiquitination of FOXO3 and hence the protein levels of this transcription factor [96]. The activation of SIRT1 resulted in enhanced degradation of FOXO3 by the proteasome system. Aging results in a loss of the activity of SIRT1, which is associated with increased levels of lysine acetylation in rat brain and skeletal muscle [42,82,100]. The level of acetylated lysine residues correlated well with that of carbonylation, which may mean that acetylated lysine could not have been ubiquitinated and degraded by proteasomes. If this happens, the half-life of proteins increases, which is believed to take place with aging, resulting in the enhanced accumulation of damaged proteins and consequently decreased physiological function [101]. However, acetylation of nonhistone proteins also takes place in a very selective manner [102].

Acetylation of histone residues by sirtuins alters the transcription from genes that are involved in redox homeostasis (Fig. 3). For instance, SIRT1–3 and 6 are involved in shaping chromatin structure, and the well-known target of SIRT1 deacetylation is the acetylated histone H3 lysine 9, which is a precursory step toward the methylation of the same lysine residue by the histone methyltransferase suppressor of variegation (Suv) 39h1 [103]. SIRT1 preserves pericentromeric heterochromatin via stabilization of Suv39h1 by preventing the polyubiquitination of lysine 87

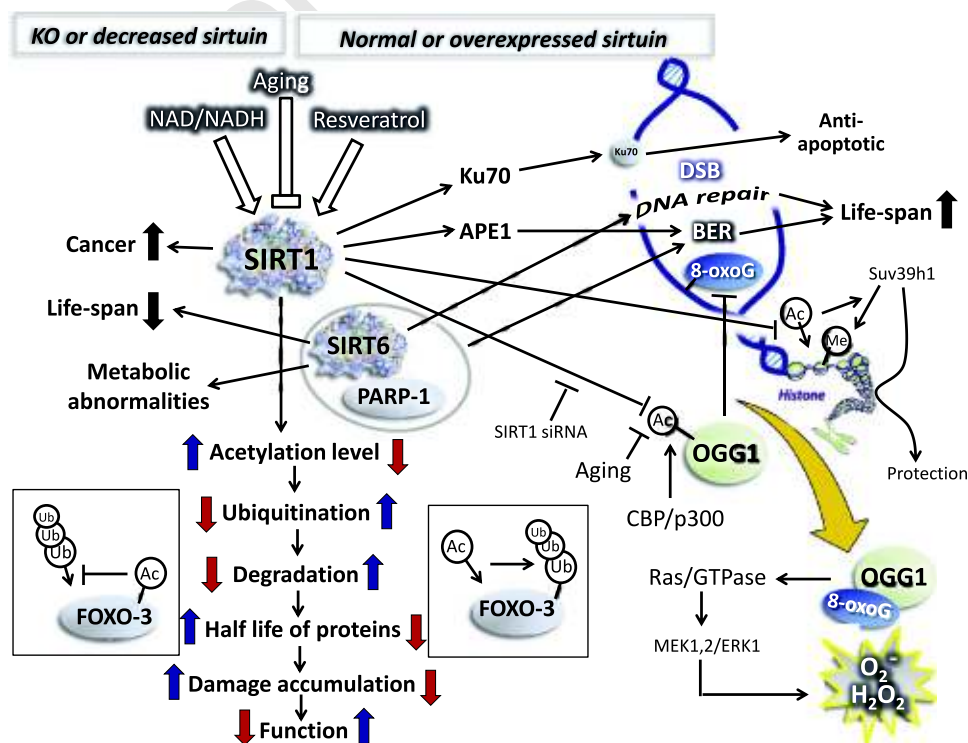


Fig. 3. Sirtuins regulate the stability of proteins, which in turn would affect cell and organ function. Modulation of DNA repair could be associated with longevity, metabolic abnormalities, cancer, and aging.

of this methyltransferase by E3-ubiquitin ligase [104]. The stabilization of Suv39h1 under stress conditions, such as oxidative stress, results in enhanced protection of chromatin structure.

Sirtuins and mammalian aging

The involvement of sirtuins in the aging of yeast, such as the silencing of rDNA, is quite different and less complex than that occurring in mammalian systems. However, even in yeast, the life extension effects of overexpression of Sir2 have been questioned recently [27]. It appears that SIRT1 regulates mammalian FOXOs more than does the bacterial homolog Sir2, and the inhibition of FOXOs and p53 results in an antiapoptotic process, which is closely related to the resistance to oxidative stress. Deacetylation of p53 by SIRT1 attenuates the DNA-binding and transcriptional activity, whereas deacetylation of FOXOs can result in suppression of transcription from proapoptotic genes and enhance activity of those that are involved in oxidative stress resistance [54]. Therefore, the effects of SIRT1 on these transcription factors are to promote cell survival in mammals. Recently, it was reported that mammalian sterile 20-like kinase (MST1) can inhibit SIRT1 by phosphorylation in response to DNA damage, which consequently leads to apoptosis through the acetylation of p53. This observation further supports the key role of SIRT1 in cellular resistance during oxidative stress [105]. The controlling role of SIRT1 on p53 can alter redox balance, because the proapoptotic role of p53 can induce genes involved in ROS production, and this transcription factor can activate enzymes in the antioxidant system [106,107]. It is suggested that low levels of deacetylated p53 suppress cellular levels of ROS, whereas high levels of acetylated p53 induce ROS production-related genes (see review in [108]).

In terms of aging, it has been shown that overexpression of SIRT1 in primary fibroblasts decreased acetylation of p53 and antagonized PML/p53-induced premature aging of cells in culture [109]. The question is whether this activity occurs at the level of the organism in vivo as well. The finding that there is an increase in the levels of p53, FOXO1, and SIRT1 in the nuclei of cells of old animals [110] may be indicative of a complex setting in mammals. In sporadic inclusion-body myositis, which is an aging-associated muscle disease, it was found that deacetylation of p53 was decreased [111]. Aging results in decreased intracellular levels of NAD⁺ and NAD:NADH ratio in various organs of old rats, and this finding was associated with decreased activity of SIRT1 and increased acetylation of p53 [33]. Moreover, aging processes resulted in a lower activity of complexes I-IV in the mitochondria and enhanced oxidative DNA damage [33]. In support, an increase in SIRT1 levels has been shown in skeletal muscle of aged animals, and this was associated with decreased activity of the enzyme along with lower levels of NAMPT and increased levels of oxidative damage [42]. During the aging process, the level of noncoding microRNA R-34a and microRNA R-93 is increased in rat liver, and these microRNAs suppress the activity of specificity protein 1 and Nrf2 transcription factors, resulting in decreased SIRT1 and microsomal glutathione S-transferase 1 expression [112]. This posttranslational repression of SIRT1 and microsomal glutathione S-transferase 1 naturally results in decreased oxidative defense during aging in the rat liver.

There is a paucity of these studies on human healthy aging and sirtuins. SIRT1 plays a role in telomere length, which is used as a marker to appraise the aging process [113,114]. A single-nucleotide polymorphism in the SIRT1 gene and another in the 3' flanking region of XRCC6 interfered with the length of the telomere in human leukocytes and aging [115]. Moreover, it was also reported that the frequency of the minor allele of SIRT1 was

enhanced in older subjects. Data from human and animal studies revealed that SIRT1 could modulate and deacetylate endothelial nitric oxide synthase (eNOS), which in turn resulted in altered NO production. The acetylation level of aortic eNOS of 30-month-old rats was many times higher than that of young rats [116]. Sirtinol was used to inhibit SIRT1 in this study, and it abolished the age-associated difference in endothelium-dependent dilatation, and the results gained from the samples of endothelial cells, obtained from the brachial artery of young and old subjects, confirmed that in healthy aging SIRT1 could play a role in endothelial dysfunction [116].

mRNA analysis revealed no age-associated changes in SIRT1 and SIRT3 expression in skeletal muscle in young vs old subjects [117]. Similar results were obtained in the rat myocardium of young and old rats [118]. It seems that, as a result of aging, SIRT1 and SIRT6 levels increase in rat skeletal muscle, whereas the activity of OGG1 is decreased [42]. Interestingly, the SIRT1 levels in the hippocampus of the same animals decreased as a result of aging, whereas we found elevated levels of SIRT3 [82]. An increased level of 8-oxoG in the hippocampus with elevated levels of OGG1 was observed in aged subjects. However, when we checked the acetylation level of OGG1, it was found that aging significantly decreased the Ac-OGG1 content. Therefore, it was suggested that the drop in OGG1 acetylation could account for the increased 8-oxoG levels in cellular DNA of hippocampus [82]. We further studied the acetylation pattern of OGG1, and our unpublished data suggested that SIRT1 interacts with and deacetylates OGG1 as in case of APE1.

Analysis of specimens from human brain regions of healthy and Alzheimer disease (AD) subjects showed that SIRT1 expression at both the RNA and the protein level is significantly decreased in the parietal cortex of AD patients, whereas cortical expression was not affected [119]. Global cognition scores obtained before death would suggest that the decrease in SIRT1 levels is related to the accumulation of amyloid- β and τ in the cerebral cortex of persons with AD [119]. On the other hand, it was also reported that in age-related cataracts in healthy older humans there was a lower level of SIRT1 expression, and the levels of SIRT1, p53, FOXO3, and FOXO4 were comparable to data from young subjects [120]. It is also clear that the antiapoptotic role of SIRT1 can contribute to increased tumor growth [121], which can be attenuated by SIRT1 inhibitors. The complexity of SIRT1 in senescence involves stem cell differentiation, which emphasizes the role of SIRT1 in controversial processes during aging [122].

Sirtuin knockout and transgenic animal models

Despite drawbacks, knockout animal models offer one of the most powerful means for studying gene function. There are powerful data showing that knockdown or overexpression of sirtuins results in shorter or longer life spans, respectively, in model organisms from yeast to mice [4,76], although the information in the literature is not unequivocal.

It has been reported that in SIRT1 knockout mice, although the oxidative damage was decreased in the brain, the life span was shorter, and CR was not effective [123]. In addition, inhibition of SIRT1 decreased insulin-like growth factor 1 (IGF-1) signaling and increased resistance against oxidative challenge. According to that report, SIRT1, under certain physiological conditions, enhances IGF-1 signaling by deacetylating insulin receptor substrate 2 (IRS2), resulting in Ras/ERK activation, increasing ROS production and altering redox signaling [123]. Knocking out SIRT1 in mice with A53T α -synuclein mutation decreased, while overexpression increased, the life span of transgenic mice [124].

Moreover, in another disease model, which was developed to mimic Huntington disease, it was found that ablation of SIRT1 caused exacerbation of brain pathology, whereas brain-specific overexpression of SIRT1 attenuated the consequences of Huntington disease, partly by the upregulation of brain-derived neurotrophic factors leading to enhanced survival [125].

On the other hand, data from another research group indicated that ablation of SIRT1 resulted in impaired memory, cognitive function, and spatial learning, and the expression of genes important for synaptic plasticity and metabolism was decreased [126]. In contrast, SIRT1-overexpressing mice showed normal cognitive functions and learning. It should be pointed out that overexpression of SIRT1 in striatum and hippocampus, under the control of the α subunit of Ca²⁺-calmodulin-dependent protein kinase II promoter, decreased glucose transporter type 4 level in skeletal muscle and increased body fat. This genetic manipulation altered the performance in an open-field test, through the deacetylation of IRS2, which induced phosphorylation of IRS2 and ERK1/2 in the striatum of these mice [127]. SIRT1 knockout mice revealed that SIRT1 is important to development, and the hyperacetylation of p53 was observed in SIRT1-ablated models, which may indicate that SIRT1 is crucial for p53-mediated apoptosis [128]. Indeed, it has been shown that SIRT1 mutant and p53 mutant mice develop tumors in multiple tissues, which also emphasizes the possible role of SIRT1 in the regulation of p53 [77].

It has been reported that SIRT1 knockout mice have greater mobility; when they were exposed to treadmill running and the rotarod test, their performance was better than that of wild-type mice [129]. It would be important to understand how the ablation of SIRT1 in this model could result in better physical performance. On the other hand, overexpression of SIRT1 in mice also resulted in better performance on the rotarod test, and their phenotype resembled those of mice that are exposed to CR [130]. Therefore, overall, data from knockout models clearly showed how complex the role of SIRT1 is in mammalian physiology and how carefully the knockout data need to be evaluated in terms of SIRT1's function. A major problem of these studies is that the proteins that are targets of SIRT1 are not apparent, in most cases, nor is how deacetylation of those proteins is mechanistically related to the outcomes observed.

Regulation of sirtuins by CR and exercise

CR is known to increase mean and maximum life spans, and regular physical exercise can also enhance mean life span; moreover, sirtuins are indicated as being involved in both cases. For example, a number of studies have shown that sirtuins, especially SIRT1, mediate CR-induced longevity. However, the role of Sir2 in CR was questioned by Kaeberlein and co-workers [131,132], but their model used yeast, which differs from mammalian models. When 12-month-old rats were subjected to CR, as 60% of the daily food allotment, the result was a downregulation of the IGF-1 pathway and SIRT1-mediated regulation of p53, Ku70, and FOXO3 [26]. This was one of the first demonstrations of the powerful effect of CR on SIRT1-associated signaling. In another rat study, in which the dietary amino acids, except methionine, were restricted to 40%, increased SIRT1 levels were found along with unchanged levels of PGC-1 α and of mtDNA damage, but with decreased levels of mitochondrial protein oxidation [133].

Thioredoxin-interacting protein (TXNIP) has been suggested to elevate sensitivity to oxidative stress through inhibition of Trx [134], having an effect opposite to that of sirtuins on life span [135,136]. Consistently, it has been shown that limited calorie availability inhibited TXNIP and slightly induced SIRT1, whereas

resveratrol had biphasic effects [137], and its inhibitory role on TXNIP was mediated through AMPK pathways.

Csiszar and co-workers [138] demonstrated that life-long CR significantly decreased age-associated vascular dysfunction, oxidative stress, and NF- κ B activity. They also showed from a relating cell culture that inhibition of SIRT1 by silencing RNA eliminated the beneficial effects of CR on inflammation and antioxidants, possibly indicating that SIRT1 is actively involved in the CR-mediated signaling process. Aging results in decreased protein levels of SIRT1 in the hippocampus [82], and this decrease can be attenuated by CR [139], which may mean that CR could act via SIRT1. Studies on cAMP responsive-element binding 1 (CREB1) knockout mice revealed that CR (given as 60% of the ad libitum normal diet) decreased SIRT1 levels in the hippocampus and cortex, whereas in wild-type animals the SIRT1 was upregulated by CR [140]. That report presented evidence that CR in the brain was mediated by CREB1, which in turn induced SIRT1 expression, and suggested that SIRT1-associated metabolic processes played a role in neuronal plasticity. In addition, SIRT1 is also involved in the effects of CR in skeletal muscle, because it has been shown that the transcription factor signal transducer and activator of transcription 3 is deacetylated and therefore inactivated by SIRT1 during CR, leading to a downregulation of insulin-stimulated phosphoinositide 3-kinase, resulting in enhanced insulin sensitivity [141]. Moreover, another study reported that SIRT1, via its interaction with AP-1, promotes macrophage function during CR [142]. Studies on CR have resulted in some controversial issues on mitochondrial biogenesis, at least those using experimental rodents [143,144]. Data from human skeletal muscle provide evidence that CR can result in enhanced mitochondrial mass and increased SIRT1 levels [145]. Therefore, it seems to be supported by a number of papers that CR indeed acts through SIRT1-controlled molecular processes in mammalian systems.

The effects of exercise, similar to those of CR, appear to be systemic and complex [146], as it has been shown that both a single bout of exercise and endurance training result in elevated levels of SIRT1 protein in the skeletal muscle of rats [147]. Prolonged moderate exercise training resulted in increased SIRT1 levels in rat hearts. Moreover, the results demonstrated an enhanced content and expression of FOXO3, along with elevated expression of GADD45 [148]. In addition, this study concluded that exercise normalizes the aging-associated alteration in heart and adipose tissue. Similar conclusions were drawn with skeletal muscle, whereby exercise counterbalanced the age-associated decline in NAMPT content and decrease in SIRT1 activity [42]. Data indicate that exercise can attenuate the age-associated decrease in mitochondrial protein synthesis in skeletal muscle of rats, which included the loss of SIRT1 and PGC-1 α levels as well [149]. Similar data have been reported for mouse liver [150].

Studies using human skeletal muscle have also shown exercise-related upregulation of NAMPT content [151], an important adaptive response to exercise-induced metabolic challenge, which can help to regulate redox balance through the NAD:NADH ratio, thus modulating the activities of sirtuins, PARP, and lactate dehydrogenase. Two weeks of high-intensity interval training in humans resulted in significant increases in mitochondrial biogenesis, increased nuclear PGC-1 α content, and increased GLUT4 content, all of which appear indicative of improved insulin sensitivity of these subjects [152]. A single bout of exercise increased the SIRT1 expression in young individuals, whereas it failed to have similar effects on the aged, and, in general, young subjects responded with greater levels of mRNA expression of mitochondrial biogenesis-related genes [117].

Exercise appears to increase SIRT1 levels in the hippocampus [153], but not in the cerebellum [100]. However, data from other studies have shown that age-associated decreases in brain

function can be alleviated by regular exercise [82,154]. Moreover, recent data indicate that the age-induced increases in Ku70 levels can be attenuated by the combined effects of IGF-1 and exercise [155]. The protein content of SIRT1 decreased, whereas SIRT3 increased, with aging. Exercise-induced redox and metabolic challenges and adaptive responses to regular activity lead us to suggest that sirtuin-mediated metabolic pathways could be important to mitochondrial biogenesis, fuel availability, antioxidant response, and DNA repair.

Conclusion

Sirtuins are master regulators of a wide range of metabolic processes in mammalian cells, and aging affects sirtuin-associated molecular pathways. Sirtuins could be on both sides of redox regulation, because the oxidative stress-related loss of NAD content would affect their activity. However, by their interactions with a number of transcription factors that target pro-oxidant and antioxidant genes, such as p53, NF- κ B, PGC-1 α , and FOXOs, sirtuins are not just dependent but also regulatory factors of the redox state. In addition, the role of sirtuins in oxidative damage repair is another pathway that could significantly alter the redox state. Accumulating data suggest that sirtuins, especially SIRT1, are potential targets for natural and pharmaceutical interventions into aging processes. Among natural interventions, it seems that CR and regular physical exercise act through sirtuin-associated processes, which has promise to improve health.

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