Seven sirtuins for seven deadly diseases of aging

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Review Article

Seven sirtuins for seven deadly diseases of aging

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ABSTRACT

Sirtuins are a class of NAD^+-dependent deacetylases having beneficial health effects. This extensive review describes the numerous intracellular actions of the seven mammalian sirtuins, their protein targets, intracellular localization, the pathways they modulate, and their role in common diseases of aging. Selective pharmacological targeting of sirtuins is of current interest in helping to alleviate global disease burden. Since all sirtuins are activated by NAD^+, strategies that boost NAD^+ in cells are of interest. While most is known about SIRT1, the functions of the six other sirtuins are now emerging. Best known is the involvement of sirtuins in helping cells adapt energy output to match energy requirements. SIRT1 and some of the other sirtuins enhance fat metabolism and modulate mitochondrial respiration to optimize energy harvesting. The AMP kinase/SIRT1–PGC-1α–PPAR axis and mitochondrial sirtuins appear pivotal to maintaining mitochondrial function. Downregulation with aging explains much of the pathophysiology that accumulates with aging. Posttranslational modifications of sirtuins and their substrates affect specificity. Although SIRT1 activation seems not to affect life span, activation of some of the other sirtuins might. Since sirtuins are crucial to pathways that counter the decline in health that accompanies aging, pharmacological agents that boost sirtuin activity have clinical potential in treatment of diabetes, cardiovascular disease, dementia, osteoporosis, arthritis, and other conditions. In cancer, however, SIRT1 inhibitors could have therapeutic value. Nutraceuticals such as resveratrol have a multiplicity of actions besides sirtuin activation. Their net health benefit and

Abbreviations: ABCA1, ATP-binding cassette transporter A1; ACADL, acyl-CoA dehydrogenase, long chain; ACSS2, acyl-CoA synthase short-chain family member 2; AgRP, agouti-related peptide; AKT, also known as protein kinase B; ALDH2, aldehyde dehydrogenase 2 family, mitochondrial; AMPK, AMP kinase; APC, anaphase-promoting complex; AROS, activator of SIRT1; ATG, autophagy-related protein; ATF4, activating transcription factor 4; ATM, ataxia telangiectasia mutated; ATP5A1, F_1F_0-ATP synthase, H^+ transporting, mitochondrial F1 complex, α subunit 1; BATF, basic leucine zipper transcription factor; BCL6, B cell lymphoma 6 protein; BCR, breakpoint cluster region gene; BMAL1, brain and muscle ARNT-like 1; CBP, CREB-binding protein; CCAR, cell cycle and apoptosis regulator; CDK, cyclin-dependent kinase; CHK, cell-cycle checkpoint kinase; ChREBP, carbohydrate responsive element-binding protein; CIITA, class II transactivator; CNS, central nervous system; CoA, coenzyme A; COX, cyclooxygenase; CPS1, carbamoyl phosphate synthetase 1; CPT1, carnitine palmitoyl transferase-1; CRTC, CREB-regulated transcription coactivator; CREB, cyclic-AMP-responsive-element-binding protein; CreP, constitutive repressor of eIF2α phosphorylation; CRP, C-reactive protein; DBC1, deleted in breast cancer 1; DNMT1, DNA (cytosine-5)-methyltransferase 1; DYRK, dual specificity tyrosine-phosphorylated and regulated kinase; E2F1, E2F transcription factor 1; eIF2α, eukaryotic initiation factor 2α; EPC, endothelial progenitor cell; eNOS, endothelial nitric oxide synthase; ER, estrogen receptor; ERK1/2, extracellular signal-regulated kinase 1/2; EVI1, ectopic viral integration site I; FMRP, fragile X mental retardation protein; FOXO, forkhead box; class O, transcription factors; FXR, farnesoid X receptor; G6P, glucose 6-phosphatase; GADD34, growth arrest and DNA damage-inducible protein 34; GDH, glutamate dehydrogenase; HBX, hepatitis B virus X protein; HIF, hypoxia-inducible factor; HISTH3, histone cluster 3, H3; HMGCS2, 3-hydroxy-3-methylglutaryl CoA synthase 2; HNF, hepatocyte nuclear factor 1; HOXA, homeobox A; HSF, heat shock factor; IDH2, isocitrate dehydrogenase 2; IGF-1, insulin-like growth factor-1; IKZF, ikaros family zinc finger protein; JNK, JUN N-terminal kinase; KU70, DNA repair factor KU70; LC3-II, microtubule-associated protein 1 light chain 3-II; LCAD, long-chain acyl CoA dehydrogenase; LKB1, liver kinase B1; LOX-1, lectin-like oxidized LDL receptor-1; LSD, methyltransferase lysine-specific demethylase; LXR, liver X receptor; MAP, mitogen-activated protein; MEF2, myocyte enhancing factor 2; MHC, major histocompatibility complex; MKP3, mitogen activated protein kinase phosphatase 3; MMP, matrix metalloproteinase; MRPL10, mitochondrial ribosomal protein L10; MYOD, myoblast determination protein; NAMPT, nicotinamide phosphororibosyltransferase; NBS1, Nijmegen Breakage Syndrome 1; NCoR, nuclear receptor corepressor; NDUFA9, NADH dehydrogenase (ubiquinone) 1α subcomplex 9; NDUFS8, NADH:ubiquinone oxidoreductase 8; NF-κB, nuclear factor-κB; NA, nicotinic acid; NMN, nicotinamide mononucleotide; NR, nicotinamide riboside; OTC, ornithine transcarbamoyltransferase; OXPHOS, oxidative phosphorylation; $P450_{SCC}$, P450 side chain cleavage enzyme; p53, tumor suppressor p53; PAR3, partitioning defective 3 homologue; PARP, poly(ADP-ribose) polymerase; PEPCK, phosphoenolpyruvate carboxykinase; PER2, period circadian protein homolog 2; PGC1α, peroxisome proliferator-activated receptor-γ coactivator 1α; Pi, inorganic phosphate; PID, peptidylpropyl isomerase D (cyclophilin D); PKA, cAMP-dependent protein kinase A; PKB, protein kinase B; POMC, proopiomelanocortin; PPAR, peroxisome proliferator-activated receptor; PRC, polycomb repressive group complex; PTEN, phosphatase and tensin homolog; RARβ, retinoic acid receptor-β; Rb, retinoblastoma protein; RIP, receptor-interacting protein; ROS, reactive oxygen species; RUNX2, runt-related transcription factor 2; SDH, succinate dehydrogenase; SDHA, SDH subunit A flavoprotein; SIRT, sirtuin; SMRT, silencing mediator of retinoid and thyroid hormone receptor; SNP, single nucleotide polymorphism; SOD, superoxide dismutase; SOST, sclerostin; SREBP, sterol regulatory element-binding protein; STAC, sirtuin activating compound; STAT3, signal transducer and activator of transcription 3; STAU, staufen; STK11, serine/threonine kinase 11 (LBK1); SUMO, small ubiquitin-like modifier; SUV39H1, a methyltransferase; TCA, tricarboxylic acid cycle; TDP-43, 43-kDa transactive response DNA-binding protein; TORC, CREB-regulated transcription coactivator; UCP2, uncoupling protein 2; USP22, ubiquitin specific peptidase 22; WRN, Werner syndrome ATP-dependent helicase; XRCC6, X-ray repair complementing defective repair in Chinsese hamster cells 6 (Ku70)

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Type 2 diabetes
Obesity
Dementia
Cancer

relative safety may have originated from the ability of animals to survive environmental changes by utilizing these stress resistance chemicals in the diet during evolution. Each sirtuin forms a key hub to the intracellular pathways affected.

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Introduction

The "Seven Deadly Sins" of old (sloth, gluttony, wrath, greed, pride, lust, and envy) have overlapping counterparts in modern society (sloth, gluttony, junk food, smoking, alcoholism, drug abuse, and psychosocial stress). These "sins" contribute to the seven deadly conditions that increase in prevalence with aging (obesity, type 2 diabetes, cardiovascular disease, cancer, dementia, arthritis, and osteoporosis).

The sirtuins are a class of NAD^+-dependent deacetylases comprising seven members in humans and other mammals [1]. These enzymes have attracted major interest because of their apparent roles as protectors, and controversially, as contributors to all or some of the life-threatening conditions of aging. More specifically, the sirtuins are important in the transduction pathways emanating from energy sensing. Their ability to regulate systems that control the redox environment has the potential to help counteract oxidative damage that is associated with common diseases of aging and that contributes to aging itself [2]. Because sirtuin malfunction likely has pathophysiological consequences in common clinical conditions of aging pharmaceutical agents targeting sirtuins have been developed [3].

The history leading up to the explosion in research on sirtuins stems from the finding that calorie restriction can extend rat life span [4,5] and later evidence that the natural flavanoid resveratrol might mimic this effect in lower organisms [6–8]. The gain in life expectancy achieved by calorie restriction was suggested to exceed that achieved by curing cardiovascular disease, cancer, and type 2 diabetes combined [9]. Since population level calorie restriction is unrealistic, many therefore asked whether sirtuin activation might mimic calorie restriction [10] or at least delay onset of age-related diseases by "compression of morbidity" [11].

Although the first sirtuin was discovered in 1984 in yeast [12], interest did not really take off until an effect on life span was noted, first in yeast in 1997 [13], then in higher eukaryotes such as the nematode worm *Caenorhabditis elegans* in 2001 [14] and the fruit fly *Drosophila melanogaster* in 2004 [15]. The nature of sirtuins as NAD^+-dependent deacetylases was recognized in 2000 [16] and immediately implicated them in the metabolic state of the cell [17]. Once their enzymatic actions in the cell started to be elucidated it was soon realized that their apparent ability to extend life span involved similar pathways as utilized by calorie restriction.

A role for the most-studied sirtuin, SIRT1, in life span has now been refuted, however. Manipulation of SIRT1 expression or activity has little or no effect on mammalian life span [18,19]. And subsequent studies in model organisms using better controls showed that SIRT1 activation or deletion had little [20,21] or no [22] effect on their life span. So too calorie restriction it seems, at least in primates, with the 25-year study of rhesus monkeys having found no effect on life span [23], in contrast to earlier findings by others that did [24]. The earlier study involved *ad libitum* feeding of controls with a different diet that notably included 28% (as opposed to 4%) sucrose, leading to overweight and diabetes. So earlier death of controls may partly explain the discrepancy. Disease onset was later and health markers were nevertheless more favorable in the calorie-restricted group of each cohort of monkeys.

Overexpression of SIRT6, however, extended the life span of male mice by 15%, in effect extinguishing the sex difference in mouse longevity [25]. This effect was seen in mice with different genetic backgrounds. While SIRT1 deacetylates numerous targets, the best-known substrates for SIRT6 are two histones (H3K9 and HK56); the targeting of these explains SIRT6's role in DNA damage response, especially during oxidative stress, and in telomere maintenance [26–28]. While SIRT1 is more similar *structurally* to the single sirtuin present in yeast, SIRT6 is more similar *functionally* to yeast sirtuin [29]. Male SIRT6 transgenic mice exhibit a female metabolic profile, most notably in fat tissue, in which changes seem linked to life span [29]. The liver of male mice showed the biggest changes in gene expression [25], which overlapped those seen in calorie-restricted mice [30,31]. Serum IGF-1 level was reduced modestly in male mice, as was IGF-1 signaling in adipose tissue [25]. Thus SIRT6 has partial feminizing effects in male mice. Loss of SIRT6 causes severe metabolic defects, rapid aging, and death at 4 weeks of age [32].

The seven mammalian sirtuins all appear to be important in suppression of such common diseases of aging as cardiovascular disease, type 2 diabetes and dementia [33–35]. Effects involving mitochondrial function are crucial to this.

Here I provide the most extensive review of sirtuins to date. This describes their function and roles in disease processes. Because there are over 2500 publications on sirtuins, only a selective overview is provided. Moreover, for simplicity, this review will use the human nomenclature "SIRT" rather than "Sirt" or "SirT" to refer to both the human and rodent sirtuin proteins and genes. All abbreviations are shown in the footnote on the previous page.

Intracellular localization

SIRT1 is localized in the nucleus [36] (Table 1), but it shuttles to the cytoplasm when required to act on cytoplasmic targets, such as during inhibition of insulin signaling [37]. During prometaphase, levels increase and SIRT1 associates with mitotic chromatin until telophase [38]. It mediates loading of histone H1 and the condensing I complex to chromatin, thus contributing to chromosomal condensation [38].

In contrast, SIRT2 is cytoplasmic (Table 1). It deacetylates tubulin microtubules [39] and transcription factors that shuttle from the cytoplasm to the nucleus [40]. During mitosis SIRT2 is required for exit of cells from the mitotic phase [41]. It localizes to chromatin and, in part by a dynamic interplay with the human ortholog of MOF [42], deacetylates acetylated histone H4K16, and thus decreases the latter during G2/M transition [43]. SIRT2 is thereby pivotal to formation of condensed chromatin when the latter must be generated anew [43]. Moreover, SIRT2, by deacetylating BubR1 kinase, may help ensure faithful chromosome separation during mitosis [44].

SIRT3, SIRT4, and SIRT5 are located in mitochondria [36,45] (Table 1). SIRT6 and SIRT7 are nuclear, being present in heterochromatin and nucleoli, respectively [36]. However, during G1, but not the S phase of the cell cycle, SIRT6 is present in nucleoli, and when overexpressed it slows down mitosis [46]. More about the function of each in their respective locations follows.

Table 1
The activity and localization of each sirtuin.

Sirtuin	Activity	Location	Targets
SIRT1	Deacetylation	Nucleus Cytosol	FOXO1, FOXO3, PGC-1α, p53. NF-κB, Notch, HIF1α, LXR, FXR, SREBP1c, etc
SIRT2	Deacetylation	Cytosol	FOXO1, PEPCK, tubulin, PAR-3
SIRT3	Deacetylation ADP-Ribosylation	Mitochondria	OXPHOS complexes, SOD2, LCAD, HMGCS2, GDH, IDH2, PIP2, ACADL, FOXO3, ACSS2, OTC, GLUD1, NDUFA9, SDHA, ATP5A1, ALDH2, MRPL10, STK11, HISTH3, XRCC6
SIRT4	ADP-ribosylation	Mitochondria	GDH
SIRT5	Deacetylation Demalonyation Desuccinylation	Mitochondria	CPS1
SIRT6	ADP-ribosylation	Nucleus	H3K9, H3K56
SIRT7	deacetylation	Nucleolus	

References to the information in this table can be found in the text and Ref. [45] in the case of mitochondrial sirtuins.

Enzymatic activity of each sirtuin

Each sirtuin has a characteristic enzymatic activity [47–49] (Table 1). SIRTs 1, 2, 3, 5, and 6 have NAD^+-dependent deacetylase activity [16,50]. Whether SIRT7 is [51] or is not [36] a deacetylase [3] has now been clarified by recent evidence pointing to an important role for SIRT7 in deacetylation of acetylated histone H3K18 [52]. Deacetylation is the most prominent activity of SIRT1 [16] and SIRT2 [39]. In contrast, SIRT4 [53] and SIRT6 [54] possess mostly mono-ADP-ribosyl transferase activity. SIRT3 [55] and SIRT 6 [26,54,56] possess both activities. SIRT5 was originally reported to deacetylate CPS1 [57], but its demalonylation [58,59] and desuccinylation [58] actions on CPS1 and other proteins now seem more important. It has therefore been suggested that sirtuins should be redefined as "deacylases" rather than deacetylases [58].

A 25 amino acid sequence in the C-terminal domain of SIRT1 is essential for SIRT1 activity, serving as an "on" switch for the deacetylase core [60]. By itself the catalytic core has low catalytic activity, but regions in the N- and C-terminal potentiate catalytic activity 12- to 45-fold [61]. NAD^+ levels increase during exercise, fasting, and calorie restriction [62,63]. During such "energy stress," NAD^+ may serve as a metabolic sensor leading to SIRT1 activation. While the 2-fold range of intracellular NAD^+ fluctuations encompasses the K_m of SIRT1, it is not clear how much NAD^+ is actually bioavailable [63].

Conversion of NAD^+ to its reduced form, NADH, lowers sirtuin activity [64]. A high-fat diet reduces the NAD^+/NADH ratio and sirtuin activity in mice [65]. NAD^+ can be synthesized from tryptophan [66] and the dietary vitamin B3 components nicotinic acid and nicotinamide [67], as well as nicotinamide ribose present in milk [68]. Although the NAD^+ precursor nicotinamide mononucleotide can activate SIRT1 and improve glucose tolerance, it is not present in the human diet [69]. Whereas at low levels nicotinamide serves as a NAD^+ precursor, thus producing an increase in SIRT1 activity, at high levels nicotinamide exerts potent, end-product inhibition of SIRT1 in a manner that is noncompetitive with NAD^+ [70,71], making nicotinamide deleterious to the cell [72]. Nicotinamide, by increasing the NAD^+/NADH ratio, induces SIRT1-mediated autophagy [73]. A conserved serine in the catalytic domain of SIRT1 can be phosphorylated in response to cAMP-mediated PKA activation, leading to increased SIRT1 activity independent of NAD^+ [74]. This may have relevance to β-adrenoceptor- or cold-mediated stimulation of fatty acid oxidation and energy expenditure [74].

Alterations in the activity of enzymes that deplete intracellular NAD^+ also affect sirtuin activity. The protein nimbrin, involved in DNA repair, is mutated in Nijmegen breakage syndrome, a condition exhibiting elevation in ROS and hyperactivation of PARP enzymes, which consume and thereby lower NAD^+, thus reducing SIRT1 activity [66]. But when PARPs are inhibited, SIRT1 is activated [75]. Since SIRT1 and PARPs are confined to the nucleus, PARP gene deletion has no effect on SIRT2 or SIRT3 [75,76]. CD38 also consumes NAD^+, and CD38 deletion increases sirtuin activity [77].

Thus sirtuins have unique and overlapping activities and their activation is exquisitely sensitive to modulation of NAD^+/NADH ratios.

Actions of nuclear sirtuins (SIRT1 and SIRT6)

These are crucial in adaptation of metabolic processes to redox state.

SIRT1

There is now considerable data on how dietary restriction exerts antiaging effects via SIRT1. Histone deacetylation was noted first [78] and then later deacetylation of nuclear receptors and the transcriptional coactivator PGC-1α [79], FOXO proteins [80–82], and transcription factors and their cofactors (see reviews [34,49,63]). The activation of SIRT1 by calorie restriction stimulates PGC-1α to induce expression of gluconeogenic genes and reduces the repressive effect of PGC-1α on glycolytic genes, thus increasing hepatic glucose output [79]. By activating PPAR-α, SIRT1 increases fatty acid oxidation [83].

Screening for transcription factors that partner with SIRT1 in response to nutrient restriction identified HNF-1α, a homeobox protein involved in regulation of β-cell and hepatocyte function [84]. The HNF-1α–SIRT1 complex binds to two sites in the promoter of the gene for the well-known disease marker C-reactive protein.

Deacetylation of FOXOs by SIRT1 [80,82] channels transcriptional regulation toward specific targets in pathways involved in stress resistance, lipid metabolism, and apoptosis [85]. Whereas deacetylation of FOXO3A by SIRT1 suppressed apoptosis genes, it activated stress resistance genes [80]. An additional stress-derived signal is required to trigger the functional interaction of FOXOs and SIRT1 in the nucleus.

PGC-1α is the master regulator of mitochondrial biogenesis, doing so by orchestration of a constellation of transcription factors needed for induction of gene expression in mitochondria [79,86]. Just as is the case for FOXOs, the coexistence of PGC-1α and SIRT1 in the nucleus does not lead to deacetylation unless additional signals concerning energy stress occur. This involves an imbalance in AMP/ATP ratio [87]. When this ratio is increased, SIRT1 and AMPK are activated [87]. The link between SIRT1 and AMPK is one of the most important connections to have emerged in recent years [64]. Each is activated under conditions of energy deprivation, such as occurs during calorie restriction. SIRT1 and AMPK work in synergy to adjust cellular physiology. AMPK phosphorylates, and thereby activates, PGC-1α [88]. This effect primes PGC-1α for deacetylation and activation by SIRT1 [64], although, *in vitro*, SIRT1 can deacetylate nonphosphorylated PGC-1α [89]. It has been suggested that the phosphorylation either modifies the nuclear localization or facilitates interaction with proteins capable of stabilizing the SIRT1–PGC-1α interaction [63]. FOXOs are also phosphorylated by AMPK in response to energy stress [90]. It was therefore suggested that a similar mechanism as for PGC-1α might explain why, despite their coexistence in the nucleus, the interaction of FOXOs with SIRT1 only occurs during energy stress. The AMPK/SIRT1/PGC-1α signaling pathway is utilized by several hormones, such as leptin, adiponectin, and fibroblast growth factor 21, to increase mitochondrial metabolism [91–93].

Hepatic effects of SIRT1 involve control of two pathways that affect gluconeogenesis in opposite ways. The activation by SIRT1 of FOXO1 and PGC-1α favors glucose production [79]. In contrast, deacetylation by SIRT1 of CRTC-2 leads to CRTC2 destabilization, with the result that glucose production is suppressed [94]. Each pathway is fine-tuned according to the length of fasting. Overall, with steady calorie restriction, the net effect is a mild increase in glucose output [64].

SIRT1 modulates carbohydrate metabolism by deacetylating other transcription factors. In the case of gluconeogenesis, which maintains blood glucose levels during fasting, regulation involves CREB, which in turn is controlled by CRTCs [95]. In the early stages of fasting CRTC2 translocates to the nucleus, where it activates CREB on promoters for genes such as PEPCK and G6P [94]. With prolonged fasting, increased NAD^+ in the liver activates SIRT1, which deacetylates CRTC2, shunting it to degradation pathways [79,94]. In this way SIRT1, by attenuating gluconeogenesis, helps prevent premature energy depletion during the fasting state. The ability of SIRT1 orthologs to decrease CRTCs may be one mechanism by which abrogation of CRTC and CREB orthologs in *C. elegans* had been thought to prolong life span [96].

After deacetylation, both PGC-1α and FOXOs increase lipid catabolism and mitochondrial respiration [63]. SIRT1 also inhibits lipid anabolism. SIRT1 forms a complex with NCoR1 [97], leading to interference with PPAR-γ signaling in white adipose tissue and LXR [98]. During fasting the suppression of PPAR-γ activity results in fat mobilization instead of storage. The SIRT1–LXR pathway appears important for reverse cholesterol transport. The beneficial effects of SIRT1 on cholesterol metabolism may involve not only LXR deacetylation but also deacetylation of SREBP-1c. As a result, the affinity of SREBP-1c for promoters of lipogenic target genes is decreased and, via ubiquitination and ensuing degradation, SREBP-1c protein levels in the cell are reduced (see review, [63]). This could explain how SIRT1 might promote beneficial effects on cholesterol metabolism with no detrimental effect on lipid accumulation in the liver. In this regard SIRT1 prevents steosis during high-fat feeding [99,100].

The processes described above explain how SIRT1 is able to orchestrate metabolism at the cellular and whole-body level in order to extract energy from sources other than carbohydrate—in particular, pathways involving mitochondrial respiration (Fig. 1).

By actions on circadian clock proteins [101], circadian rhythm appears connected to SIRT1's deacetylation reactions that affect aging and metabolism. Feeding/fasting cycles influence the circadian clock via NAD^+ levels, that then influence SIRT1 activity and thus metabolism [63].

SIRT1 assists in stress resistance via p53, HIF-1α, and HIF -2α, as well as HSF-1 [102]. It regulates the cellular response to oxidative and genotoxic stress by binding to eIF-2α, as well as effects involving the mediators of eIF2α dephosphorylation, growth arrest, and GADD34 protein and CreP [103].

SIRT1 is intimately linked to the formation of facultative and constitutive heterochromatin. This effect is crucial during the cellular response to stress. In the case of facultative heterochromatin formation, SIRT1 deacetylates the acetylated forms of histones H4K16, H3K9, and H1K26 [104]. It also promotes methylation of H3K9 in conjunction with the histone methyltransferase SUV39H1 [105]. SIRT1 is important for histone gene expression, which takes place during the S phase of the cell cycle. Histone gene regulation involves recruitment of SIRT1 and the CBP–p300 coactivator complex to histone promoters [106]. The role of SIRT1 in DNA repair involves actions on NBS1, PARP-1, Ku70, and WRN [102]. In the case of constitutive heterochromatin formation, SIRT1 enhances SUV39H1 protein levels and turnover, thus leading to greater genomic integrity during the stress response [107].

There are also targets for SIRT1 in the cytosol. Cytosolic acetyl-CoA synthetase 1 is only deacetylated, and thereby activated, by SIRT1 [108], although the physiological role of this is unclear. SIRT1 activates eNOS in the cytoplasm [109]. This increases NO, leading to vasodilatation, increased blood flow, and nutrient delivery to tissues.

Cytosolic SIRT1 regulates autophagy by deacetylation of key components of the autophagic machinery ATG5, ATG7, and ATG8 [110]. This prevents accumulation of damaged organelles, especially mitochondria, during starvation [110], consistent with SIRT1 being a master metabolic switch that drives the cell to derive energy from sources other than carbohydrate.

SIRT1 represses NF-κB-dependent expression of STAT3, thus lowering mitochondrial respiration [111]. FOXO3A, by inhibiting c-MYC, represses a large number of nuclear-encoded mitochondrial genes, leading to reduction in mitochondrial copy number, mitochondrial proteins, respiratory complexes, and mitochondrial respiratory activity [112]. FOXO3A inhibits the increase in ROS and HIF-1α stabilization normally seen during hypoxia [112].

A hallmark of most diseases of aging is inflammation. Not surprisingly, SIRT1 is involved in countering inflammatory processes [63,64]. This involves negative regulation of NF-κB [113].

SIRT1 activity in cells is also altered by factors that regulate its transcription and thus its intracellular concentration, by post-transcriptional regulation and posttranslational modifications, as will be discussed later.

SIRT6

Deficiency of this other nuclear sirtuin in mice leads to low IGF-1, severe hypoglycemia, and early death [32]. SIRT6-deficient cells show increased HIF-1α, glucose uptake, and glycolysis, and diminished mitochondrial respiration [56]. The high glucose uptake by muscle and brown adipose tissue leads to severe, potentially fatal hypoglycemia [56]. Loss of SIRT6 is accompanied by genome instability. This is because during oxidative stress SIRT6 is recruited to sites of DNA double-strand breaks, where it stimulates DNA repair through both nonhomologous end-joining and homologous recombination [114]. SIRT6 binds to, and mono-ADP-ribosylates,

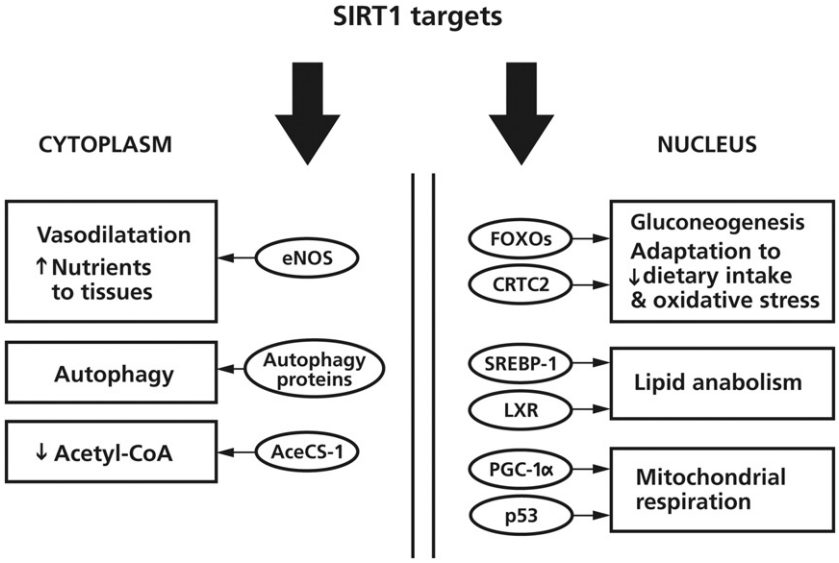

Fig. 1. Proteins that are targeted by SIRT1 for deacetylation and the metabolic processes that these regulate. Protein targets in the nucleus that are implicated in transcriptional metabolic adaptations are shown on the right, and those in the cytoplasm are shown on the left. (Modified from [63]).

PARP-1, leading to increased PARP poly-ADP-ribosylase activity and DNA repair [114]. Overexpression of SIRT6 in "middle aged" and presenescent cells strongly stimulates PARP1-dependent homologous recombination repair, suggesting that in aging cells the precise homologous recombination pathway becomes repressed, giving way to a more error-prone nonhomologous DNA end-joining pathway [115]. Mice in which deletion of SIRT6 is neural specific are small at birth owing to low growth hormone and IGF-1, but exhibit catch-up growth and become obese in adulthood [116]. Moreover, histone H3 in the brain becomes hyperacetylated [116]. The occurrence of the latter in regions involved in neuroregulation points to a central role of SIRT6 in obesity prevention via modulation of neural chromatin structure.

SIRT6 is a transcriptional regulator. A deficiency of SIRT6 alters expression of hundreds of genes [117–119]. Like SIRT1, SIRT6 is able to repress NF-κB activity [117]. In response to TNF-α, SIRT6 exhibits a highly dynamic response on mouse promoters across the genome, with more than half of SIRT6 target genes only becoming apparent in response to stress-induced signaling that involves NF-KB [118]. The RelA subunit of NF-κB recruits SIRT6 and is responsible for SIRT6 relocalization [118]. The epistatic interaction between RelA and SIRT6 determines the shaping of diverse temporal patterns of gene expression, including prominent genes involved in cell senescence and aging [118].

Actions of mitochondrial sirtuins (SIRT3, SIRT4, and SIRT5)

These sirtuins help the cell adapt to reduced energy consumption [45].

SIRT3

SIRT3 is the major mitochondrial deacetylase [120]. It regulates the acetylation status, and thus activity, of metabolic enzymes such as acetyl CoA synthetase 2 [45]. During prolonged fasting, upregulation of SIRT3 in liver and brown adipose tissue leads to activation of long-chain acyl dehydrogenase and ornithine transcarbamoylase involved in β-oxidation of fatty acids and the urea cycle [121,122]. It could be that involvement of sirtuins in the production of ATP from catabolism of fat rather than carbohydrates may protect against ROS production and aging [123]. In support of this, SIRT3 deletion exacerbates diet-induced obesity [124]. Another target of SIRT3, HMGCS2 [125], regulates ketone body production, thus helping supply energy to the brain during fasting. During calorie restriction SIRT3 activates the TCA cycle components IDH2 [126,127] and GDH [120,126]. GDH then promotes metabolism of glutamate and glutamine to generate ATP, which results in insulin secretion.

SIRT3 maintains basal ATP levels and regulates mitochondrial electron transport by stimulatory effects on mitochondrial respiration [128]. SIRT3 targets involved in OXPHOS were identified by a proteomic screen [129]. These included electron transport chain complex I components (NDUFA11 and NDUFS8) [128,129], complex II components (SDHA and SDHB) [129], complex III component (56-kDa core I subunit) [130], and complex V components (ATP5A1, ATP5B1, and ATP5F1) [129]. Others targeted were proteins that bind to SIRT3 [129]. Thus SIRT3 controls the final stage of mitochondrial aerobic respiration and may be an important regulator of SDH activity in cells and mouse brown adipose tissue [129]. Despite these effects, SIRT3-deficient mice exhibit normal basal metabolic rate and adaptive thermogenesis [120].

SIRT3 protects cells from oxidative stress in several ways. One is by reducing ROS, since ROS production is elevated in SIRT3 knockout mice [130]. Another is SIRT's ability to deacetylate and activate SOD2, a major mitochondrial antioxidant enzyme [131–133]. The activation of IDH referred to above [127] increases NADPH generation, leading to in an increase in the ratio of reduced-to-oxidized glutathione in mitochondria and protection against oxidative stress-induced cell death via enhanced detoxification of ROS. SIRT3-dependent mitochondrial adaptations, by stimulating pathways associated with calorie restriction, might be a central component of age retardation in mammals [127]. Cardiac protection by SIRT3 involves activation of FOXO3A, MnSOD, and catalase, thus reducing ROS, a reduction in activation of Ras, and downstream signaling through the MAPK/ERK and PI3K/AKT (also known as PKB) pathways, blocking hypertrophy and interstitial fibrosis [134]. The ability of SIRT3 to reduce superoxide is accompanied by increased genomic stability [135]. Thus the SIRT3-mediated increase in efficiency of OXPHOS protects against the damaging effects of ROS.

Other protective effects involving SIRT3 include prevention of cell death caused by hypoxia and staurosporine. SIRT3 does this by preventing loss of mitochondrial membrane potential, intracellular acidification, and the accumulation of ROS [136]. Senescence of human diploid fibroblasts induced by high glucose is reduced when the SIRT3–FOXO1 signaling pathway is activated [137].

SIRT3 also acts as a tumor suppressor [135]. This involves suppression of ROS. Consistent with this, SIRT3 is inactivated in many tumors [135,138]. When overexpressed in tumor cell lines SIRT3 reverses the Warburg effect [138], which is the metabolic reprogramming that occurs in cancer cells to enforce the production of ATP by glycolysis. SIRT3 appears to achieve this by reducing HIF-1α, thus suppressing genes for glycolysis and angiogenesis [138].

Stimulation by resveratrol of the enzyme involved in the first step of adrenal steroid hormone synthesis, $P450_{SCC}$, located in the mitochondrial inner membrane, involves SIRT3 and SIRT5 [139]. Interestingly, another enzyme required for cortisol synthesis, $P450_{11\beta}$, is also stimulated by resveratrol [139].

SIRT4

This mitochondrial sirtuin appears to be involved primarily in metabolism. In pancreatic β-cells SIRT4, via ADP-ribosylation, represses GDH [53]. Thus SIRT4 and SIRT3 have opposing roles in GDH regulation [53,120,126]. They also have opposing roles in fatty acid oxidation [121,140]. What is not known is how SIRT3 and SIRT4 integrate similar nutrient states into opposite responses. The decline in SIRT4 in β-cells and liver during calorie restriction increases glutamine availability for catabolic metabolism, as well as glucose production in the liver. Depletion of SIRT4 in hepatocytes and myotubes, or *in vivo*, increases mitochondrial and fatty acid metabolizing enzymes, as well as SIRT1, which mediates the effect on fatty acid oxidation [140]. Thus inhibition of SIRT4 may have implications for type 2 diabetes, a condition in which ectopic lipid storage is elevated.

SIRT5

The effects mediated by this mitochondrial sirtuin include activation of the first and rate-limiting enzyme in the urea cycle—CPS1 [57,141]. This facilitates the disposal of ammonia during calorie restriction, when amino acids are used as fuel sources. As stated earlier, the main enzymatic activity of SIRT5 is, however, demalonylation and desuccinylation [59], including of CPS1 [58].

Actions of the cytosolic sirtuin (SIRT2)

SIRT2 deacetylates FOXO1 [142] and FOXO3 [143,144], thus implicating it in the diversity of processes that these key transcription factors regulate.

SIRT2 overexpression delays cell cycle progression [41]. It colocalizes with microtubles in the cytoplasm, where it deacetylates α-tubulin [39,145]. During mitosis SIRT2 increases [41]. It migrates transiently into the nucleus during G2/M transition [39], where it deacetylates histone H4 to modulate chromatin condensation during metaphase [43,146]. SIRT2 also deacetylates histone H3 [147]. These effects implicate SIRT2 in cell cycle regulation [146,148].

A specific target of SIRT2 is the acetyl transferase p300 [149]. Autoacetylation of p300 is a transcriptional regulatory checkpoint. Deacetylation by SIRT2 of a lysine in the catalytic domain of p300 restores binding of p300 to the preinitiation complex, thus affecting preinitiation complex assembly [149]. In turn, p300 is able to acetylate SIRT2 [150], thus attenuating its deacetylase activity and relieving the inhibitory effect of SIRT2 on p53 transcriptional activity [150].

SIRT2 binds to 14-3-3 β and γ protein isoforms [151], which are involved in control of a multitude of signaling molecules such as kinases and phosphatases, as well as transmembrane receptors [152]. The SIRT2–14-3-3β/γ interaction enhances the AKT-dependent deacetylation of p53 by SIRT2, thus reducing the effect of p53 on transcription [151]. The homeobox transcription factor HOXA10 is another SIRT2 binding partner [153], although more work is required to ascertain the biological role of this interaction.

By deacetylating the p65 subunit of NF-κB, SIRT2 regulates the wide repertoire of NF-κB-dependent genes [154]. It stabilizes PEPCK, the rate-limiting enzyme for gluconeogenesis, by deacetylation [155]. SIRT2 affects Schwann cell myelination by deacetylation of the cell polarity protein PAR-3 [156].

Actions of the nucleolar sirtuin (SIRT7)

SIRT7 associates with active ribosomal RNA genes and binds to histones and RNA polymerase I to stimulate transcription [157]. A proteomic analysis using HEK293 kidney cells identified 462 proteins bound by SIRT7, including 257 in the nucleus, 189 of which were nucleolar [158]. Its interaction with RNA polymerase I and upstream binding factor was confirmed, and new associations included 150 proteins involved in transcriptional processes involving both RNA polymerase I and II, as well as 32 associated with chromatin remodeling complexes, particularly members of the B-WICH complex known to associate with the RNA polymerase I machinery to facilitate rDNA transcription, and 30 relevant to proteosome/ubiquination processes [158]. By chromatin immunoprecipitation and sequencing, 276 binding sites for SIRT7 were identified in the genome, 241 of which were at protein-coding genes [52]. Most (74%) of the latter were close to the transcription start site where histone H3K18 binds and where H3K18 was shown to be a deacetylation target of SIRT7 [52]. Almost 60% of the SIRT7-binding sites contain the MAP kinase-signaling-dependent ETS transcription factor, ELK4 [52].

Mice with SIRT7 deletion develop inflammatory cardiomyopathy, cardiac hypertrophy, fibrosis, increased collagen III accumulation, hyperacetylation of p53, increased apoptosis, and reduced resistance to oxidative stress [51].

Transcriptional regulation of sirtuin genes

Most is known about SIRT1, whose expression is induced during low energy states such as nutrient or calorie deprivation [159], and repressed during energy excess, such as high-fat feeding [160].

Fig. 2 shows transcription factors and targets in the SIRT1 promoter. Activators include FOXO1 [159], PPAR-α [161] and PPAR-β (also known as PPAR-δ) [162], CREB [163], and the cell-cycle and apoptosis regulator E2F1 [164]. Repressors include PPARγ [165], ChREBP [163], HIC1 [166] (via CTBP [167]), and PARP-2 (involved in DNA repair, apoptosis, and transcription) [76].

SIRT3 transcription is stimulated by ERRα which binds to the transcriptional coregulator PGC-1α, leading to changes in expression of genes for development and function of brown adipose tissue [168].

The outcome of the various transcriptional effects will be discussed in subsequent sections.

Posttranscriptional regulation

The well-known posttranscriptional regulator HuR binds to the 3′UTR of mRNA for many different genes and enhances mRNA stability. HuR increases SIRT1 mRNA and protein levels [169]. Under oxidative stress the cell-cycle checkpoint kinase CHK2 phosphorylates HuR causing it to dissociate from SIRT1 mRNA causing HuR to decay [169].

Micro (mi) RNAs target specific sequences in the 3′UTR of mRNAs to cause mRNA decay [170]. Sixteen microRNAs can bind to the SIRT1 3′UTR [171]. The miRNAs miR-34a [172] and miR-199a [173] destabilize SIRT1 mRNA. More on miRNA effects will be discussed later.

Posttranslational regulation

Various posttranslational modifications of sirtuins 1 to 6 take place [174]. These direct sirtuins to specific targets. They can also increase activity, as occurs after phosphorylation of SIRT1 [175]. The pattern of SIRT1 phosphorylation by the cyclin B–CDK-1 complex affects the cell cycle and cell proliferation [175]. JNK1-mediated phosphorylation during oxidative stress increases nuclear localization of SIRT1 and orients SIRT1 to substrates such as histone H3, although not p53 [176]. Curiously, in obesity and metabolic syndrome, while SIRT1 activity is reduced [160], JNK activity is elevated [177]. In contrast, deacetylation of p53 occurs when SIRT1 is phosphorylated at threonine 522 by DYRK-1 and DYRK-2 [178]. This prevents apoptosis during genotoxic stress,

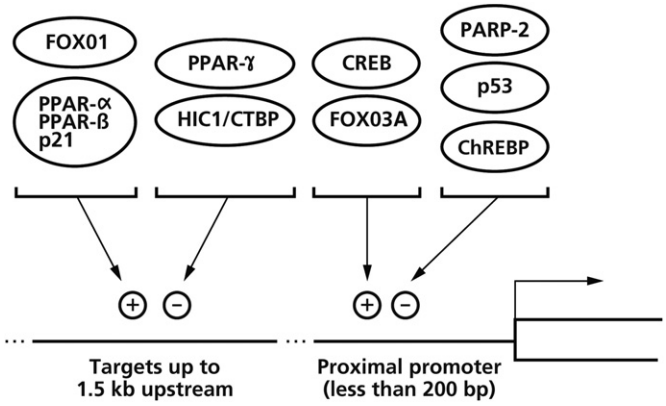

Fig. 2. Transcriptional regulation of the SIRT1 gene. Shown is the SIRT1 promoter and several of the transcription factors that influence transcriptional activity by acting on either proximal or distal regions of the promoter. Once synthesized, the SIRT1 protein can enhance the activity of some positive regulators such as FOXOs, and inactivate repressor complexes such as p53, PPAR-γ, and HIC1/CTBP. (Modified from [63]).

thus promoting cell survival [178]. Many other kinases phosphorylate SIRT1 at sites that differ between species.

A ubiquitin-specific peptidase, USP22, stabilizes SIRT1 by removing polyubiquitin chains conjugated to it [179]. Since USP22 is one of the 11 death-from-cancer signature genes and this effect leads to suppression of p53, USP22 may have critical roles in embryonic development and cancer.

SIRT2 phosphorylation by cyclin E–CDK2, cyclin A–CDK2, and p53–CDK5 inhibits SIRT2 activity [180]. SIRT2 is dephosphorylated by the dual specificity protein phosphatase CDC14B, which decreases SIRT2 abundance [41]. SIRT2 phosphorylation occurs late in G2, during mitosis, and into the period of cytokinesis [41]. CDC14B may provoke exit from mitosis when ubiquitination of SIRT2 results in its proteosomal degradation [41].

SUMO proteins can interact with ("SUMOylate") human, but not mouse, SIRT1, thus increasing its activity [181]. This affects apoptosis, leading to speculation that SUMOylation serves as a switch between the survival and the death of a cell [181].

Desomoylation, which involves SENP protease, inactivates SIRT1, leading to cell death [181].

Posttranslational modifications may help specify particular substrates according to the circumstances. Moreover, SIRT1-mediated deacetylation of PGC-1α requires phosphorylation of the latter by AMPK [63]. A considerable amount of work is, however, needed to decipher the various mechanisms by which SIRT1 specificity is regulated.

Formation of complexes with other proteins

Sirtuin action is modulated SIRT1-binding proteins. Binding of the cell-cycle apoptosis regulator E2F1 inhibits SIRT1 activity [164]. This effect may regulate the induction of apoptosis in response to DNA damage.

The protein AROS binds to the N-terminus of SIRT1, but not other sirtuins, to double its activity and inhibit p53 [182]. AROS reduction increases apoptosis [182]. In contrast, SENP-1 inactivates SIRT1 by binding to the C-terminus and desumoylating it, thus increasing p53 activity [181].

During fasting, NCoR1 and SMRT bind to SIRT1 and PPAR-γ to downregulate adipogenesis mediated by PPAR-γ [97] (Fig. 3A).

In response to genotoxic stress DBC1 binds to the catalytic domain of SIRT1 and inhibits its activity [183,184] in mice on a high-fat diet, but not during fasting [185] (Fig. 3B). DBC1 loss leads to increased SIRT1 activity, SIRT1-mediated deacetylation of p53, and a reduction in p53-mediated apoptosis [183,184]. DBC1 gene knockout increases SIRT1 activity 2- to 4-fold in various tissues, causes p53 hypoacetylation, and protects against hepatic steosis and liver damage in mice on a high-fat diet [185]. This phenotype was noted in liver-specific SIRT1 knockout mice [83,186]. DBC1 was, moreover, able to disrupt a complex of SIRT1 with the histone methyltransferase SUV39H1, thus inactivating each enzyme [187]. The interaction of DBC1 with SIRT1 in response to DNA damage and oxidative stress requires ATM-dependent phosphorylation of DBC1 at threonine 454, which creates a second binding site for SIRT1 [188]. DBC1 binds to ER-α and cooperates synergistically with the important coactivator of estrogen-induced gene expression and estrogen-dependent growth of breast cancer cells, CCAR1, to enhance ER-α function [189]. In contrast DBC1 reduces binding of SIRT1 to ER-α and thus lowers ER-α deacetylation [189]. Binding of SIRT1 to DBC1 disrupts the binding of DBC1 to CCAR1 [189]. In this reciprocal manner, SIRT1 and DBC1 exert major effects on ER-α activity.

The histone methyltransferase LSD1 binds to the catalytic domain of SIRT1 resulting in convergent repression of Notch target genes (Fig. 3C). The latter involves SIRT1-mediated deacetylation of acetylated histones H4K16 and H1K26, and LSD1-mediated demethylation of H3K4 [190]. Notch, once activated, blocks the interaction of LSD1 with SIRT1 [190] (Fig. 3C). SIRT1 is a component of the PRC4 complex [191]. At least one of the other components, EZH2, is a histone methyltransferase, which, when overexpressed, upregulates all other PRC4 components [191].

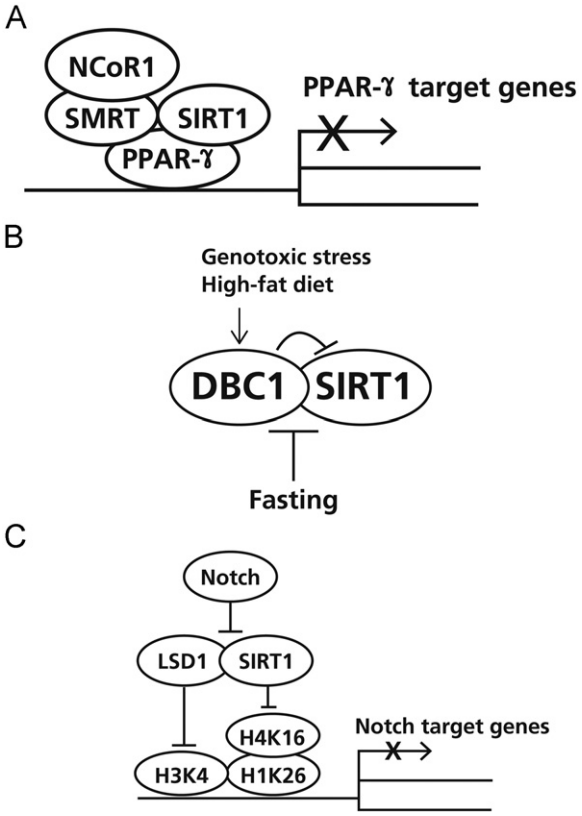

Fig. 3. Proteins that form complexes with SIRT1 affecting its activity. (A). Binding of the NCoR1–SMRT complex to SIRT1 leads to blockade of the transcriptional effect of PPAR-γ on PPAR-γ target genes. (B). Genotoxic and metabolic stress caused by a high-fat diet results in DBC1 forming a complex with SIRT1, leading to SIRT1 inhibition. (C). LSD1 demethylates, while SIRT1 deacetylates, specific histone genes (H3K4, H4K16, and H1K26) leading to repression of transcription of genes targeted by Notch. Activation of the Notch pathway reverses this effect. (Adapted from [192]).

These and other proteins that bind to SIRT1 should affect the ability of SIRT1 to deacetylate other SIRT1 substrates, including PGC-1α and FOXOs [63]. SIRT1, via its deacetylase activity, may also influence corepressor and coactivator complexes and thus the binding properties of these.

In the case of SIRT7 a proteomic analysis referred to earlier has identified 462 proteins that bind to this sirtuin [158]. A major theme for many of these was regulation of transcription of RNA polymerase I.

Roles for sirtuins in tissue functions

Sirtuins have a multiplicity of different actions in most tissues in the body. Those involving SIRT1 are summarized in Fig. 4.

Liver

Fig. 5 depicts the action of each sirtuin in the regulation of hepatocyte function. SIRT1 activates gluconeogenesis and inhibits glycolysis [192]. Studies *in vitro* show that SIRT1 increases fat oxidation and downregulates the master regulator of fatty acid synthesis, SREBP-1c [100,193].

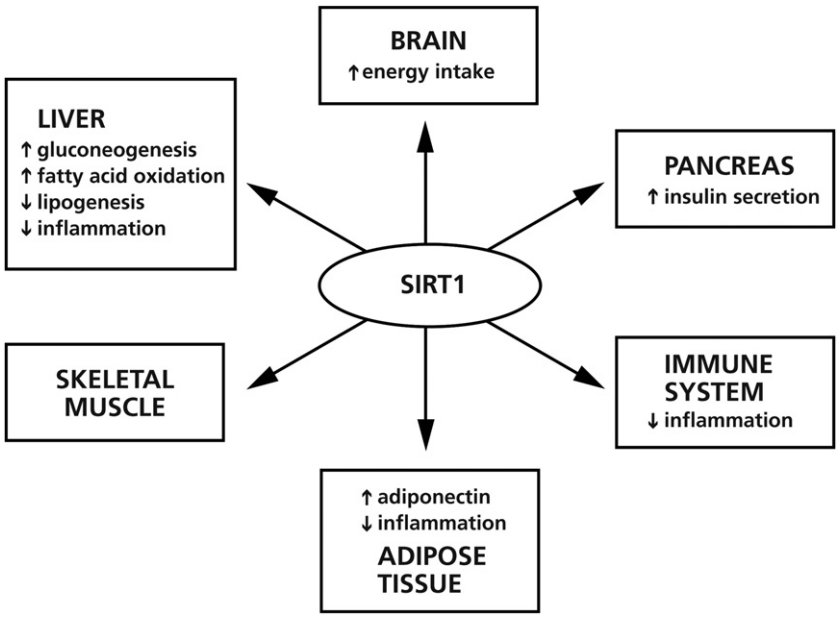

Fig. 4. Major tissues targeted by SIRT1 and key effects that result. (Adapted from [323]).

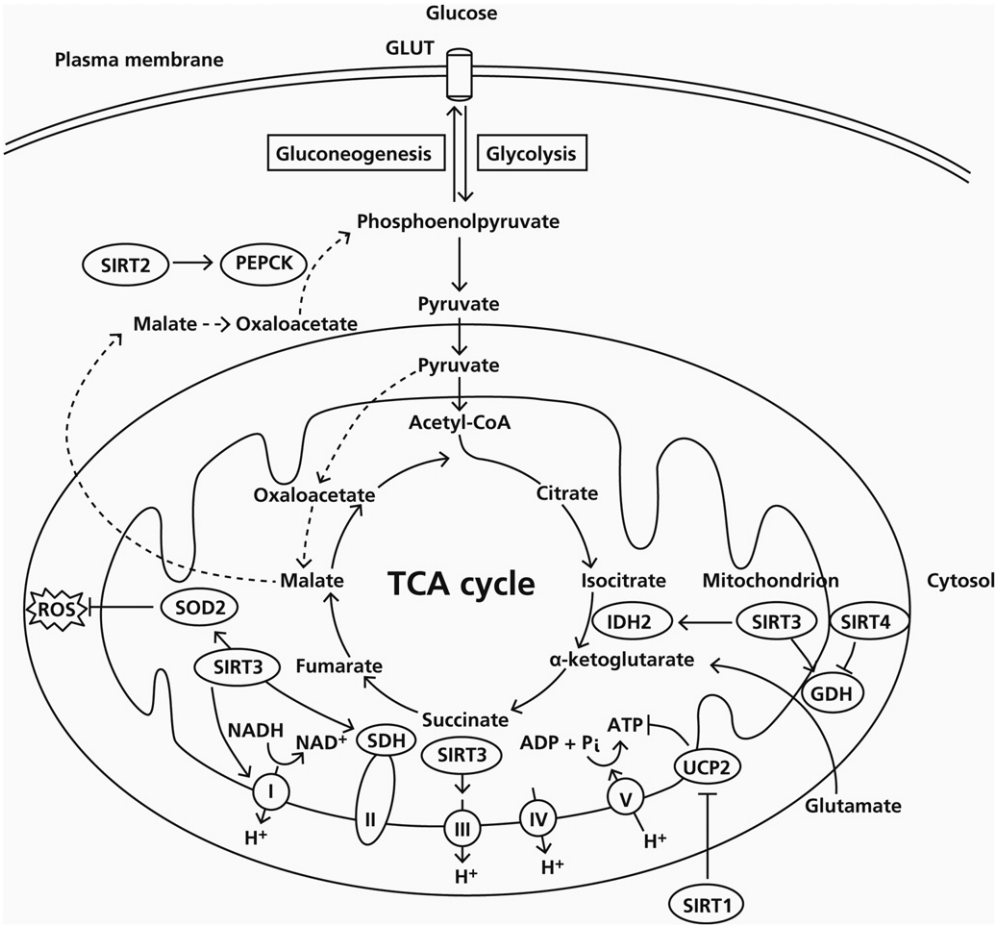

Fig. 5. The roles of the various sirtuins in glucose metabolism. Glucose taken up by the cell via the glucose transporter (GLUT) is catabolized by glycolysis into pyruvate, which enters the TCA cycle in mitochondria to generate energy. Under conditions in which energy supply is limited, glucose production by gluconeogenesis (dashed arrows) increases. This involves the conversion of pyruvate to oxaloacetate and subsequently to malate. In the cytoplasm malate is converted back to oxaloacetate, which is used by PEPCK to produce phosphoenolpyruvate. The latter is then available for conversion to glucose. In β-cells SIRT1 increases insulin secretion by repressing UCP2 transcription. In liver cells SIRT2 stimulates gluconeogenesis by deacetylating and increasing the stability of PEPCK. SIRT3 stimulates SOD2, thus decreasing ROS production, and also enhances cellular respiration by increasing the activities of complex I, complex II (via SDH), complex III, and IDH2. SIRT3 deacetylates GDH, stimulating its activity to affect gluconeogenesis and insulin secretion. By ADP-ribosylation SIRT4 inhibits GDH. (Modified from [192]).

Studies of liver-specific SIRT1-null mice show that the gluconeogenic G6K and PEPCK genes are normally downregulated by a signaling pathway in which SIRT1 positively regulates the gene for Rictor, a component of the mammalian TOR complex 2 (mTORC2), to trigger a cascade of phosphorylation of AKT and FOXO1 [194]. The hepatic overproduction of glucose, chronic hyperglycemia, and increased ROS production in SIRT1 null mice leads to oxidative stress-mediated impairment in mTORC2/AKT signaling in other insulin-sensitive organs, and consequent insulin resistance [194]. The latter is reversed by treatment with antioxidants.

Knockdown of SIRT1 mRNA by delivery of adenoviral vectors containing SIRT1 short hairpin RNAs has no effect on hepatic triglyceride accumulation, but severely affects glucose homeostasis and gluconeogenic capacity [193]. This is not, however, seen in the liver-specific SIRT1 knockout mice above [194]. While there has been speculation that SIRT1 boosts gluconeogenesis by PGC-1α activation, leading to coactivation of CREB on the promoters of genes for gluconeogenesis [195,196], this was based on artificial overexpression of PGC-1α, whereas the evidence for such a mechanism during physiological modulation of PGC-1α is weak [197]. In fact, as evident from liver-specific SIRT1 knockout [194], under most circumstances SIRT1 activation in liver is associated with a reduction in gluconeogenesis [198].

SIRT1 binds to PXR which, apart from being a xenobiotic-sensing nuclear receptor with a major role in drug metabolism, is upregulated in liver during fasting and serves as a modulator of hepatic energy metabolism [199]. This SIRT1 interaction disrupts PXR binding to PGC-1α [199].

SIRT2 activates gluconeogenesis by deacetylation and increased stability of PEPCK [155] (Fig. 5).

SIRT3 has several targets in hepatocytes [192] (Fig. 5). SIRT3 knockout mice respond to a high-fat diet by accelerated obesity, insulin resistance, hyperlipidemia, and hepatic steosis [124]. After a week on a high-fat diet SIRT3 expression is elevated, but by 13 weeks is decreased [124]. The lipogenic enzyme SCD1 was highly induced in these mice, and SCD1 deletion rescued the mice from liver steosis and insulin resistance [124]. During calorie restriction SIRT3 stimulates [120,126] and SIRT4 inhibits [53] GDH and thereby gluconeogenesis (Fig. 5).

Less is known about the function of SIRT6, although in the Otsuka Long-Evans Tokushima Fatty rat, rosiglitazone ameliorates hepatic stenosis by activating SIRT6, AMPK, PGC-1α, FOXO1, LKB1, and adiponectin, thus reducing hepatic lipid accumulation [200].

Pancreas

SIRT1 stimulates insulin secretion from β-cells [201,202] only in young mice [203]. This might be explained by the reduction in NAD^+ that occurs with age [204]. In β-cells UCP2 affects the ability of glucose to modulate the ADP/ATP ratio and cause insulin secretion [205]. By reducing UCP2, SIRT1 permits better coupling and production of ATP in response to high glucose [202]. The ability of SIRT1 to stimulate insulin secretion involves other mechanisms further downstream [202]. SIRT1 levels in β-cells are modulated by miR-9, which reduces SIRT1 mRNA stability [206]. Glucagon-like peptide 1 lowers NAD^+/NADH ratio, thus reducing SIRT1 activity and binding to FOXO4, leading to increased FOXO4 activity [207]. FOXO4 activation stimulates β-cell growth, thus increasing insulin secretion. These experiments show that SIRT1 is a negative regulator of β-cell proliferation.

SIRT3 activates GDH [120,126], but an effect on insulin secretion is yet to be shown. Mitochondrial SIRT4 inhibits insulin secretion via suppression of GDH activity in β-cells, thereby downregulating insulin secretion in response to amino acids [53]. These effects are alleviated during calorie restriction. In insulinoma cells, SIRT4 is overexpressed, leading to a decrease in insulin secretion in response to glucose [208].

Muscle

SIRT1 regulates mitochondrial metabolism in cultured muscle cells [209]. Muscle-specific knockout SIRT1 mice show that SIRT1 is not needed for deacetylation of PGC-1α nor for mitochondrial biogenesis induced by exercise [210]. Acute exercise leads to increased nuclear SIRT1 activity, but not protein, accompanied by increased expression of genes activated by PGC-1α, as well as of mitochondrial biogenesis, possibly via AMPK activation [211]. SIRT1 overexpressing mice have a normal phenotype under basal conditions [212]. During calorie restriction SIRT1 enhances insulin sensitivity of skeletal muscle by deacetylation and inactivation of the transcription factor STAT3, leading to decreased expression of the p55α/p50α subunits of PI3K [213]. This results in more efficient PI3K signaling in response to insulin. SIRT1 also binds to the p85 adaptor subunit of PI3K to stimulate PI3K-mediated insulin signaling in muscle cells [214]. During exercise muscle temperature can rise to 40 °C. Mild heat stress induces mitochondrial biogenesis and this correlates with activation of the AMPK–SIRT1–PGC-1α pathway [215]. The muscle wasting induced by glucocorticoid administration can be prevented by resveratrol-mediated activation of SIRT1 [216].

Calorie restriction reduces age-associated muscle atrophy by lowering oxidative stress, even when CuZnSOD (SOD1) is absent [217]. SIRT3 and MnSOD are increased in these mice [218]. SIRT3 is highly expressed in slow oxidative type I soleus muscles compared to fast type II muscles, and exercise training increases SIRT3 expression, phosphorylation of CREB, and upregulates PGC-1α in skeletal muscle [218]. While fasting and calorie restriction increase SIRT3 protein levels, a high-fat diet suppresses SIRT3 [218]. Germ-line SIRT3 knockout mice exhibit reduced phosphorylation of AMPK and CREB, as well as lower PGC-1α expression [218]. Mice with muscle-specific deletion of SIRT3 exhibit marked hyperacetylation of mitochondrial proteins, but do not have the high-fat diet-induced mitochondrial dysfunction nor the metabolic abnormalities seen in germ-line SIRT3 knockout mice [219]. In resting muscle SIRT3 is localized to subsarcolemmal and intermyofibrillar mitochondria and with chronic stimulation SIRT3 is upregulated in an AMPK-independent manner [220].

Adipose tissue

Lipid metabolism and homeostasis are affected by actions of sirtuins on various tissues, particularly white adipose tissue, liver, and skeletal muscle [192]. Fig. 6 shows the sirtuin targets and mechanisms involved. In fat cells, SIRT1 inhibits adipogenesis and increases lipolysis by suppressing PPAR-γ activity [97]. This involves the ability of SIRT1 to promote the assembly of a corepressor complex that includes NCoR1 and SMRT on the promoters of PPAR-γ target genes, thus repressing their transcription (Fig. 3A) and opposing fat storage during calorie restriction and fasting [97]. SIRT2-mediated inhibition of adipogenesis and promotion of lipolysis involves deacetylation-mediated activation of FOXO1 during nutrient deprivation [40]. SIRT2 enhances the binding of FOXO1 to PPAR-γ and represses the transcription of the PPAR-γ gene [40,142].

Brain and nerves

SIRT1 sensitizes neurons to oxidative damage but also has neuroprotective effects [221]. Deletion of SIRT1 in neurons does not affect development of the CNS, although because of a deficiency in growth hormone the mice are smaller [222]. While glucose tolerance is affected little in younger mice, defects

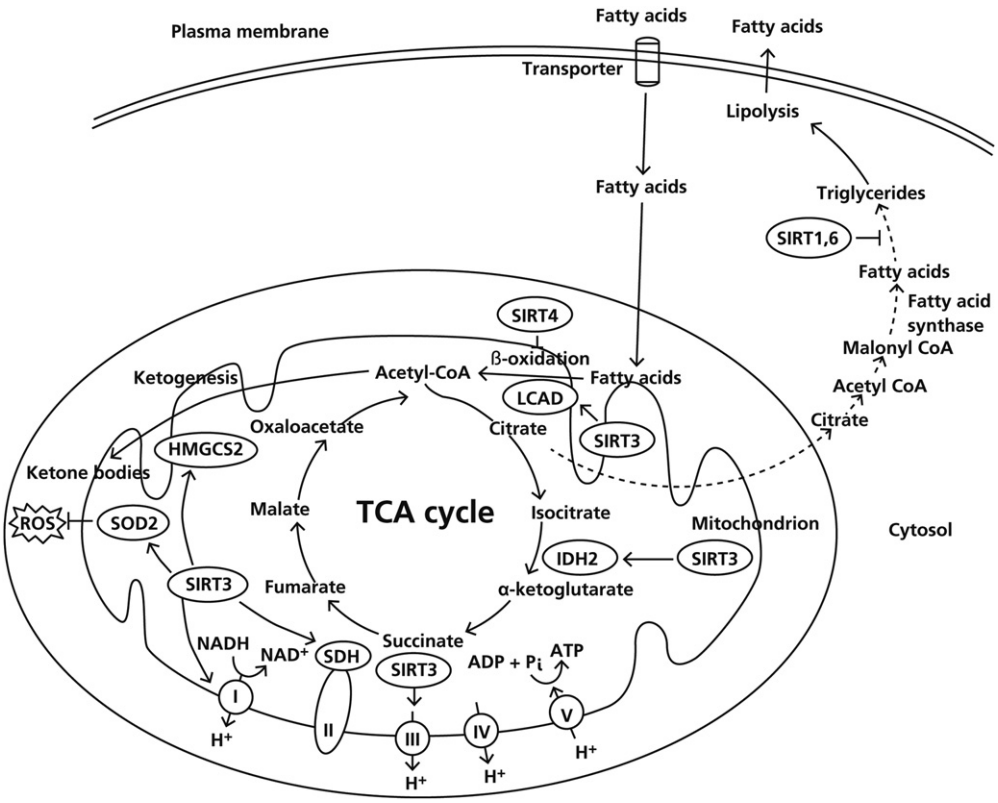

Fig. 6. The roles of the various sirtuins in lipid metabolism. Fatty acids are taken up by the cell by fatty acid transporters, and then transported to mitochondria, where their oxidation leads to ATP production. HMGCS2 breaks down fatty acids to generate ketone bodies. In the cytosol, lipogenesis (dashed arrows) leads to synthesis of fatty acids from malonyl CoA by fatty acid synthase, after which they are converted to triglycerides. When energy demand is high, triglycerides can be broken down to free fatty acids by lipolysis, mostly in fat tissue. These are then released into the bloodstream. By enhancing lipolysis through the inhibition of PPAR-γ and by decreasing fatty acid synthesis through SREBP1c, SIRT1 reduces fatty acid storage. SIRT6 represses expression of genes involved in fatty acid synthesis. SIRT3 activates LCAD to stimulate β-oxidation. By activating HMGCS2, SIRT3 stimulates ketone body formation. SIRT3 stimulates SOD2 to decrease ROS. SIRT3 enhances cellular respiration by increasing the activities of complex I, complex II, complex III, and IDH2. SIRT4 appears to be a negative regulator of transcription of genes involved in fatty acid oxidation. (Modified from [192].).

became greater as the mice age [222]. SIRT1 and SIRT5 proteins increase in cerebral tissue of rats in response to calorie restriction, and their rise is accompanied by enhanced cognitive ability [223]. SIRT1 mediates anxiety level, which is probably an adaptive response to environmental changes affecting food availability [224]. The mechanism involves deacetyaltion of brain-specific helix-loop-helix transcription factor NHLH2, thus stimulating monoamine oxidase A and reducing serotonin in the brain [224]. The prevention of aging-related neurodegeneration and impaired neuronal plasticity, memory, and social behavior by calorie restriction involves forebrain CREB-1 [225]. CREB directly regulates SIRT1 transcription [163] and recruits SIRT1 to assist it in the regulation of PGC-1α and nNOS transcription [225]. Loss of such functions may explain accelerated brain aging during overnutrition and diabetes. SIRT1 binds to and stimulates promoter activity of the presenilin1 gene [226]. Resveratrol stimulates both SIRT1 and presenilin expression, as well as proliferation of neuronal stem cells in the hippocampus of rats [226]. This points to a role for SIRT1 signaling in hippocampal plasticity. By deacetylating the critical epigenetic regulator methyl-CpG-binding protein 2, SIRT1 controls binding of this protein to BDNF in the hippocampus [227]. Treadmill exercise improves various important parameters in the rat CNS, especially the hippocampus, and is associated with increased SIRT1, mitochondrial biogenesis, and AMPK activation [228].

The hypothalamus is crucial for metabolic actions of SIRT1 [229]. In arcuate neurons of the hypothalamus, necdin, by formation of a stable ternary complex with SIRT1 and FOXO1, is crucial for FOXO1 deacetylation and for modulation of the hypothalamo–pituitary–thyroid axis [230].

SIRT2 is the most abundant sirtuin in the brain. It is expressed in virtually all brain cells, but is highest in cells that produce myelin, namely the oligodendrocytes [145,231]. SIRT2 is severely depleted from the myelin proteome in PLP knockout mice, in which PLP and DM20 (which arise from the same primary mRNA transcript by alternative splicing) are absent [232]. PLP regulates SIRT2 protein levels via the QKI-dependent pathway [231]. By deacetylation of α-tubulin, SIRT2 decelerates cell differentiation of oligodendrocyte precursor cells [145]. The transcription factor Nkx2.2, via HDAC-1, binds to the start codon of the SIRT2 gene, and suppresses SIRT2 transcription in oligodendroglial cells, thus enhancing cellular differentiation [233]. In myelin sheaths SIRT2 acts at sites of microtubule remodeling [234,235]. In postmitotic hippocampal neurons SIRT2 inhibits neurite outgrowth and growth cone collapse [180]. There are, however, no studies to date in support of a direct role of SIRT2 in neuronal differentiation [148]. But since SIRT2 can deacetylate FOXO3, which can regulate the neuronal stem cell pool by maintaining senescence [236], it is possible that SIRT2 might be involved in the latter process [148]. SIRT1 also deacetylates FOXO3A and thereby modulates differentiation of neuroblastoma cells [237]. Since p53 can regulate neuronal growth and p53 can be deacetylated by SIRT2 *in vitro*, a role for SIRT2 in p53-dependent neuronal function is possible [148].

SIRT2 transcripts undergo alternative splicing [238]. The full-length SIRT2 protein (SIRT2.1) is only moderately expressed in the central nervous system, the levels being comparable to those in other tissues. SIRT2.2 concentrations are, however, much higher, both during postnatal development and in adulthood. In the aged brain, an age-dependent increase in SIRT2.3 was observed [238].

In peripheral nerves, elaboration of myelin by Schwann cells is dependent on SIRT2 [156]. SIRT2 deacetylates the master regulator of cell polarity, PAR-3, thus lowering activity of the polarity signaling component atypical protein kinase C during the myelination program [156]. This may be relevant to peripheral neuropathy in diabetes [156]. Moreover, stimulation of SIRT2 may help in the treatment of multiple sclerosis, a disease involving progressive loss of myelin [148].

Mitochondrial SIRT3 serves as a prosurvival factor by protecting neurons exposed to NMDA-induced excitotoxicity [239]. The latter involves PARP-1 mediated NAD^+ depletion. The ensuing rise in ROS is responsible for the increase in SIRT3.

Sirtuins in metabolism

Mice with whole-body overexpression of SIRT1 are leaner, more metabolically active, and glucose tolerant [240,241]. This phenotype resembles calorie restriction. The mice were moreover resistant to diet-induced hyperglycemia, metabolic syndrome, and fatty liver [242,243]. Although they were protected against diseases of aging such as metabolic syndrome and cancer, there was no significant increase in their life span [19]. The higher SIRT1 activity achieved by its overexpression confirmed that NAD^+ is sufficiently abundant in cells not to be rate limiting for SIRT1 activity.

Physiologically, an increase in SIRT1 activity occurs during nutrient deficiency and exercise [159]. This correlated with elevated expression of nuclear-encoded genes for mitochondrial proteins and higher energy expenditure [159,244]. In early studies nutrient deprivation was found to increase expression of FOXO3A in concert with SIRT1 promoter stimulation [159]. p53 is another transcription factor that normally represses SIRT1. During nutrient depletion FOXO3A is activated and binds to p53, thus extinguishing its inhibitory effect [159]. SIRT1 and p53 negatively regulate each other by a homeostatic loop involving deacetylation and by transcriptional effects. Deacetylation of FOXO3A by SIRT1 enhances FOXO3A action, and this includes FOXO3A-mediated stimulation of SIRT1 transcription. Whereas the effect of FOXO3A on the SIRT1 promoter is indirect (via binding of p53), FOXO1 binds to DNA target sites in the SIRT1 promoter to activate transcription directly [245] (Fig. 2). By their separate actions both FOXO3A and FOXO1 generate a feed-forward mechanism involving SIRT1 expression and activity. It is notable, moreover, that AMPK also activates FOXOs [90] and increases SIRT1 expression [246].

The protein HIC1 binds to SIRT1, forming a corepressor complex on the SIRT1 promoter [166]. This action of HIC1 is regulated by its binding to the energy and redox stress sensor CTBP [167]. Since the binding of CTBP to HIC1 is increased by NADH, when glycolysis is low NADH decreases, leading to destabilization of CTBP/HIC1/SIRT1 inhibitory complexes, with the result that SIRT1 transcription increases. The consequent elevation in SIRT1 results in metabolic adaptation favoring utilization of energy sources other than carbohydrate.

Chronic inflammation represses SIRT1. This involves induction by interferon-γ of CIITA, which, with HIC1, attaches to the SIRT1 promoter to repress SIRT1 expression [247].

In obese mice and humans SIRT1 is reduced [160,248]. Nutrient overload activates PPAR-γ, which then binds to PPAR-γ response elements in the SIRT1 promoter to repress SIRT1 transcription [161,165] (Fig. 2). PPAR-α and PPAR-β/δ can also bind to PPAR-γ response elements, although for PPAR-β/δ at least, the elevation in SIRT1 expression that ensues involves instead binding to p21, which has a stimulatory binding site in the SIRT1 promoter [162] (Fig. 2). The effects of each on lipid metabolism differ. PPAR-γ causes lipid anabolism and inhibits SIRT1 expression, whereas PPAR-α and PPAR-β/δ are involved in fatty acid oxidation and increased SIRT1 expression. More research is needed to understand whether differential sensing of lipid species may direct pathways in one direction or the other.

The NAD^+-dependent protein PARP-2 binds to the SIRT1 promoter, thus inhibiting transcription [76] (Fig. 2). PARP-2 increases PPAR-γ transcription, but not that of PPAR-α and PPAR-β/δ [249]. PARP-2 deletion has a similar effect on bodily processes as SIRT1 activation [76]. This includes protection against diet-induced obesity, elevation in mitochondrial number, enhanced oxidative metabolism, and insulin resistance.

During fasting, glycogenesis and glycogenolysis in the liver are activated early, followed by ketogenesis when fasting is prolonged. The fasting hormones glucagon and norepinephrine increase cAMP signaling, which elevates CREB. Gluconeogenesis is activated by at least two transcriptional pathways [250] (Fig. 7). One involves glucagon-mediated dephosphorylation of CRTC2 in the cytoplasm, causing it to move to the nucleus and serve as coactivator for CREB [94]. At the same time glucagon-mediated activation of cAMP-dependent PKA phosphorylates CREB (Fig. 7).

With longer fasting (12–18 h in mice), SIRT1 deacetylates CRTC2, permitting ubiquitination and degradation of CRTC2, thus attenuating CREB [94]. During this period, SIRT1 activates FOXO1 and its coactivator PGC-1α [79,80], which maintains gluconeogenesis after the loss of CRTC2.

The regulation of SIRT1 transcription during fasting involves a short promoter sequence containing overlapping sites for CREB and ChREBP (Fig. 2), known to be activated by feeding and which mediate energy disposal and storage [251]. ChREBP suppresses SIRT1 transcription, whereas CREB activates it [163]. During fasting ChREBP translocates to the cytosol, meaning that its binding site on the SIRT1 promoter becomes exposed. Binding of CREB within this composite response element then stimulates SIRT1 transcription [163]. This effect of CREB occurs by 18 h of fasting and coincides with activation of SIRT1 as a result of the elevation in NAD^+ generated by gluconeogenesis. SIRT1 limits the early phase of gluconeogenesis by deacetylating CRTC2 and, by activating PGC-1α, promotes the onset of the prolonged phase of fasting. At this time deacetylation of SREBP1 represses SREBP1 activity, thus shutting down fat and cholesterol synthesis [99].

By 24 h of fasting SIRT1 transcription returns to normal, most likely as a result of CRTC2 destruction, and such normal SIRT1 transcription appears to be sufficient for ongoing gluconeogenesis during prolonged fasting (Fig. 7).

Gluconeogenesis then needs to be switched off quickly when feeding resumes. This involves insulin. Upon refeeding, insulin signaling stimulates AKT, leading to phosphorylation of FOXO1, which causes FOXO1 to shuttle to the cytoplasm. During this time ChREBP moves to the nucleus, binds to a site in the SIRT1 promoter that overlaps the CREB site, and represses SIRT1 transcription [163] (Fig. 7). Thus when insulin is high and glucagon is low PGC-1α and CREB actions are turned off.

The SIRT1 substrate PGC-1α activates not only gluconeogenesis but also biogenesis of mitochondria, and PPAR-α, another substrate activated by SIRT1, stimulates mitochondrial fatty acid oxidation [83]. Thus SIRT1 upregulation early in fasting would boost oxidative metabolism in cells, thus assisting in the use of fatty acids, amino acids, and acetate as energy sources involving the gluconeogenic pathway.

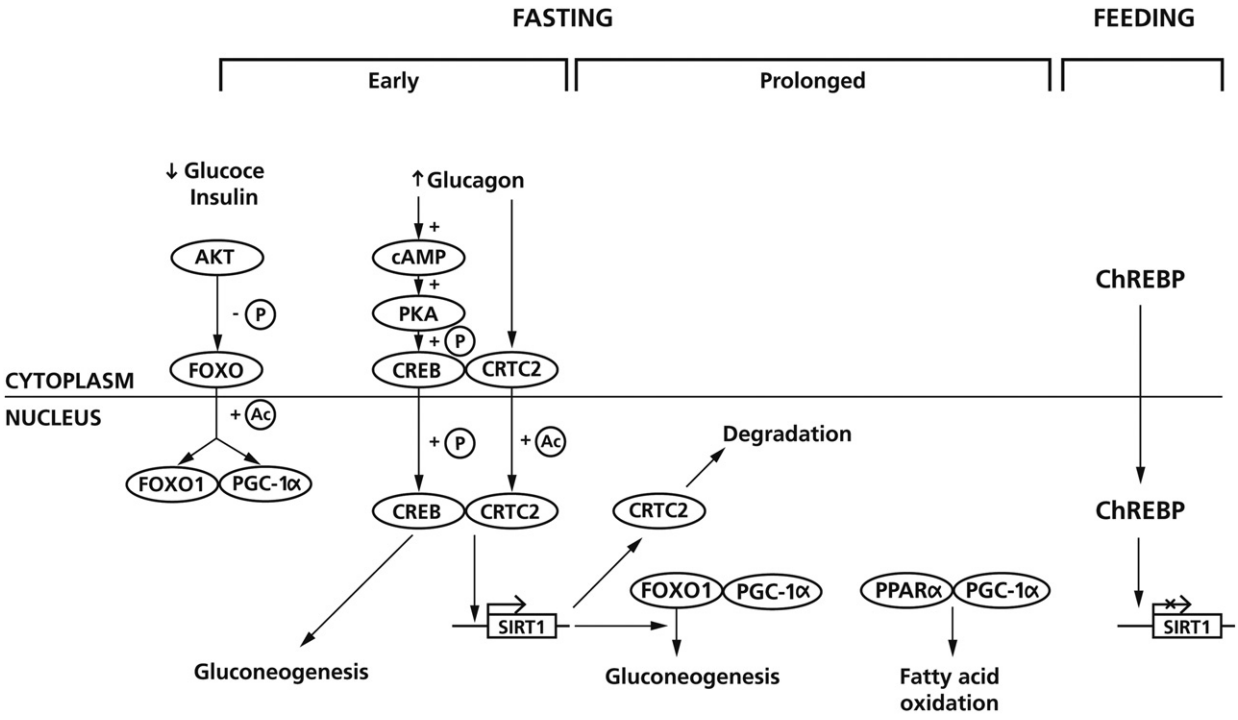

Fig. 7. Nutrient availability regulates SIRT1-mediated metabolic response. Short-term (or early phase of) fasting causes a decrease in blood glucose and insulin levels, and a concomitant rise in glucagon. Both of these hormonal changes initiate cellular pathways that induce gluconeogenesis. Low insulin signaling results in the dephosphorylation of the transcription factor FOXO1 and its translocation to the nucleus. Glucagon produces an increase in cyclic AMP levels and activation of PKA, which phosphorylates CREB and drives its translocation to the nucleus. Glucagon also causes the dephosphorylation of the CREB coactivator CRTC2 to trigger its translocation to the nucleus. The transcriptional complex CREB–CRTC2 activates the transcription of genes for gluconeogenesis and SIRT1. In a second phase of fasting, after more prolonged deprivation of food, increased SIRT1 deacetylates CRTC2 and targets it for ubiquitination and degradation by the proteasome, thereby terminating the transcriptional activity of CREB. SIRT1 also deacetylates PGC-1α, FOXO, and PPAR-α increasing their transcriptional activity. PGC-1α coactivates PPAR-α to induce the expression of fatty acid oxidation genes, and FOXO1 to maintain the expression of genes for gluconeogenesis. In the fed state, SIRT1 expression is repressed by the activity of the transcription factor ChREBP, which translocates to the nucleus.

Fasting also leads to upregulation of mitochondrial SIRT3 [121], which then deacetylates enzymes for oxidative metabolism and for detoxification of ROS [252]. SIRT3 knockout mice exhibit decreased oxygen consumption, accompanied by increased production of ROS and oxidative stress in skeletal muscle, leading to activation of JNK, along with impaired insulin signaling [130].

The microRNA miRNA-34a is elevated in diet-induced and genetically obese mice [253] and serves to negatively regulate SIRT1 expression at the posttranscriptional level by binding to the 3′UTR of SIRT1 mRNA [172,253].

Chemical compounds that activate sirtuins

Given the health benefits of sirtuins, it makes sense that chemicals that activate sirtuin activity might prove useful in pharmacotherapy [3,35,254–256]. The natural polyphenol, resveratrol, has long been known to confer health benefits. The first demonstration of this was its ability to prevent skin cancer in mice [257]. Then a decade ago, screening an array of chemical compounds for the ability to activate SIRT1 and extend *C. elegans* life span led to the identification of not just resveratrol but also quercetin, piceatannol, and several other natural polyphenols with this ability [6]. In 2007, large-scale screening of thousands of unselected compounds identified several small molecular activators of SIRT1, including SRT1720, that were up to 1000 times more potent than resveratrol in their ability to activate SIRT1 and exert beneficial effects [258,259], including quenching of the attenuated life span of mice fed a high-fat diet [259]. As noted in the Introduction, however, the ability of resveratrol to increase SIRT1 activity by a direct effect has been questioned [260–262]. Earlier claims of direct activation were shown to be assay artifacts emanating from the fluorophore attached to the peptide substrate used in the assay [261–263]. Although, by use of peptide substrates lacking any chemical modifications, direct binding and activation of SIRT1 were subsequently demonstrated, the sequence of amino acids surrounding the lysine 382 residue of the short polypeptide substrates related to p53 that were used did not match the native sequence [264]. This means there is no compelling evidence for direct SIRT1 activation physiologically. The current view is that SIRT1 activation may involve an upstream effect [265]. Resveratrol activates AMPK [265–269]. The metabolic effects of dietary resveratrol are, moreover, lost in AMPK knockout mice [270]. In addition, resveratrol inhibits mitochondrial ATP synthase activity [271] by binding to its γ-subunit [272]. By interfering with mitochondrial respiration in this way, resveratrol causes an increase in the ratio of AMP to ATP, which then causes AMPK activation [273] (Fig. 8). Such upstream effects probably explain how the various chemicals activate SIRT1 [274].

Resveratrol competitively inhibits phosphodiesterases responsible for degradation of cAMP [275]. The rise in cAMP activates the cAMP-regulated guanine nucleotide exchange factor EPAC1, which in turn increases intracellular Ca^{2+} concentration [275]. This then activates phospholipase C and ryanodine receptor Ca^{2+} release channel, leading to activation of the calcium/calmodulin-dependent protein kinase 1 and hence AMPK [275]. The ensuing rise in NAD^{+} activates SIRT1. Inhibition of the phosphodiesterase PDE4 by rolipram confers metabolic benefits similar to resveratrol, *i.e.*, prevention of diet-induced obesity, increased physical stamina, glucose tolerance, and improved mitochondrial function [275]. Rolipram is,

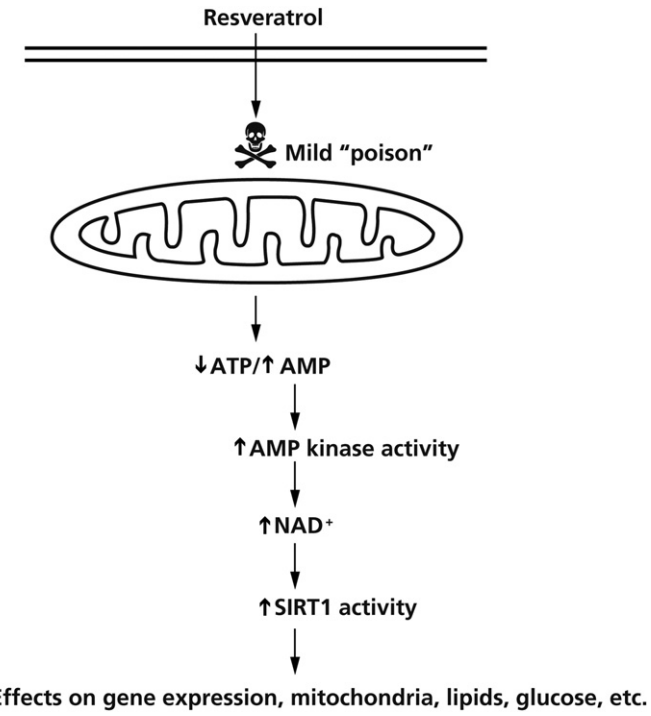

Fig. 8. The action of resveratrol leading to increased SIRT1 activity. Some consider that resveratrol or its metabolites act on mitochondria as a mild "poison." This then reduces ATP synthesis. As a result, the ratio of ATP to AMP decreases. The increase in AMP activates AMP kinase, which in turn increases NAD^+, which activates SIRT1. This then leads to the various effects of SIRT1. (Modified from [63]).

however, far more potent than resveratrol at blocking phosphodiesterases [276]. Moreover, SIRT1 activation by the SRT compounds is claimed not to involve phosphodiesterase inhibition [276]. AMPK can phosphorylate PGC-1α directly, thus triggering downstream effects without a requirement for SIRT1 activation [88]. SIRT1 appears, however, to be required for activation of AMPK by lower doses of resveratrol [3]. At higher doses of resveratrol AMPK activation is independent of SIRT1. In cultured cells at least, there is ample evidence that many of the effects of resveratrol and SRT1720 depend on activation of SIRT1 [3].

SIRT1 and AMPK are mutually reinforcing and interdependent for their full effects [277]. But whereas AMPK activation occurs within minutes, activation of SIRT1 requires hours, consistent with the latter being a downstream event [63]. Whereas resveratrol can activate AMPK in tissue from SIRT1 knockout mice [267,270], it is unable to activate SIRT1 in the absence of functional AMPK [270,277]. Activation of AMPK leads to activation of fatty acid oxidation, which increases NAD^+ and hence SIRT1 [64]. The rise in NAD^+ is sustained by an increase in NAMPT expression, leading to increased NAD^+ synthesis from nicotinamide via the NAD^+ salvage pathway [278].

A randomized, double-blind cross-over trial in humans found that the beneficial effects of 30 days of resveratrol were achieved at 150 mg/day [279], which was an order of magnitude lower than needed in mice. Just as in other species, the effects seen were generally similar to calorie restriction, and included reduction in metabolic rate and sleeping, activation of AMPK in muscle, as well as stimulation of SIRT1 and PGC-1α levels, increased citrate synthase activity, and improved mitochondrial respiration on a fatty acid-derived substrate [279]. Circulating glucose, triglycerides, alanine-aminotransferase, markers of inflammation, and blood pressure were reduced, and HOMA markers, that estimate β-cell function and insulin sensitivity, were improved [279].

Analysis of the exometabolome of HepG2 cells has shown that resveratrol causes a metabolic switch from glucose and amino acid utilization to fat utilization for energy production, consistent with an effect via AMPK and SIRT1 activation [280]. The cell cycle was slowed in the S phase without inducing apoptosis.

Resveratrol can modulate alternative splicing of some primary mRNA transcripts, but does so by a SIRT1-independent effect [281].

As discussed in the Introduction, the life span-extending effects of resveratrol seen in lower organisms [282] are not supported by more recent findings. Furthermore, while resveratrol prevented mice on a high-fat diet from dying prematurely [244,265], it was unable to significantly increase the life span of mice on a normal diet [283]. In mice [244], but not humans [279], resveratrol increased mitochondrial content. The ability of SRT1720 to prevent diet-induced obesity and diabetes [258,259,269], as well as fatty liver [284], seems, moreover, to be indirect, probably involving AMPK activation [269]. Another study also suggested that SRT1720 and resveratrol work by indirect effects, but found, however, that SRT1720 was lethal to mice fed a high-fat diet, and that while insulin was decreased, plasma glucose was not and mitochondrial capacity did not improve [261]. SRT1720 and SRT2183 can, moreover, decrease p53 acetylation in the absence of SIRT1, apparently by inhibiting the histone acetyltransferase p300 [285].

It should be obvious that the most direct way of activating all sirtuins, not just SIRT1, is by raising intracellular NAD^+ levels. The main source of NAD^+ is probably from salvage pathways of other metabolites of adenine nucleotides such as nicotinamide and nicotinamide riboside [66]. The enzyme NAMPT is rate limiting in conversion of nicotinamide to NMN, which is then converted to NAD^+ by NMN adenylyltransferase [68,286] (Fig. 9). NMN administration to mice protects against age-related diabetes, in part through SIRT1 activation [69]. Although nicotinic acid and nicotinamide supplementation can raise intracellular NAD^+, albeit in a tissue-specific manner, an increase in sirtuin activity has yet to be described [63]. Niacin is composed of nicotinic acid and nicotinamide and is used to treat tryptophan deficiency as well as hypercholesterolemia. It would make sense that sirtuin activation might be involved in niacin's beneficial effects, especially as niacin causes white adipose tissue to secrete adiponectin [287,288], which activates AMPK in muscle and liver [92]. The increase in fat deposition caused by adiponectin involves elevation in SIRT1, FOXO3A, and PGC-1α mRNA [289]. On a normal or high-fat diet SIRT1 overexpression increased adiponectin approximately 40% [242]. The nicotinamide ribose-induced increase in NAD^+ in mammalian cells led to activation of SIRT1 and SIRT3, increased oxidative metabolism, and protection against high-fat diet-induced metabolic abnormalities [290]. As noted earlier, the presence of nicotinamide ribose in cow's milk points to a potential dietary therapy [68].

Although stimulation of NAMPT increases NAD^+ and SIRT1 activity [278,291–293], this could, at least in part, be a result of greater nicotinamide clearance [70]. A NAMPT inhibitor reduces NAD^+ in the cytoplasm, but not in mitochondria, indicating that mitochondria lack the canonical NAD^+ rescue pathway [294].

Another way to boost NAD^+ availability for sirtuin activity could be via inhibition of other enzymes that utilize NAD^+, the major families being the PARPs and the cADP-ribose synthases CD38 and CD157 [66]. SIRT1 activity is downregulated during PARP-1 activation [75,295], and since SIRT1 is not directly affected by poly-ADP-ribosylation [75], it seems that PARP-1 and SIRT1 compete for a limited pool of NAD^+ in the cell. Owing to the very low K_m and high V_{max} of PARP-1 [296], PARP may limit SIRT1 activity, but the opposite will not apply. Inhibition of PARP-1 leads to higher SIRT1 activity, which then elevates mitochondrial oxidative metabolism, suggesting

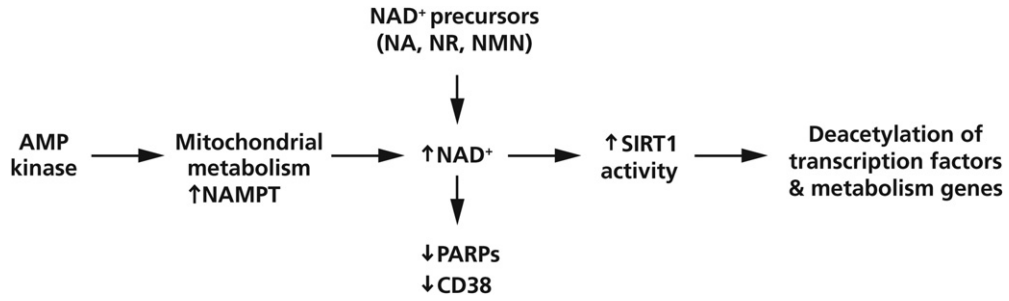

Fig. 9. The position of NAD^+ at a crucial hub of pathways that affect SIRT1 activity and thus metabolic regulation. NAD^+ is most likely rate-limiting for SIRT1 activity and therefore its actions on various pathways under divergent metabolic conditions. Activation of AMP kinase increases NAD^+ biosynthesis through precursor (nicotinic acid [NA], nicotinamide riboside [NR], and nicotinamide mononucleotide [NMN]) supplementation, or through inhibition of other enzymes that consume NAD^+ (*e.g.*, PARPs or CD38), leading to an increase NAD^+ levels. (Modified from [63]).

a means of protecting against metabolic diseases such as those caused by diet-induced obesity [75]. PARP-1 inhibition also increased mitochondrial gene expression [75]. Drugs that inhibit PARP-1 [297] could block the progression of cancer in part by boosting oxidative metabolism and opposing the Warburg effect referred to earlier (*i.e.*, the reliance of cancer cells on anaerobic glycolysis even when oxygen is plentiful, an effect crucial for malignant transformation). By their ability to inhibit PARP-1, such drugs would, via increased NAD^+, raise SIRT1 activity, and by inhibiting PARP-2, would increase SIRT1 gene expression. The ability of sirtuin activators to induce a switch from glycolysis to oxidative metabolism [138,298] would similarly contribute to an antitumor action.

Inhibition of PARPs leading to increased SIRT1 activity should mimic calorie restriction, and thus the beneficial effects of SIRT1 on healthy aging [299]. The elevated PARP activity in aged tissues, accompanied by a reduction in SIRT1, supports this premise [63,204]. So too does the fact that glucose homeostasis is impaired, age-related diseases are elevated, and life span is attenuated in mice expressing an extra copy of the human PARP-1 gene [300].

Only SIRT1 is activated in response to PARP inhibition, there being no effect on SIRT2 or SIRT3 [75]. This is probably because only SIRT1 is located in the nucleus and there are different pools of NAD^+ specific for different intracellular compartments, with each pool being regulated independently [301].

A further NAD^+-consuming class of enzymes, the cADP-ribose synthases referred to above, are another group of molecules that compete more effectively than SIRT1 for intracellular NAD^+, CD38 having a very low K_m for NAD^+ [302]. Since approx. 100 molecules of NAD^+ are required to yield one cADP ribose molecule [303], even low levels of CD38 are likely to have a profound effect on intracellular NAD^+ [63]. Although CD38 is mainly extracellular, small quantities are also present inside the cell, including the nucleus, endoplasmic reticulum, sarcoplasmic reticulum, and mitochondria [304,305], where CD38 would compete with SIRT1 for available NAD^+ [306]. In mice lacking CD38 the tissue level of NAD^+ is elevated 10- to 20-fold [305] and CD38 in nuclear extracts is several fold higher [306]. The elevation in NAD^+ in such mice was accompanied by a doubling of SIRT1 activity and consequent deacetylation of targets of SIRT1 [306]. Use of specific CD38 inhibitors [307] could also be relevant to treatment of diet-induced diabetes and other conditions [63].

Activation of SIRT1 changes the genome-wide transcription profile to resemble in many ways that induced by calorie restriction [308,309]. The potential for SIRT1-activating compounds (STACS) in treatment and prevention of common diseases of aging [47] was what prompted the extensive molecular screening, irrespective of structure, for SIRT1 activators [258]. Thus STACs, by direct and/or indirect actions, should engender metabolic fitness. STACS are now undergoing clinical trials.

The six other sirtuins are also likely to be valuable therapeutic targets. Their activation will likely involve chemicals that differ from those that lead to SIRT1 activation. But, as noted, since NAD^+ activates all sirtuins, modulation of NAD^+ levels seems a strategy worthy of exploration [63].

Since SIRT1 is critical for embryonic development, studying the role of SIRT1 on whole-animal physiology and biochemistry cannot be done in germ-line knockout animals. These complications were overcome by developing mouse strains in which whole-body SIRT1 deletion could be induced after the mice reach adulthood [310]. The usual increase in mitochondrial biogenesis and function, AMPK activation, and increased NAD^+ in skeletal muscle in response to a moderate dose of resveratrol did not occur in these knockout mice. But at high doses resveratrol did activate AMPK. These experiments showed that dose is critical for AMPK activation. In mice lacking SIRT1 there was, however, no improvement in mitochondrial function irrespective of dose of resveratrol. Glucose homeostasis and liver function did not differ between SIRT1 knockout and wild-type mice in response to resveratrol. Whether this means SIRT1 has only a minimal role in liver or whether there is either residual SIRT1 in the knockout mice or compensation from extrahepatic influences is unclear. As noted, AMPK is an important mediator of resveratrol action [270,310]. It seems that at low doses resveratrol stimulates SIRT1 upstream of AMPK by deacetylation of the AMPK activator LKB1 [310] and, as discussed above, inhibition by resveratrol of PDE4, thus elevating cAMP and CamKK2 [275]. It was argued, however, that the latter mechanism might only occur with high doses of resveratrol [310]. Once activated, AMPK stimulates enzymes for NAD^+ production and the rise in NAD^+ increases SIRT1 activity [64,270]. Based on experiments with C2C12 myoblasts, it could be that at lower doses resveratrol stimulates SIRT1-dependent phosphorylation of AMPK, whereas higher doses activate AMPK by a mechanism that does not depend on SIRT1 [310]. Interestingly, at lower doses of resveratrol, NAD^+ and ATP are increased by 12 h, but at higher doses (50 μM) each is reduced [310]. It may be that the higher doses inhibit mitochondrial respiration, thus lowering ATP, and that this then leads to AMPK activation, independently of SIRT1 [310]. Since PGC-1α is the ultimate beneficiary of the signaling pathways induced, it will be important to determine whether SIRT1-dependent deacetylation of PGC-1α occurs prior to, coincident with, or after AMPK activation [311]. In this regard, it is of interest that a specific AMPK agonist, AICAR, increases NAD^+, SIRT1 activity, and PGC-1α deacetylation [64]. Clearly SIRT1 is a mediator of the effects of resveratrol, increased PGC-

1α protein and activity being in large part what then lead to prevention of diseases involving mitochondrial dysfunction and aging [19,64,265,283]. But whether the rise in NAD^+ is an adaptation to drive SIRT1 effects long term or to replenish NAD^+ consumed by the initial increase in SIRT1 activity is another issue to be resolved [311].

In insulin target tissue, resveratrol decreases iNOS and NO production in skeletal muscle and white adipose tissue, but not liver, of endotoxin-treated mice [312]. This was also seen in each cell type *in vitro* and involved activation of AMPK, but not SIRT1 [312].

Other SIRT1 activators include oxazolo[4,5–b]pyridines and related heterocyclic analogs [313], as well as imidazo[1,2–b]thiazole derivatives [314]. Whereas there are chemicals with a wide range of core structures that inhibit the other sirtuins [256], sometimes selectively, as for SIRT2 [315] and SIRT3 [316], activators are less common. It is therefore of interest that placement of a benzyl group at N1 of the scaffold structure of 1,4-dihydropyridine conferred potent SIRT1, -2, and -3 activation [317]. SIRT3, AMPK, and mitochondrial biogenesis are activated by the natural stilbene viniferin [318]. Viniferin also attenuates the huntingtin-mediated loss of SIRT3 in neurons [318]. SIRT3 was, moreover, essential for the neuroprotective effect exerted by viniferin.

Clinical trials of resveratrol and SRT compounds in patients with various conditions are in progress and the limited results to date highlight the need for care in relation to dose [3,319]. A small clinical study with resveratrol has provided promising results for obesity [279]. For treatment of cancer, inhibitors of SIRT1 and SIRT2 have been proposed [256], but there is a long way to go before the various chemicals available reach the clinical trials stage.

Obesity

In obesity SIRT1 activity is low, consistent with a causative role. The mechanism of SIRT1's effect could, in part, involve hypothalamic control of food intake [229]. Genetic deletion of SIRT1 in orexigenic Ag-RP neurons of the hypothalamus and administration of the SIRT1 inhibitor EX527 reduce food intake and body weight via the melanocortin pathway [320,321], whereas knockout of SIRT1 in anorexigenic POMC neurons of the hypothalamus leads to obesity [322]. Perhaps SIRT1 is required for homeostasis, for example, by suppressing NF-κB. Thus, by disrupting the normal biology of different cells, deletion of SIRT1 in an anorexigenic cell type would cause obesity, whereas deleting it in an orexigenic cell type would lead to loss of body weight [323]. Mice lacking SIRT1 in SF1 neurons exhibit reduced energy expenditure and hypersensitivity to diet-induced obesity [324]. The converse is true for mice overexpressing SIRT1. It has been pointed out that to be effective in prevention of weight gain, SIRT1-activating compounds must be found that target only SF1 and POMC neurons, avoiding AgRP-expressing neurons [229]. Doing so would also avoid psychotropic side effects typical of current anorectic antiobesity drugs [325]. Various hormones control appetite by hypothalamic effects. Impairments in the action of orexin-A and leptin have, moreover, been implicated in the causation of obesity in SIRT1-deficient mice [324].

Transgenic SIRT1 overexpression in the striatum and hippocampus upregulates adipogenic genes in white adipose tissue and increases fat accumulation [326]. The mice exhibit decreased energy expenditure and lower mitochondrial gene expression in muscle, accompanied by impaired lipid and glucose metabolism, motor function, and hypothalamic hormone axis [326].

SIRT1 in fat cells inhibits adipocyte differentiation and suppresses inflammation by targeting PPAR-γ and NF-κB [327]. The latter two factors might be expected to be increased when SIRT1 is knocked out. However, SIRT1 null mice exhibit lower body weight, smaller adipocytes, reduced adipocyte differentiation, reduced extracellular matrix content, lower adiponectin and leptin, decreased capillary density, lower angiogenic factors (apart from VEGF), reduced macrophage infiltration and inflammatory cytokine expression in adipose tissue, and lower macrophage differentiation of embryonic fibroblasts [327]. Perhaps SIRT1 controls adipose tissue function via regulation of angiogenesis, which is also controlled by inflammation. SIRT1 suppresses transcription of genes for inflammation, thus reducing inflammation in adipose tissue [328].

Both glucose control and control of fat synthesis involve subtle effects. SIRT1 knockout was found to predispose fat-fed mice to weight gain, hepatic triglyceride accumulation with fasting, and hepatic steosis in some studies [83,185,186,329], but not in another, in which mice with liver-specific SIRT1 deletion gained less weight, had better glucose tolerance, and were protected against hepatic steosis [62]. The latter study found no specific changes in response to a regular diet and the mice responded normally to calorie restriction [62]. Hepatic steosis and lipid accumulation in heterozygous SIRT1 knockout mice fed regular chow worsened with age [186].

Extendin-4 decreases body weight, free fatty acids, triglycerides, and heptic steosis by stimulating SIRT1 expression and downstream effectors such as AMPK in hepatocytes *in vivo* [330]. Lipolysis is stimulated when SIRT1 activation results in deacetylation and thereby activation of FOXO1, which binds to the promoter of the rate-limiting lipase for lipolysis, ATGL [331].

SIRT2 is low and HIF-1α is high in visceral adipose tissue from obese subjects [332]. Activation of HIF-1α in visceral white adipocytes is critical for diet-induced obesity, glucose intolerance, insulin resistance, and cardiomyopathy. Suppression of SIRT2 transcription leads to diminished PGC-1α and genes for β-oxidation and mitochondrial function [332].

As referred to above, screening of a vast array of compounds identified the natural phytoalexin, resveratrol, as a potent SIRT1 activator [6]. Although resveratrol can extinguish virtually all of the adverse effects of a high-fat diet in ob/ob mice, a strain that lacks leptin, obesity persisted [244,265]. Nevertheless diabetes, cardiovascular disease, cancer, hepatic steosis, and premature death were prevented [244,265]. As well, a shift in muscle fiber composition meant the mice could run twice as far before exhaustion [244]. Whereas life span shortening is averted in obese mice, resveratrol did not significantly extend their life span [283].

The beneficial effects of resveratrol in obese mice have been replicated in healthy obese humans [279]. As noted earlier, the dose needed (2 mg/kg/day) was 10-fold lower than that required in obese mice. The plasma levels achieved were, however, similar. After 30 days of treatment the subjects had reduced metabolic rate, as well as increased mitochondrial OXPHOS and respiration in skeletal muscle, but not number of mitochondria. There were increases in SIRT1 and PGC-1α protein, as well as activation of AMPK in skeletal muscle. Lipid was reduced in liver but increased in muscle, and plasma glucose, triglycerides, alanine-aminotransferase, and markers of inflammation were all decreased. Insulin sensitivity improved and systolic blood pressure was reduced by 5 mm Hg. The metabolic changes observed were reflected in the changes in gene expression seen by genome-wide expression profiling, with 219 genes upregulated and 250 downregulated [279]. The pathways affected were similar to those seen in obese mice treated with resveratrol [244]. There were no adverse effects.

α-Lipoic acid activates SIRT1 in mice [333]. This occurs upstream of AMPK activation and leads to lowering of plasma

lipids and intracellular triacylglycerol accumulation [333]. The beneficial effects of α-lipoic acid on mitochondrial function and oxidative stress in high-fat fed obese mice involve stimulation of SIRT3 as well as SIRT1, leading to increased deacetylation of FOXO3A and PGC-1β and a reduction in hepatic steosis [334].

The microRNA miR-132, by decreasing SIRT1, lowers deacetylation of the p65 subunit of NF-κB, thus activating the latter and inducing IL-8 and MCP-1 transcription [335]. The p65 subunit is also a target of SIRT2, which, by causing its deacetylation in the cytoplasm, is involved in the regulation of NF-κB-dependent genes as well [154]. miR34a increases with disease severity in hepatic steosis and suppresses SIRT1 expression [336].

Maternal obesity of gestation is accompanied by diminished SIRT3 expression and mitochondrial function [337]. The resultant reduction in fatty acid oxidation in liver likely contributes to the obesity and associated comorbidities of this condition.

Type 2 diabetes

Sirtuins have important roles in diabetes. Transgenic overexpression of SIRT1 prevents diabetes in various mouse models of this condition, as well as diabetes that occurs during normal aging [240,242]. Similarly, chemical activation of SIRT1 has antidiabetic and other beneficial effects. SRT1720 is one chemical being examined in clinical trials. The key NAD^+ intermediate NMN, in part via activation of SIRT1, ameliorates type 2 diabetes seen with aging and caused by a high-fat diet in mice [69]. In addition to actions on liver, muscle, and fat cells, diabetes can be ameliorated by effects of SIRT1 in the hypothalamus, which is a major regulator of feeding behavior and energy expenditure [212,229,322]. The ability of resveratrol to improve glucose homeostasis involves in large part a SIRT1-dependent pathway in the arcuate nucleus of the mediobasal hypothalamus, which has a pivotal role in integrating responses to peripheral alterations in nutrients and hormones [338].

SIRT1 increases insulin sensitivity by repressing the tyrosine phosphatase PTP1B [242,244,258,265,339] and increasing insulin secretion by suppressing UCP2 [202]. The antidiabetic drug metformin activates SIRT1 and AMPK, accompanied by a reciprocal reduction in p53 protein, which becomes deacetylated at a SIRT1 target site [340]. In diabetic retinopathy, metformin suppresses the metabolic "memory" of hyperglycemia stress in small and large blood vessels [341]. SIRT1 is intimately involved in mediating metformin's beneficial effect. Resveratrol, by activating AMPK, SIRT1, and NF-κB, reduces retinal inflammation in diabetes [342].

Advanced glycosylation end products derived from food have a prooxidant effect and their contribution to insulin resistance in type 2 diabetes may involve suppression of SIRT1 expression [343].

The microRNAs miR-34a and miR-93 are increased in liver in middle and old age [344]. SIRT1 in hepatocytes is downregulated posttranscriptionally by miR-34a [253,344], miR-93 [344], and miR-181a [344] via a target site in the 3′UTR of SIRT1 mRNA. miR-34a and miR-93 also regulate Sp1, a transcription factor that regulates SIRT1 expression [344]. Insulin-resistant liver cells and serum from diabetic patients exhibit elevated miR-181a [344]. Overexpression of miR-34a in pancreas inhibits insulin secretion [344] and overexpression of miR-181a causes insulin resistance in liver cells [345]. Thus inhibition of miR-34a and miR-181a could help in the treatment of insulin resistance and type 2 diabetes.

The peptide obestatin, derived from ghrelin, prevents apoptosis in 3T3-L1 preadipocytes by stimulating PI3K/AKT and ERK12 signaling, leading to phosphorylation of AMPK, induction of adiponectin, suppression of leptin, and inhibition of lipolysis [346]. Obestatin stimulates SIRT1 expression, which mediates the stimulation by obestatin of glucose uptake [346]. In mice fed a high-fat diet, obestatin ameliorates metabolic dysfunction [346].

Dietary restriction, by increasing SIRT1 levels and reducing inflammation, ameliorates renal injuries in diabetic Wistar fa/fa rats [347]. SIRT1 mediates in part the protective effect of resveratrol against mitochondrial function in renal mesangial cells, thus reducing oxidative stress and providing a possible therapy for diabetic nephropathy [348]. Each of NAD^+, resveratrol, and rapamycin can oppose mesangial cell senescence induced by high glucose, and this effect involves SIRT1 [349]. Downregulation of SIRT1, followed by an increase in acetylated FOXO4 and expression of the proapoptosis gene Bcl2I11, leads to apoptosis of glomerular podocytes [350]. The authors of this study therefore advocated stimulation of SIRT1 for prevention of podocyte loss in diabetic nephropathy [350]. Podocyte injury and mitochondrial damage caused by aldosterone can be prevented by SIRT1 activation and this preventive effect involves upregulation of PGC-1α [351].

Fig. 10 shows several of the SIRT1 targets associated with type 2 diabetes and the other conditions that will be discussed in the following sections.

Cardiovascular disease

By various means the actions of SIRT1 protect against cardiovascular disease processes [352,353]. Humans who restrict their caloric intake exhibit a very favorable cardiovascular risk profile [354]. Calorie restriction, by increasing SIRT1, leads to deacetylation and thereby activation of eNOS [355]. The ensuing rise in NO causes vasodilatation and vascular protection. This may contribute to the ability of resveratrol (150 mg/day for 30 days) to reduce systolic blood pressure by 5 mm Hg [279].

SIRT1 is involved in protection against atherosclerosis [356]. Long-term calorie restriction in Rhesus monkeys delays the onset of cardiovascular and other diseases of aging [23,24]. Transgenic overexpression [357] or resveratrol-mediated activation [358] of SIRT1 reduces plaque formation. Susceptibility to atherosclerotic lesion formation is reduced by SIRT1-mediated activation of eNOS in endothelial cells [109] and inhibition of proinflammatory NF-κB signaling [113]. Moreover, the ability of AMPK to phosphorylate eNOS is needed for SIRT1 to deacetylate eNOS and thus affect NO generation [359]. Thus eNOS seems to be an important factor mediating the beneficial effect of SIRT1 on the cardiovascular system. Interestingly, SIRT1 expression in both endothelial and smooth muscle cells of resistance-sized vessels in the brain seems important for endothelium-dependent relaxation [360].

The protective effect of SIRT1 against atherosclerosis involves a reduction in macrophage foam cell formation [361]. This effect of SIRT1 is due to suppression of NF-κB signaling, which results in a decrease in expression of LOX-1 [361]. In fact transgenic mice with endothelium-specific blockade of NF-κB signaling exhibit reductions in markers of oxidative stress, diet- and age-related insulin resistance, vascular senescence, metabolic deterioration, as well as improvement in locomotor activity, upregulation of mitochondrial sirtuins, and increased life span [362].

While loss of SIRT1 in vascular endothelial cells does not affect the vasculature during embryonic development, it impairs revascularization in response to ischemia [363]. This includes a reduction in endothelial cell branching and proliferation, a decrease in blood vessel density, and a defect in the ability to form vascular networks. Part of the effect involves loss of SIRT1-mediated deacetylation of FOXO1, where normally SIRT1 deacetylation of FOXO1 opposes its antiangiogenic action [363]. FOXO1, -3, and -4 are essential for keeping vascular endothelial cells quiescent [364]. SIRT1 also blocks Notch and thus the ability of Notch to

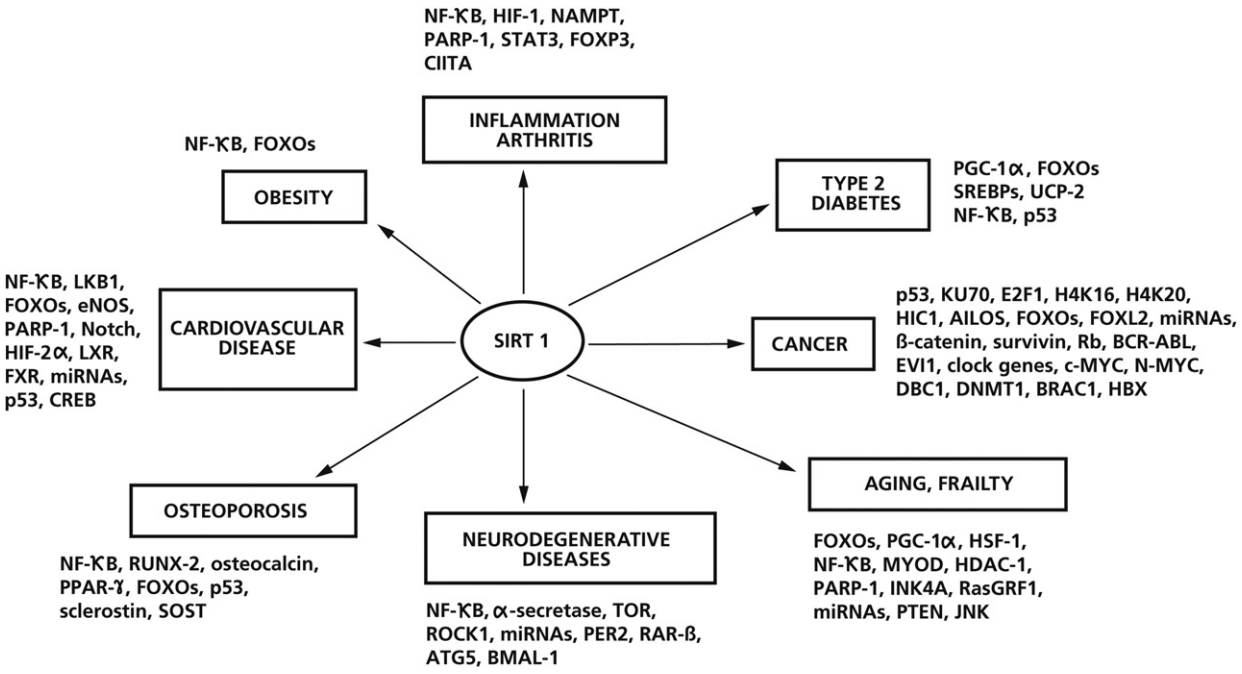

Fig. 10. The numerous SIRT1 targets and regulatory proteins that are involved in various diseases of aging. (Expanded and modified from [3]).

coordinate essential steps in blood vessel morphogenesis [365]. An alteration in the interaction of SIRT1 and Notch signaling may contribute to bicuspid aortic valves, present in over 1% of the population, and that are a risk factor for aortic dissection and aneurysm [366].

Vascular remodeling induced by hypertension appears to involve SIRT2, which deacetylates microtubules, leading to redistribution of endothelial cells in response to angiotensin II and mechanical stretch [367].

SIRT1 expression is enhanced by pulsatile flow-induced shear stress [359]. Resveratrol by activating SIRT1 (indirectly) activates the MAP kinase-5/MEF2-dependent signaling pathway, thus leading to upregulation of the transcription factor Krüppel-like factor 2 that controls expression of flow-responsive genes [368]. Activation of SIRT1 signaling pathways by resveratrol also inhibits the oxidative stress-dependent pathology of endothelial cells in response to high shear stress and proinflammatory factors [369].

Part of the vascular protection provided by SIRT1 appears to involve inhibition of expression of the angiotensin II receptor AGTR1 [370]. Knockout of AGTR1 reduces oxidative organ damage and increases renal SIRT3 and NAMPT [371]. The reduction in ROS was in fact mediated by the elevation in SIRT3. The life span of these mice was increased by 26%.

SIRT1 plays an important role in preserving cell viability during hypoxia. In response to hypoxia SIRT1 activity and expression increase [372]. This increase is HIF-dependent. SIRT1 activates HIF-2α and promotes degradation of PHD2 [173,373], which controls the various HIF isoforms that are transcription factors involved in orchestrating adaptive responses in expression of genes for cell survival, metabolism, and angiogenesis in response to alterations in oxygen tension [374,375]. In contrast, SIRT1 inhibits HIF-1α [298]. SIRT6, which, like SIRT1, is located in nuclear chromatin, is a corepressor of HIF-1α [56]. In mitochondria SIRT3 inhibits HIF-1α via effects on ROS [138].

SIRT1 prevents premature senescence induced in endothelial cells by oxidative stress [376]. This effect of SIRT1 is mediated by the major stress sensor p53 [376]. Other actions of SIRT1 that protect endothelial cells from senescence involve deacetylation of LKB1, leading to degradation of the latter, thus preventing prolonged LKB1-induced AMPK signaling [377]. SIRT1 also suppresses $p66^{SHC}$, an adaptor protein, which, when inactivated, protects mice from vascular disease associated with aging [378]. SIRT1 does this by binding to the promoter and lowering histone H3 acetylation, thus blocking transcription. During oxidative stress, binding of SIRT1 to FOXO3A shifts its effects on transcription from apoptosis to stress resistance [80]. In endothelial cells FOXO3A, via an interaction with PGC-1α, stimulates transcription of enzymes such as MnSOD that are involved in scavenging ROS [379].

SIRT1 activation provides neuroprotection during cerebral ischemia by inducing autophagy [380]. NAMPT, which is rate limiting in NAD^+ production, causes an elevation in SIRT1 activity, which then affects TSC2–TOR–S6K1 signaling to induce the autophagic proteins LC3-II and beclin-1 [380].

Hyperhomocysteinemia, a major risk factor of cardiovascular disease, is associated with increased oxidative stress leading to endothelial cell dysfunction. A reduction in methylenetetrahydrofolate reductase leads to increased ROS generation, eNOS uncoupling, reduced NO, lower homocysteine, impaired differentiation and increased senescence of endothelial progenitor cells, decreased telomere length, and downregulation of SIRT1 expression [381].

miR-34a, by downregulating SIRT1 mRNA, induces senescence of endothelial cells [382] and EPC-mediated angiogenesis [383]. miR-34a's effect on senescence also involves modulation of FOXO1 [383]. The therapeutic benefit of atorvastin on coronary artery disease may involve inhibition of miR-34a, leading to upregulation of SIRT1 in EPCs [384]. Lysosomal dysfunction, via cathepsin-induced proteolytic cleavage of SIRT1, appears to play a critical role in stress-induced premature senescence of EPCs [385].

Another means by which SIRT1 protects against atherosclerosis is by effects on lipid and glucose, as well as metabolism in liver. SIRT1 increases HDL cholesterol by activating LXR [386] and the nuclear bile acid receptor FXR [387]. LXR activation in liver upregulates ABCA1, which then increases the transport of reverse

cholesterol from peripheral tissues [386]. LXR also activates the SREBP1 gene in liver. SREBP1 is a transcription factor that is highly active in the fed state and works to promote expression of lipogenic and cholesterogenic genes, thus increasing synthesis of fat and cholesterol. SIRT1, by deacetylating SREBP1 and thus suppressing its activity, inhibits lipid and cholesterol synthesis [100]. FXR activation results in its dimerization with RXRα, leading to favorable outcomes on lipids and glucose metabolism [387]. In a curious finding, one group found a proatherogenic effect of SIRT1 overexpression in mice fed an atherogenic diet [388]. This involved deacetylation of CREB. The ability of SIRT1 to improve glucose metabolism and insulin resistance in type 2 diabetes is another means by which SIRT1 counteracts atherosclerosis.

Thus in various ways SIRT1 potently protects blood vessels against inflammatory and procoagulant stress signals.

Sirtuins also exert cardiac benefits. For example, overexpression of SIRT1 protects the heart against pathological cardiac hypertrophy [389]. In heart failure, genes regulating mitochondrial function are downregulated. Cardiac hypertrophy and failure involves in part binding of PPAR-α to SIRT1, thus recruiting SIRT1 to the estrogen-related response element and repressing mitochondrial genes in a RXR-dependent manner, just as occurs during fasting [390]. Activation of SIRT1 by resveratrol improves cardiac contractility, as well as left ventricular function, in trauma-hemorrhage [391]. PGC-1α and ATP increase, and c-MYC, cytosolic cytochrome c, and plasma TNF-α decrease [391]. The protective effect of SIRT1 on the heart during ischemia–reperfusion injury involves eNOS, NF-κB, and stimulation of autophagy [392]. Overexpression of IGF-1 propeptide helps the heart recover from an infarct and this may involve JNK-1-signaling pathway-mediated stimulation of SIRT1 expression [393].

Postinfarction heart failure is associated with reduced SIRT3 as well as lower mitochondrial respiratory control index and higher mitochondrial permeability transition pore opening [394]. SIRT3, via modulation of mitochondrial homeostasis and proteins that mediate energy metabolism and adaptation to mitochondrial redox stress, is able to impede cardiac hypertrophy [395].

Overexpression of SIRT6 can suppress angiotensin II-induced cardiomyocyte hypertrophy by binding to the p65 subunit of NF-κB, thus blocking the effects of NF-κB on transcription [396]. The NAD^+ regulating enzyme NMNAT2 is downregulated in cardiac hypertrophy, and NMNAT2 overexpression blocks angiotensin II-induced cardiac hypertrophy in a SIRT6-dependent manner [397].

In the case of SIRT7, homozygous knockout leads to cardiac hypertrophy, inflammatory cardiomyopathy, and reduced life span [51].

The kidney is important for blood pressure regulation. The protective effects of calorie restriction on the kidney with aging in mice are mediated by SIRT1 and activation of FOXO3 [398]. SIRT1 protects the tubular epithelium by maintaining peroxisome number and function, upregulating catalase, and reducing ROS [399]. Renal medullary cells are particularly prone to metabolic stress owing to local hypoxia and hypertonicity. Here too SIRT1 has a protective function, in part via induction of COX2 expression [400]. Overexpression of SIRT1, or activation of SIRT1 with resveratrol, upregulates PGC-1α, leading to improvement in mitochondrial function and epithelial–mesenchymal transition in renal tubulointerstitial fibrosis [401]. In a mouse model of renal lipotoxicity, SIRT3 reverses ROS production and inflammation in proximal tubule cells [402].

Neurodegenerative diseases

A functional deficit in sirtuin activity appears to be involved in a variety of neurological diseases that increase in frequency during aging. The cognitive decline that occurs with aging may be countered by sirtuin activation.

SIRT1 improves learning and memory in mice [403,404]. Its expression in the hippocampus is crucial to such actions. This involves effects on dendritic branching, branch length, complexity of neuronal dendritic arbors, ERK1/2 phosphorylation, and its ability to alter expression of hippocampal genes involved in synaptic function, lipid metabolism, and myelination [404]. SIRT1 also represses miR-134 that normally downregulates CREB and BDNF to impair synaptic plasticity [403]. Another microRNA, miR-34a, regulates neuronal stem cell differentiation in part by regulation of SIRT1 expression, but also p53 [405]. In a positive feedback loop, p53 induces expression of miR-34a, which suppresses SIRT1, thus preventing SIRT1-mediated deacetylation of p53, and thereby increasing p53 activity [406].

The most common serious neurodegenerative disorder is Alzheimer's disease. In a model of this condition SIRT1 activation prevented axonal degeneration [407] and neurodegeneration [408]. SIRT1 is upregulated in mouse models of Alzheimer's disease produced by transgenic overexpression of p25 (a toxic coactivator of CDK5), as well as in cultured neurons subjected to neurotoxic insults [408]. This could possibly represent a defense mechanism. By inhibiting NF-κB signaling in microglia SIRT1 protects against β-amyloid toxicity [409]. The increase in tau protein aggregates (''tangles'') in Alzheimer's disease can be reversed by SIRT1's ability to deacetylate tau, thus destabilizing this protein and reducing tangles [410]. In a mouse model of Alzheimer's disease, overexpression of SIRT1 in the brain reduces toxic β-amyloid generated by proteolytic cleavage of the amyloid precursor protein [411]. A major aspect of this effect involves stimulation of α-secretase gene expression via SIRT1-mediated deacetylation of RAR-β, the transcriptional activator of ADAM10 (the α-secretase gene) [411,412]. As a consequence the amyloid precursor is directed along an alternate pathway that does not lead to β-amyloid production. In mice overexpressing both β-amyloid and SIRT1, less plaque is formed. In addition, cleavage of the membrane-bound Notch receptor by α-secretase leads to upregulation of genes for neurogenesis [413]. The amelioration of neuronal stress in the brain, and in cultured neurons, by SIRT1 involves suppression of the serine/threonine Rho kinase ROCK1, the action of which is to inhibit α-secretase and thereby amyloid precursor protein processing in brain [414]. SIRT1 also promotes growth and survival of neurons in the CNS by downregulating TOR [415]. Leptin activates AMPK and SIRT1 in neuronal cell lines; thus the association of low plasma leptin with increased risk of Alzheimer's disease may involve a reduction in SIRT1 [416]. Leptin's ability to reduce phosphorylation of tau and production of β-amyloid is, moreover, sensitive to inhibitors of AMPK and sirtuins [416].

Vascular lesions have been implicated in age-related brain pathology [417]. The ε4 variant of apolipoprotein E is strongly associated with Alzheimer's disease. Unlike APOE3, APOE4 is unable to prevent inflammation in pericytes and the consequent disruption of the blood–brain barrier leads to leakage of blood-borne molecules that are toxic to neurons [418]. The vascular protection afforded by sirtuins may add to its role in the interplay between the vascular and the nervous systems in Alzheimer's and other neurodegenerative disorders. It therefore seems reasonable to suggest that SIRT1 activation in the brain might help prevent or impede the progression of Alzheimer's disease [419].

Loss of neurons with age is also seen in amyotrophic lateral sclerosis (ALS). In human postmortem ALS samples, while SIRT1 and SIRT2 were reduced in white and gray matter of the primary motor cortex, in cortical layers 1–VI of the precentral gyrus and ventral/dorsal horn of the spinal cord SIRT1, -2, and -5 were increased [420]. SIRT1 is upregulated in a mouse model of ALS

involving transgenic overexpression of mutant SOD1 [408]. In this model the increase in SIRT1 occurs in cerebral cortical pyramidal cells, hippocampal pyramidal cells of area CA1–3 and dentate gyrus cells, thalamus, and spinal cord [421]. By activation of SIRT1, resveratrol protects against neurodegeneration in cell models of ALS [408,422].

In a mouse model of Huntington's disease, overexpression of SIRT1 improves neuropathology, increases BDNF expression, and enhances survival, whereas brain-specific knockdown of SIRT1 exacerbates pathology [423,424]. SIRT1 activates TORC1 and promoted its interaction with CREB [423]. SIRT1 rescues Huntington's disease neurons from the interference caused by mutant huntingtin protein on the TORC1–CREB interaction that represses BDNF transcription [423,424]. BDNF transcription was also a key effect of SIRT1 and TORC1 in normal neurons. Enhanced cell survival involved SIRT1-mediated FOXOA deacetylation [424].

Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy involve a pathogenic effect of the protein α-synuclein. In models of Parkinson's disease, resveratrol, by stimulating SIRT1 and autophagy, causes degradation of α-synuclein, further confirming the importance of SIRT1 in neuroprotection [425]. SIRT1 overexpression in brains of mice with an α-synuclein mutation increases life span [426]. This effect involves deacetylation of molecular chaperones HSF1 and HSFV, thus increasing the activity of each [426].

SIRT1 protects neurons against neurotoxicity induced by prion protein 106–126 [427]. By inducing autophagy, SIRT1 protects against the reduction in mitochondrial membrane potential caused by this prion. SIRT1 also protects against prion-induced Bax translocation to mitochondria and release of cytochrome *c* into the cytosol [427]. The beneficial effects of SIRT1 were in part mediated by ATG5 and the definitive marker of autophagy, LC3-II [427].

TDP-43 is a major pathological marker in an array of neurodegenerative diseases. TDP-43 forms a functional complex with FMRP and STAU-1 [428]. The complex binds to the 3′UTR of SIRT1 mRNA in neuronal cells to increase SIRT1 mRNA stability, and thus SIRT1 levels and cell survival [428].

Sirtuin agonists have potential as therapeutics for neurological diseases and neurological decline with aging. Such agents need to be able to cross the blood–brain barrier, however. While SIRT1 activation is neuroprotective, upregulation of the major sirtuin in brain, SIRT2, is toxic to neuronal cells. Chemical or siRNA-mediated inhibition of SIRT2 rescued cells from toxicity induced by α-synuclein in a cell model of Parkinson's disease [429]. In an animal model of Parkinson's disease SIRT2 deacetylated FOXO3A, thus increasing Bim, which stimulates apoptosis [430]. This points to the use of SIRT2 inhibitors in the treatment of Parkinson's disease. SIRT2 inhibition protects dopaminergic neurons against cell death in a *Drosophila* model and in a cell model of Parkinson's disease [429]. Neuroprotection in response to SIRT2 inhibition is seen in a cell model and in a huntingtin-challenged *Drosophila* model of Huntington's disease [431,432]. Inhibition of SIRT2 reduces sterol levels via a decrease in trafficking of SREBP-2 [432]. Manipulation of cholesterol biosynthesis at the transcriptional level mimicks SIRT2 inhibition, thus establishing that the metabolic effects of SIRT2 inhibition are sufficient to reduce mutant huntingtin toxicity [432]. *In silico* screening identified a brain-permeable SIRT2 inhibitor possessing these neuronal effects [433].

SIRT2 could have adverse effects in nerves by inhibiting lysosome-mediated autophagic turnover, increasing accumulation and aggregation of amyloid-β and other proteins, and thus making cells more vulnerable to the cytotoxic effects of such aggregates [434]. Thus SIRT2 could be a valuable therapeutic target for a range of neurodegenerative diseases.

Roles for the five other sirtuins in neurological function and disease will require further research.

Cancer

There seems to be little doubt that sirtuins are involved in carcinogenesis. But owing to the complexity and diversity of their effects, the mechanisms remain elusive and there is as yet no consensus on precisely what the role of each is in cancer [435]. Overall, however, it would appear that SIRT2 and SIRT6 may be tumor suppressors, whereas, depending on the context, SIRT1 seems to be either a tumor suppressor or an oncogenic factor [435].

Does SIRT1 promote cancer?

SIRT1 is overexpressed in several types of human tumors, and could serve to silence tumor suppressors, so promoting cancer development [436,437]. Increased SIRT1 expression has been reported in breast cancer [166,438], prostate cancer [439], colon cancer [440], hepatocellular carcinoma [441,442], pancreatic cancer [443], nonmelanoma skin cancers [444,445], acute myeloid leukemia [446], and in adult T-cell leukemia-lymphoma patients and cell lines [447], consistent with, but not necessarily proving, an oncogenic role.

There are a multitude of possible mechanisms that might be involved. The basic reasons for SIRT1 overexpression in cancer are largely unknown [435]. Also not clear is whether SIRT1 overexpression causes or is a consequence of tumorigenesis [448].

SIRT1 overexpression is the most likely explanation for the loss of global acetylation of histone H4K16 and methylation of histone H4K20 seen in human tumors [449]. SIRT1 localizes to the promoters of tumor suppressor genes in which 5′ CpG islands are hypermethylated [450]. This leads to their transcriptional silencing. Inhibition of SIRT1 in breast and colon cancer cells increases H4K16 and H3K9 acetylation at the promoters of tumor suppressor genes and their reexpression, in the absence of an effect on promoter methylation [450]. SRT1720 administration activates SIRT1 in breast cancer cells implanted subcutaneously into mice, leading to tumor cell migration and pulmonary metastasis [451].

A likely reason for the elevated SIRT1 expression in cancer is loss of a transcriptional repressor. One is HIC1. Mice lacking HIC1 are prone to cancer and exhibit SIRT1-dependent resistance to apoptosis in response to DNA damage [166]. Another negative regulator of SIRT1 expression is IKZF3 (also known as AILOS), loss of which leads to lymphoma accompanied by increased SIRT1 [452].

In various cancers, loss of p53 is seen. p53 binds to the SIRT1 proximal promoter (Fig. 2) to suppress SIRT1 expression [159]. So loss of p53 would increase SIRT1 expression. Also, SIRT1 might cause cancer by its ability to deacetylate p53 at one lysine residue, inhibiting p53 activity on the promoter of p21, and blocking p53-dependent apoptosis [50,453]. SIRT1 inhibition reduces tumor growth and inhibition of p53 seems to be a common theme in mediating this effect [454]. For example, SIRT1 inhibition in chronic myelogenous leukemia (CML) reduces growth of quiescent leukemia stem cells both *in vitro* and *in vivo* in a p53-dependent manner [455]. The Philadelphia translocation involving formation of a fusion gene of part of the BCR gene and the ABL1 gene is a cause of CML. BCR-ABL kinase is constitutively active and, via STAT5 signaling, activates SIRT1 in hemopoietic progenitor cells, thus promoting CML cell survival and proliferation, accompanied by deacetylation of SIRT1 substrates such as FOXO1, p53, and KU70 [456]. Inhibition of BCR-ABL partially reduces SIRT1 expression, and knockout of SIRT1

prevents BCR-ABL-mediated transformation of mouse bone marrow cells and CML-like disease, as does SIRT1 inhibition [456]. Interestingly, SIRT1 is dispensable for maintenance of the hemopoietic stem cell compartment in mice [457]. Casein kinase-2 inhibitors, by downregulating SIRT1, sensitize glioma cells to TNFα-induced apoptosis [458].

An alternatively spliced form of SIRT1 lacking exon 8, and as a result its deacetylase activity, is expressed in human and mouse tissues [459,460]. The splice variant retains its ability to bind p53 [460]. The alternative splicing of SIRT1 that generates this version is, moreover, regulated by p53 [459]. The SIRT1 splice variant is overexpressed in various cancers lacking wild-type p53, and has been suggested as a possible therapeutic target [460]. As well as low deacetylase activity, the alternatively spliced SIRT1 is sensitive to stress, and differs from SIRT1 in its protein and RNA stability, as well as its interaction with other proteins [459]. Research is needed to determine the role of the alternatively spliced SIRT1 in cancer.

Studies *in vivo* have, however, suggested that SIRT1 does not affect p53-dependent functions [461]. Nevertheless, p53 can be downregulated by other sirtuins, namely SIRT2 [462] and SIRT3 [463].

Another effect of SIRT1 is deacetylation of the tumor suppressor protein Rb, reducing its activity, and thus stimulating the cell cycle [464]. In diffuse large-cell lymphoma and Burkitt lymphoma SIRT1 binds to and deacetylates the oncogene BCL6, so activating it [465]. Cambiol inhibits SIRT1 and SIRT2, leading to hyperacetylation and inactivation of BCL6 [465]. Since cambiol is well tolerated, it may be suitable for therapy.

The protein EVI1 causes proliferation and abnormal differentiation of hemopoietic stem cells [466]. In cell lines and chronic myeloid leukemia patient samples containing EVI1, SIRT1 is upregulated and this involves the binding of EVI1 protein to a site 1 kb upstream of the SIRT1 gene [467]. Moreover, SIRT1, by binding to and deacetylating EVI1, can negatively regulate EVI1 activity [467]. Thus SIRT1 may have a role in EVI1-positive neoplasms.

The oncogene c-MYC can bind to the SIRT1 promoter to induce SIRT1 expression [468]. SIRT1 then deacetylates c-MYC to reduce c-MYC activity, target gene expression, and cellular transformation [468]. It was suggested that this mechanism could block tumor initiation in premalignant cells.

The pathogenesis of neuroblastoma involves N-MYC, which stimulates SIRT1 transcription, followed by binding of SIRT1 to N-MYC, which serves as a transcriptional repressor complex on the MKP3 promoter [469]. The importance of SIRT1 in tumorigenesis of neuroblastoma was demonstrated by inhibition of SIRT1 with cambiol [469]. It was suggested that cambiol could serve in prevention and treatment of this cancer. In brain cancer, neuroinflammation causes breakdown of the blood–brain barrier. While resveratrol reduces markers of neuroinflammation (MMPs and COX-2) in human brain microvascular endothelial cells, SIRT1 appears not to be involved [470].

Expression of c-MYC is elevated in colorectal cancer and, by inducing NAMPT expression, raises NAD^+ and thus SIRT1 activity [471]. Moreover, c-MYC, by sequestering the SIRT1-binding protein DBC1 from SIRT1, increases SIRT1 protein [471]. Human fibroblasts immortalized with c-MYC undergo senescence and apoptosis in response to downregulation of SIRT1 [471]. SIRT1 binds to c-MYC and deacetylates it, thus increasing c-MYC stability [471]. SIRT1 also stimulates c-MYC gene expression [471]. Thus, in the context of deregulated tumors, this positive feedback loop may contribute to the development and maintenance of tumors. SIRT1, by triggering c-MYC transcription, increases the recruitment of c-MYC to the hTERT promoter, leading to enhanced life span of HUC-F2 fibroblasts [472]. In colorectal cancer a reduction in SIRT1 expression is associated with changes in clock gene expression, with lower PER3 and higher ARNTL1, CLOCK, PER1, PER2, CRY1, TIPIN, and CSNKIE [473]. Induction of SIRT1 leads to different changes in PER1 in different colorectal cancer cell lines [473].

Expression of both DBC1 and SIRT1 is associated with poor prognosis of gastric cancer [474] and breast cancer [438,475]. The negative regulation of SIRT1 by DBC1 may retard tumorigenesis, suggesting that the balance in expression of each may have prognostic significance [475]. High SIRT1 expression in gastric carcinoma correlates with lymphatic metastasis [476]. Multidrug resistance in gastric cancer involves binding of ATF4 to the SIRT1 promoter to activate SIRT1 expression [477].

In hepatocellular carcinoma SIRT1 promotes tumorigenesis by activating the PTEN/PI3K/AKT signaling pathway, leading to mitotic entry, cell growth, and caspase-3 and caspase-7-mediated inhibition of apoptosis [478].

SIRT1 regulates DNMT1, a key enzyme responsible for DNA methylation [479]. Depending on which lysines are deacetylated, DNMT1 activity can increase or decrease. Complete deacetylation impairs cell cycle G2/M transition. SIRT1 reduces the ability of DNMT1 to silence genes for the tumor suppressors ER-α and cadherin-1 [479]. Oxidative damage causes SIRT1 to relocalize to CpG islands in promoters [480]. This could explain aberrant DNA methylation and transcriptional silencing in specific cancers.

In ovarian cancer, FOXL2 downregulation or mutation is seen in granulosa tumor cells [481]. FOXL2 reduction is pivotal to tumorigenesis, ovarian aging, and premature ovarian failure [481]. SIRT1 suppresses FOXL2 activity on targets involved in the cell cycle and in DNA repair, so that inhibition of SIRT1 by nicotinamide limits proliferation by increasing FOXL2 [481].

As discussed earlier, more than 16 microRNAs modulate SIRT1 mRNA stability by binding to its 3′UTR [171]. The first to be identified as a SIRT1 regulator, miR-34a [172], serves as a tumor suppressor gene in neuroblastoma [482]. Expression of miR-34a is reduced in drug-resistant DLD-1 colon cancer cells, and introduction of miR-34a induces apoptosis in colon cancer cells by downregulating SIRT1 [172,483]. This miRNA also downregulates SIRT1 in pancreatic, prostate, brain, and liver cancers [171]. p53 induces miR-43a expression and this causes SIRT1 downregulation [171]. Thus mutation of p53 leads to increased SIRT1 expression. In human fibrosarcoma HT1080 cells, miR-520c and miR-373 suppress SIRT1 and TOR mRNA translation by targeting the 3′UTR of each [484]. This results in activation of the Ras/Raf/MEK/ERK signaling pathway and NF-κB, leading to increased MMP9 and thus elevation in cell migration and growth [484]. SIRT1 upregulation in breast cancer samples is associated with a reduction in miR-200a, another microRNA that targets the 3′UTR of SIRT1 mRNA [485]. In oligodendrogliomas and glioblastomas expression of 26 microRNAs differs from control brain tissue and 7 of these could discriminate each type of tumor [486]. SIRT1 was one of the miRNA targets differentially expressed in gliomas [486].

Patients with leukemia exhibit aberrant histone deacetylase activity. Treatment of leukemia cells with inhibitors of histone deacetylase causes upregulation of the proapoptotic BCl2 family member Bax, whose translocation to mitochondria is normally prevented by SIRT1 [487]. Since NAD^+-independent histone deacetylase activity and sirtuins cooperate in leukemia cells to avoid apoptosis, a combination of sirtuin inhibitors such as sirtinol, cambiol, and EX527 with histone deacetylase inhibitors leads to synergy in exerting an antileukemic effect [487].

In PC3 and DU145 prostate cancer cells, studies with sirtinol have shown that SIRT1 upregulation promotes cell growth and chemoresistance [488]. Moreover, studies involving sirtinol treatment of LNCaP prostate cancer cells reveal pleiotropic effects that involve pathways other than merely sirtuin inhibition [489].

SIRT1 deacetylates cortactin, a cell motility stimulating protein overexpressed in various cancers. Inhibition of SIRT1 or cortactin expression attenuates migration and invasion of DU145 prostate cancer cells [490]. By inhibiting cell proliferation and promoting apoptosis, FOXO3 functions as a tumor suppressor [364]. Deacetylation of FOXO3 by SIRT1 and SIRT2 causes the E3 ubiquitin ligase subunit SKP2 to bind to FOXO3, thus promoting its ubiquination and degradation [144]. Downregulation of FOXO3 in malignant PC3 and DU145 prostate cancer cells is a result of elevation of both SKP2 and SIRT1 [144]. Thus loss of FOXO3 caused by SIRT1 and SIRT2 upregulation might be considered pathogenic in some cancers. Inhibition of SIRT1 activity by a mutant version of the 25 amino acid C-terminal SIRT1 activator sequence, via its binding to the deacetylase core of SIRT1, increases the chemosensitivity of androgen-refractory prostate cancer cells [60]. This is consistent with a role for SIRT1 in supporting such prostate cancers. Both NAMPT, which generates NAD^+, and SIRT1 were overexpressed in prostate cancer cells, accompanied by increases in FOXO3A, catalase, and MnSOD, consistent with a role in carcinogenesis [491].

Interestingly, rather than being localized mostly in the nucleus, in cancer cells SIRT1 is located primarily in the cytoplasm [492]. This marked location aberration in cancer cells results from an elevation in stability of SIRT1 protein as a result of PI3K/IGF-1 receptor signaling. SIRT1 was, moreover, shown to be required for PI3K-mediated cancer cell growth [492]. The cytoplasmic location of SIRT1 was said to greatly contribute to its cancer-specific function.

An anticancer role for SIRT1?

There is also a body of *in vivo* evidence pointing to a tumor suppressor role for SIRT1 [19,436,492,493]. SIRT1 suppresses growth of intestinal and colon cancers [494], liver cancer [19], ovarian cancer, glioblastoma, and bladder cancer [436]. SIRT1 has a protective effect against breast cancer in mouse models [495] and colon cancer in human tumor specimens [494]. The latter involves an ability of SIRT1 to move an oncogenic form of β-catenin from the nucleus to the cytoplasm, limiting β-catenin signaling in colon cancer [494]. In response to SIRT1 activation, proliferation of pancreatic cancer cells is inhibited by downregulation of β-catenin and its target molecule cyclin-D1 [496]. In El4 lymphoma cells, resveratrol upregulates SIRT1 and downregulates NF-κB, thus promoting apoptosis, and having the potential to suppress tumor growth [497]. Resveratrol at 50 μM, but not 25 μM, inhibits SIRT1 expression and activity in Hodgkin lymphoma L-428 cells [498]. Both doses increase p53. These findings support the anticancer effect of resveratrol. Another small molecule, Inauhzin, by inhibiting SIRT activity, promotes p53-dependent apoptosis of lung cancer cells without exerting genotoxic stress [499].

Mice transgenic for SIRT1 exhibit a global decrease in carcinomas and sarcomas, but no alteration in lymphoma incidence [19]. These mice had less DNA damage and a reduction in $p16^{INK4A}$. Calorie restriction is well known to reduce cancer incidence, yet in p53-deficient mice engineered to have 2- to 3-fold overexpression of SIRT1, the increased cancer protection and life span extension that calorie restriction conferred were no different than in p53-deficient mice lacking SIRT1 overexpression, suggesting only a limited role for SIRT1 in cancer protection in response to calorie restriction [500].

In breast cancers associated with mutant BRCA1, SIRT1 is low and the apoptosis inhibitor survivin is high [495]. The wild-type form of BRAC1 binds to the SIRT1 promoter to increase SIRT1 expression, and SIRT1 then suppresses survivin. Since survivin inhibits apoptosis, resveratrol, by activating SIRT1, reduces survivin and thereby increases apoptosis [495].

Although SIRT1 might appear to be causative in cancer by inhibiting p53-dependent apoptosis [50,453], it may be that the other posttranslational modifications that p53 undergoes determine its specific activity. This has led to the concept that a "complex barcode" underlies p53's actions [501], and to the suggestion that the term p53 "specification" rather than "activation" should be used [63]. In support of this Cantó and Auwerx have used as an example the effect of p53 on mitochondrial metabolism. They pointed out that activation of p53 increases mitochondrial oxidation, whereas deacetylated p53 is associated with defective mitochondrial respiration. Yet, in the context of DNA damage, activated p53 decreases mitochondrial biogenesis [502]. Thus biological context and "barcode" of posttranscriptional regulation could be important determinants of the effect of p53, and no doubt other transcription factors.

The ability of SIRT1 to suppress tumors seems to include deacetylation and inactivation of HIF-1α and thus genes targeted by the latter [298].

It has been speculated that if SIRT1 elevation in many cancers is a consequence, rather than a cause, of cancer then, given the ability of SIRT1 to inhibit senescence and apoptosis, it might be that some tumors could become "addicted" to SIRT1 [435].

Anticancer drugs and SIRT1

HBX protein, by binding to SIRT1, attenuates SIRT1 binding to β-catenin, thereby upregulating β-catenin [503]. Stimulation of SIRT1 should therefore avoid resistance to anticancer drugs in HBX-related hepatocellular carcinoma [503].

The cancer drug doxorubicin causes genotoxic stress in both cancer cells and noncancer cells. By inhibiting AMPK it caused SIRT1 dysfunction and accumulation of p53 [504]. Thus pharmacological activation of AMPK and SIRT1 by resveratrol should lower the side effects of doxorubicin [504]. A side effect of doxorubicin is cardiovascular dysfunction. This involves a PARP-2-mediated reduction in SIRT1 in blood vessels [505]. Inhibition of SIRT1 increases the sensitivity of breast tumor cell lines to the chemotherapeutic agent paclitaxel, doing so via activation of caspase-2-dependent cell death by causing dissociation of 14-3-3ζ from caspase-2 [506]. The SIRT1 activator SRT1720 increases metastasis of breast cancer cells to the lung irrespective of cisplatin treatment [451]. Renal injury induced by cisplatin could be ameliorated by SIRT1 activation, which reduced acetylation of the p65 subunit of NF-κB induced by cisplatin, thus lowering inflammation [507].

Many of the proteins involved in the effects of SIRT1 in cancer are depicted in Fig. 10.

SIRT2 in cancer

Most evidence points to a role for SIRT2 as a tumor suppressor [435]. A protective role of SIRT2 against cancer is supported by its mitotic checkpoint function in the presence of mitotic stress, suggesting a role for SIRT2 activators in cancer therapy [41,146,508].

In gliomas, SIRT2 expression is reduced [509]. It may be that SIRT2 itself is lost, since its locus, at 19q13.2, is often deleted in gliomas [509]. SIRT2 inhibits colony formation in glioma cell lines [509]. This appears to involve an effect on microtubule regulation [509]. Overexpression of a SIRT2 phosphorylation site mutant in a glioblastoma cell line exposed to mitotic stress reduces hyperploid cells [510]. On the other hand, C6 glioma cells undergo apoptosis when SIRT2 is inhibited, pointing to a pathological role

of SIRT2 in this cancer, and possibly to a benefit from pharmacological inhibition of SIRT2 [511]. Similarly, HeLa cervical carcinoma cells undergo apoptosis in response to SIRT2 downregulation [462]. SIRT2 expression is also reduced in esophageal and gastric adenocarcinomas [512]. In melanoma, an inactivating mutation has been identified in the catalytic domain of SIRT2 [513].

The ability of SIRT2 to act as a tumor suppressor is supported by the development of hepatocellular carcinoma in male mice deficient in SIRT2 and mammary tumors in $SIRT2^{-/-}$ females [514]. SIRT2 is, moreover, reduced in human mammary and hepatocellular carcinomas [514]. SIRT2 regulates APC/cyclosome (a member of the RING family of ubiquitin ligases) by deacetylation of its coactivators APC^{CDH1} and CDC20 [514]. Loss of SIRT2 increases regulators of mitosis, including Aurora-A and Aurora-B that direct centrosome amplification, aneuploidy, and mitotic cell death. Further evidence that SIRT2 acts as a tumor suppressor is the hyperacetylation of the SIRT2 target lysine 53 of histone H3 in cancer cells [147].

In acute myeloid leukemia cells, however, SIRT2 and NAMPT are upregulated and participate in the aberrant proliferation and survival of leukemic cells [515].

SIRT3 in cancer

The mitochondrial sirtuin SIRT3 regulates metabolism and oxidative stress [252]. There is some evidence that SIRT3 acts as a mitochondrial tumor suppressor [135]. Loss of SIRT3 results in an elevation in ROS that increases stabilization of HIF-α and thereby increases expression of HIF-dependent genes, metabolic reprogramming toward glycolysis, and a drive toward tumor phenotype [138]. But whether SIRT3 serves as a tumor suppressor or promoter is controversial [516]. For example, SIRT3 levels are reduced in breast cancer, consistent with it being a tumor suppressor [135], but in malignant, lymph node-positive, breast cancer SIRT3 is increased [517]. SIRT3 is downregulated in hepatocellular carcinoma tissue [518]. Adenovirus-mediated overexpression of SIRT3 inhibited hepatoceullar carcinoma cell growth and induced apoptosis [518]. This was associated with NAD^+ suppression, reduction in ERK1/2 signaling, activation of AKT and JNK signaling, and, by downregulation of NDM2, results in reduced p53 degradation and thus increased p53 levels [518]. Oral squamous cell carcinoma cells exhibiting resistance to anoikis (a form of programmed cell death) have elevated SIRT3 expression, which may have arisen from reduced RIP [519]. Inhibition of SIRT3 inhibits anoikis resistance in this cancer and lowers tumor incidence [519]. The ability of SIRT3 to protect cells from oxidative stress is dependent on inhibition of the mitochondrial matrix protein IDH2, which is a major factor in cancer [520].

SIRT4 in cancer

Photodamage induces upregulation of SIRT1 and SIRT4 in skin cells, followed by loss of sirtuin proteins, then later accumulation of SIRT4 in particular [445]. In squamous cell carcinoma, expression of all 7 sirtuin genes is increased [445].

SIRT6 in cancer

SIRT6 appears to be a tumor suppressor. It deacetylates histones H3K9 and H3K56 [26–28] and is involved in DNA repair at double-stranded breaks [521,522]. H3K56 is hyperacetylated in skin, thyroid, breast, liver, and colon cancers [147]. Induction of SIRT6 overexpression in a variety of cancer cell lines results in massive apoptosis [523]. This involves its mono-ADP-ribosyltransferase activity, but not its deacetylase activity, leading to activation of p53 and p73 apoptotic signaling cascades [523].

SIRT7 in cancer

SIRT7 levels are elevated in breast cancer generally, including node-positive breast cancer [517] and thyroid cancer [435]. While SIRT7 might not be involved in cancer onset, it seems to be crucial for maintaining the cancer phenotype [524]. It does so by deacetylating acetylated histone H3K18 at lysine 18 [52]. Of 276 binding sites for SIRT7 in the genome, 74% are in the proximal promoter of various genes where H3K18 is found and, by SIRT7-mediated deacetylation, the transcription of these is repressed [52]. This action of SIRT7 leads to anchorage-independent growth and escape from contact inhibition, consistent with progression of tumors to aggressive phenotypes and to poor prognosis. Moreover, depletion of SIRT7 markedly impedes tumorigenicity [52].

How might the dilemma be resolved?

Normally SIRT1 responds to stress by promoting cell survival. This involves its ability to induce cell cycle arrest, DNA repair, and inhibition of apoptosis. But under extreme conditions SIRT1 [525], SIRT2 [143], and SIRT3 [526] act to protect the organism by inducing cell senescence or apoptosis. When overexpressed, SIRT1 inhibits senescence caused by oncogenes [527], and SIRT1 inhibition leads to senescence-like growth arrest [528]. So it seems that if the stress is chronic or the level of damage exceeds a certain threshold then SIRT1 may cause cell senescence. But when such chronic stress or DNA damage is accompanied by loss of a tumor repressor or loss of another checkpoint factor, then an imbalance might ensue, resulting in SIRT1 being overexpressed beyond a critical limit. By stimulating cell growth and inhibiting apoptosis this would contribute to transformation and tumorigenesis. Clearly, however, more research is needed to understand the role of sirtuins in cancer.

Inflammatory arthropathies

While a role for sirtuins in inflammatory diseases has been known for a while, only more recently has it become apparent that sirtuins are involved in the acute inflammatory response as well. This involves a switch from increased glycolysis to increased fatty acid oxidation as early inflammation converts to late inflammation. SIRT6 reduces glycolysis, while SIRT1 increases fatty acid oxidation [529]. Mediators include PPAR-γ coactivators PGC-1α and PGC-1β that increase CD36 in the external plasma membrane and, in mitochondria, the fatty acid transporter CPT1 [529].

Chronic inflammation is not only a major characteristic of autoimmune diseases, but is a general feature of most common diseases of aging. SIRT1 suppresses both innate and adaptive immunity. SIRT1 expression is highly induced in anergic T cells. The early response genes 2 and 3 bind to the SIRT1 promoter, synergize with FOXO3A, and activate SIRT1 transcription [530]. T-cell anergy can be reversed by IL-2, which, via activation of the PI3K–AKT pathways, sequesters FOXO3A, thus reducing SIRT1 transcription [530]. In regulatory T cells, SIRT1 deacetylates FOXP3, a protein crucial for ensuring a balanced immune response [531]. This leads to FOXP3 polyubiquination and proteosome-mediated degradation. TNF-α upregulates proinflammatory MMP-9, IL-1β, IL-6, and iNOS in NIH/3T3 fibroblasts [532]. Resveratrol, by activating SIRT1, ameliorates these effects by causing deacetylation of the NF-κB subunit RelA/p65 and attenuating phosphorylation of TOR and S6 ribosomal protein [532].

SIRT1 also deacetylates CIITA, a protein pivotal to MHC II activation and increased MHC II gene transcription in macrophages [533]. The activation and ensuing effects of SIRT1 can be triggered by NAMPT, which increases NAD^+ [533].

SIRT1 is upregulated in synovial tissue and fibroblasts from patients with rheumatoid arthritis and appears to contribute to proinflammatory cytokine production, as well as in opposing apoptosis [534].

In osteoarthritis the proinflammatory mediator TNF-α induces cathepsin B-mediated cleavage of SIRT1 to inactivate it, and this action correlates with reduced cartilage-specific gene expression [535]. A key mediator of cartilage destruction in osteoarthritis is IL-1β. IL-1β induces NAMPT expression, which then activates SIRT1, leading to induction of ERK and p38 kinase activities that have a positive feedback effect on SIRT1 activity [536]. SIRT1–ERK is involved in IL-1β-induced chondrocyte dedifferentiation, which is mediated by SOX-9 [536]. Based on data from heterozygous SIRT1 knockout mice, SIRT1 may prolong the viability of articular chondrocytes [537]. The SIRT1 pathway is also involved in the cytoprotective and anti-inflammatory effects of melatonin, as shown in an oxidative stress-stimulated chondrocyte model of osteoarthritis [538]. Resveratrol has anti-inflammatory effects on articular chondrocytes stimulated with IL-1β and this involves SIRT1-mediated deacetylation of p65, followed by suppression of the nuclear translocation of NF-κB and a reduction in iNOS and NO [539].

The different roles of SIRT1 and roles for other sirtuins in inflammatory conditions clearly require more research.

Osteoporosis

Activation of SIRT1 by resveratrol produces expression in mesenchymal stem cells of the bone-specific transcription factor RUNX2 [540,541]. Osteocalcin is also upregulated [540], whereas genes for adipo-lineage proteins PPAR-γ [540,541] and leptin [540] are downregulated. This leads to spontaneous osteogenesis, mediated by an increase in SIRT1/FOXO3A complex formation and increased FOXO3A-dependent transcriptional activity, which includes binding of FOXO3A to a site in the RNX2 promoter [540]. PPAR-γ expression is reduced as part of the activation and differentiation of mesenchymal stem cells to osteoblasts in osteogenic repair that occurs in response to bone injury [541]. SIRT1 contributes to maintenance of the stem cell pool by ensuring prematurity of hemopoietic stem cells via activation of FOXOs, inhibition of p53, and elimination of ROS [542]. SIRT1 regulates bone mass by inhibiting sclerostin [543]. Thus in various ways SIRT1 activation may help in the treatment of osteoporosis

Reproductive function

SIRT1 affects fertility. A point mutation that disrupts SIRT1 activity, but with no effect on SIRT1 gene transcription, produces a milder phenotype than that seen in SIRT1 knockout mice [544]. Female mice were fertile rather than sterile, although male mice lacking either SIRT1 activity or transcription were sterile and hypermetabolic [544]. In human ovarian tissue the ability of resveratrol to increase mRNAs for SIRT1, lutenizing hormone receptor, steroidogenic acute regulatory protein, and P40 aromatase points to a role for SIRT1 in the luteal phase of the ovarian cycle [545]. Androgen depletion results in an increase in SIRT1 and eNOS expression in corpus cavernosum tissue [546]. The findings do not, however, appear to explain the effects of aging on structural changes in the corpus cavernosum [546].

Other medical conditions

The critical role of CD8 T-cells in protection against viral infections involves SIRT1-mediated regulation of epigenetic remodeling and energy metabolism during promotion, by BATF, of CD8 T-cell differentiation [547].

In the lung, SIRT1 activation by SRT172 or elevated SIRT1 gene expression protects against emphysema via a FOXO3A-dependent mechanism [548]. In response to air pollution, SIRT1 prevents Kruppel-like factor 2-mediated expression of the thrombomodulin gene, thus lowering lung coagulation caused by exposure to air-born particulate matter [549]. Furthermore, a dimeric derivative of resveratrol, by upregulating SIRT1 and FOXO3A, can inhibit cigarette smoke-induced autophagy in human bronchial epithelial cells [550].

Inhibition of SIRT1 by ethanol is crucially involved in alcoholic fatty liver disease, and this involves overexpression of miR-217 [551]. miR-217 impairs SIRT1-regulated genes for lipogenic or fatty acid oxidation, as well as the important lipid regulator lipin-1 [551]. By activating AMPK and SIRT1, resveratrol can prevent alcoholic fatty liver [552]. SIRT3 also ameliorates alcohol-induced mitochondrial hyperacetylation in a mouse model of alcoholic liver disease [553].

Skin damage by UV radiation and H_2O_2 involves downregulation of SIRT1 in human keratinocytes [554]. Resveratrol can reverse this process in a SIRT1-dependent manner.

Resveratrol, by activating SIRT1 in skeletal muscle, helps prevent pathological changes caused by hind limb uploading in rats, a model of microgravity of space-flight [555]. Resveratrol is therefore a physical exercise mimetic. This finding adds to the observations noted earlier that resveratrol can increase oxidative type I muscle fibers and resistance to muscle fatigue, thus allowing mice to run twice as far before exhaustion [244].

A beneficial effect in tendinitis is also apparent, in that activation of SIRT1 inhibits inflammation and apoptosis in human tenocytes [556].

In the eye, SIRT1 preserves retinal ganglion cells during hypoxia [557]. The neuroprotective effect of SIRT1 involves an interaction with apoptotic signaling proteins to reduce hypoxia-induced apoptosis [557].

Aging

Sirtuins protect against many of the various aging-associated conditions discussed above [19]. These conditions are often interrelated. For example, liver cancers can be induced by inflammation associated with the metabolic syndrome [19]. The role of SIRT1 during aging seems to involve the orchestration of different stress response pathways [558]. This involves targeting of multiple transcriptional regulators, such as p53, FOXO, and HSF1.

SIRT1 activity is modulated by nutrient availability and declines with aging. The decline in SIRT1 in liver with aging is mediated by binding of repressive CCAT/enhancer binding protein/HDAC-1 complexes to the SIRT1 promoter [559].

The kinase TOR has an important role in aging. TOR is part of an evolutionarily conserved signaling pathway linking extracellular stimuli with intracellular processes such as cellular growth, metabolism, translational control, proliferation, and inhibition of autophagy [558]. Stress triggers protein misfolding and this depletes the pool of chaperones inside cells. TOR is able to sense and respond differentially to mild and severe depletion of different chaperones [560]. As a result, there is continuous integration of levels of nutrients available to the cell with protein homeostasis within the cell. The efficiency of the heat shock response pathway is impaired with aging. This leads to changes in

chaperone levels, and such changes adversely affect autophagosome clearance [558]. Thus the levels SIRT1 and TOR are central hubs of the stress response network connecting metabolism, autophagy, DNA damage, protein homeostasis, and heat shock response [558].

A characteristic feature of aging is telomere shortening. Telomeres protect the ends of chromosomes and shorten with each cell replication event [561]. Such shortening is opposed by telomerase. SIRT1 depletion leads to telomere dysfunction [562]. SIRT1 is a positive regulator of telomere length. In mice engineered to contain additional copies of the SIRT1 gene [243] telomere shortening with aging is attenuated [563]. SIRT1 interacts with the telomeric repeat sequences and promotes recombination at different chromosomal regions, including telomeres, centromeres, and chromosome arms. This effect is dependent on telomerase activity [563].

Aging may be a metabolic condition, since defective energy metabolism seems to be intimately intertwined with DNA damage. Dysfunction of telomere maintenance, via a decrease in PGC-1α and thence p53 activation, can affect mitochondrial biogenesis and function and thereby metabolic homeostasis [564]. As discussed earlier, SIRT1 serves as a NAD^+ sensor that converts alterations in metabolic or redox state into adaptive transcriptional responses [17]. NAD^+ is rate limiting in SIRT1-mediated activation of PGC-1α, which coactivates transcription of nuclear-encoded mitochondrial genes [79]. The interplay between PARPs and sirtuins complements this mechanism [299]. PARP-1 binds DNA structures and nucleosomes, so affecting DNA repair, chromosome stability, and regulation of transcription [565]. PARP-1 uses NAD^+ as a cosubstrate. NAD^+ is converted to nicotinamide in generating polymers of ADP-ribose, which attach to acceptor proteins. When DNA is damaged strong stimulation of PARP-1 occurs, causing NAD^+ levels to fall [565]. PARP-2 makes a smaller contribution. Thus deletion of PARP-1 or PARP-2 causes SIRT1 activation and thence mitochondrial biogenesis and fatty acid oxidation. During oxidative stress PARP-1 is activated, thus resulting in NAD^+ consumption, meaning that less NAD^+ is available for SIRT1 activity, so SIRT1 activity falls [75]. Thus by limiting NAD^+, PARP-1 is a gatekeeper for SIRT1 activity.

The interaction between PARP-1 and SIRT1 affects not only metabolic regulation, but most likely aging. Mice deficient in PARP-1 or PARP-2 only exhibit DNA damage when exposed to genotoxic stress [566,567], and dual knockout of these PARPs is embryologically lethal, demonstrating that either alone can compensate for loss of the other [568]. It has long been known that maximal PARylation capacity arising from intrinsic PARP activity correlates with life span of multiple mammalian species [569]. With age, PARP activity of leukocytes decreases [569,570]. However, rather than high PARP activity being able to enhance life span, high PARP may be a consequence of longevity [299]. In support of this, high PARP-1 can in fact cause age-related diseases and premature death [300]. These mice exhibit elevated fat mass, body weight, and glucose intolerance. Thus perhaps low PARP activity might be prolongevity. The effect on life span of PARP deletion in mice differs, moreover, in different strains [75].

With age, endothelial-derived NO declines as a result of impaired eNOS activity and rising inactivation of NO caused by elevation in superoxide [571]. This leads to vascoconstriction, impaired perfusion, vascular inflammation, atherosclerosis, and impeded endothelial repair [572]. As discussed earlier, SIRT1 stimulates eNOS activity by deacetylating it, thus causing a rise in NO [109]. Moreover, during calorie restriction NO derived from eNOS increases and stimulates SIRT1 expression [355].

With aging, miR-34a [172,382] and miR-217 [573] increase and downregulate SIRT1 by posttranscriptional effects on SIRT1 mRNA stability, leading to endothelial senescence, apoptosis, and impaired angiogenic signaling.

SIRT1 delays senescence of human bone marrow-derived and adipose-derived mesenchymal stem cells [574]. The effect of SIRT1 involves in part its ability to delay the proaging factor $p16^{INK4A}$ [574]. Glucose restriction of lung fibroblasts can serve as a calorie restriction mimetic [575]. This results in an increase in SIRT1 expression, activation of AKT/p70S6K1, histone deacetylation, and methylation of the INK4A promoter, decreasing $p16^{INK4A}$ expression, thus leading to chromatin remodeling and inhibition of cellular senescence [575]. Conversely, overexpression of $p16^{INK4A}$ leads to early replicative senescence [575]. Another calorie restriction mimetic, 2-deoxyglucose, has beneficial effects on human fibroblast life span by increasing NAD^+ and SIRT1 activity [576].

As discussed earlier, SIRT1 is involved in maintenance of chromosome stability during mitosis. Thus the loss of SIRT1 activity that occurs during aging and tumorigenesis may lead to aneuploidy and genomic instability [38].

With age, the decline in SIRT1 activity and expression in skeletal muscle are accompanied by increased inflammation, oxidative stress, and reduction in ability to rebuild muscle after injury or in response to exercise [577]. This is yet another example of the role of SIRT1 in the decline in bodily function with aging. Overexpression of the sirtuin target PGC-1α in skeletal muscle increases life span by over 30% and counteracts molecular changes as well as conditions of aging [578]. SIRT1 expression is, moreover, increased, pointing to a positive feedback mechanism.

An increase in SIRT1 expression is seen in mice with deletion of the Ras-guanine nucleotide exchange factor RasGRF1 [579]. These mice exhibit partial attenuation of aging and an increase in maximum life span by 20%, thus linking Ras signaling to life span [579].

A mouse model of aging in which senescence is accelerated exhibits neuronal deterioration accompanied by cognitive decline that is SIRT1 dependent and preventable by resveratrol and melatonin [580]. In this mouse model oxidative stress in the hippocampus could be ameliorated by testosterone, which stimulated SIRT1 expression and eNOS activity, leading to suppression of endothelial senescence [581]. Senescent endothelial cells promote neuronal senescence via a hormonal mechanism. The decline in hippocampal SIRT1 with aging could be ameliorated by calorie restriction of old rats, seemingly by a posttranscriptional mechanism [582]. In models of Alzheimers' disease, activation of SIRT1 by resveratrol or overexpression of SIRT1 in the hippocampus reduces hippocampal degeneration and lowers cognitive deficit [408]. This is accompanied by a reduction in acetylation of PGC-1α and p53 [408]. It may be that in Alzheimer's disease aerobic glycolysis in the mediotemporal lobe of the brain depletes NAD^+ and thus, by lowering SIRT1 activity, contributes to amyloidogenesis [419]. Loss of heteromeric acetyl choline receptors in brain has been found to result in premature brain aging [583]. The associated cellular stress that results may upregulate SIRT1 expression as an adaptive response to counter neurodegeneration [583].

Aging is associated with a loss of wake neurons in the basal forebrain and brainstem coinciding with the progressive loss of SIRT1 and accumulation of lipofuscin [584]. SIRT1 appears to be a critical neuoprotectant for wake neurons early in life, but its loss with aging renders these neurons more vulnerable to metabolic insults.

A reciprocal decrease in blood of SIRT1 and an increase in the SIRT1 mRNA regulator miR-34a represent accessible biomarkers of aging, and in particular of impending neuronal decline [585].

The DNA methylome differs between newborns and centenarians [586]. Global hypomethylation is seen in centenarian DNA,

and, in the case of regulatory DNA, the most hypomethylated sequences are in CpG island promoters. Of genes implicated in aging, differentially methylated regions occur in promoters of SIRT5 and SIRT7 [586]. Epigenetic factors could, moreover, have a role in life span. Nutrients such as folate, riboflavin, cobalamin, choline, betaine, and methionine that are components of one-carbon metabolism affect DNA methylation by regulating levels of *S*-adenosyl-L-methionine, a methyl group donor, and *S*-adenosyl-L-homocysteine, an inhibitor of DNA methylation enzymes [587]. It has been argued that SIRT1 mediates the effects of resveratrol on DNA methylation patterns [588,589]. The active metabolite of vitamin D recruits histone acetylases; resveratrol, by activating SIRT1, causes deacetylation, as well microRNA effects that are either oncogenic or tumor suppressing [587,590].

SIRT6, by mono-ADP ribosylating PARP-1, promotes DNA repair in response to oxidative stress [591]. This effect appears relevant to aging and age-related diseases.

The regenerative potential of embryonic stem cells has potential application in rejuvenation research in aging. In mouse embryonic stem cells, SIRT1 appears to have an important role in priming the PTEN/JNK/FOXO1 pathway to respond to ROS [592].

Although mice expressing 3-fold higher levels of SIRT1 under the control of the mouse SIRT1 promoter do not live longer, they exhibit healthier ageing [19]. This includes improvements in glucose homeostasis, bone mineralization, fewer carcinomas and sarcomas, less DNA damage [19], and a reduction in molecular markers of aging such as $p16^{INK4A}$ [19], which is also causally involved in aging [593–595]. The various diseases of aging are reduced in frequency. SIRT1 activation also participates in the beneficial effects that calorie restriction confers.

The AMPK/SIRT1–PGC-1α–PPAR axis that plays such a vital role in activating mitochondrial function declines in activity in older age, suggesting that a dominant negative regulatory mechanism is involved [596]. The decline in each component is accompanied by an increase in SMRT with age, and the ability of SMRT to negatively regulate mitochondrial function that promotes aging and aging-related disease suggests potential avenues for alleviating the decline in bodily function in general with aging [596].

Clinical molecular genetics

Several SNPs in the SIRT1 gene have shown associations with a variety of factors and disease conditions. These include associations with SIRT1 gene expression [597], energy expenditure [244], body fat [598], body mass index [597,599,600], obesity [597], blood pressure [598], metabolic syndrome (which involves a coding variant) [124], a reduction in acute response in insulin to a glucose load [601], risk of type 2 diabetes [601], anxiety disorders [224], major depressive disorder [602], telomere length [603], and no difference [604] or an increase [603] in longevity. The minor allele of the same variant that conferred protection of telomere length was associated with longevity [603]. A lack of association with type 2 diabetes has also been observed, although in this Dutch Famine Birth Cohort the minor alleles of 2 of the 3 SNPs studied were associated with a lower diabetes prevalence in individuals who had been exposed to famine prenatally [605]. For Alzheimer's disease, both association [606] or lack of association [607] have been reported. No association has been found with bipolar disorder [606].

In the case of the SIRT2 gene, a SNP was associated with increased risk of Alzheimer's disease in cases that lacked the APOE e4 genotype [608].

In an Italian population a SIRT3 exon 3 SNP was associated with longevity [609], and, in exon 5, a VNTR polymorphism that downregulated SIRT3 expression was associated with reduced life span [610]. Subsequently more extensive studies have mostly failed to replicate associations at the SIRT3 locus, suggesting that any effect is at best weak [611]. An association of SIRT3 variants with carotid media-intima thickness has been reported [612].

An SNP in the SIRT6 gene was associated with carotid plaque, and interactions were found with SNPs in the SIRT1, SIRT3, and SIRT5 genes [613].

Of other genes in sirtuin pathways, the FOXO3A gene has proved to be of particular interest. Over 10 studies, beginning in 2008 [614], have shown that polymorphisms in the FOXO3A gene are associated with human longevity. Exonic polymorphisms do not explain the association [615], whereas 2 SNPs found to be associated with longevity are located in the 3′UTR, suggesting a mechanism involving differential effects of alleles of these on FOXOA mRNA stability [616]. A site in intron 2, where other genetic variants associated with longevity are located, contains enhancer sequences targeted by p53 [617]. p53 also binds to a site 4 kb upstream leading to transactivation of FOXO3A by p53, and also p73, in mouse liver [618]. The ability of p53 to transduce FOXO3A expression in response to DNA damage and environmental conditions such as nutrient deprivation, growth factor deprivation, and hypoxia may also involve promoter binding of other transcription factors, including E2F1 [619] and HIF1-α [620]. There is as well evidence for an indirect inhibitory effect of p53 during oxidative stress, via induction of serum- and glucocorticoid-inducible kinase 1 [621], or via a direct effect on the FOXO3A promoter [622]. p53 and FOXO3A interact at multiple levels, including physically [159,623], sharing common target genes [624,625], stabilization by FOXO3A of the p53 protein [626], and indirect p53 activation by FOXO3A via upregulation of $p19^{ARF}$, which inhibits the p53 ubiquitin ligase MDM2 [627]. Clearly FOXO3A and p53 are part of a transcriptional regulatory network that involves sirtuins, with important roles in aging and diseases of aging.

Conclusion

It can be seen from this extensive detailed review that sirtuins exert a wide repertoire of actions on intracellular processes. Apart from roles in adapting cellular physiology to changes in nutrient conditions, there is now compelling evidence for their role in common diseases of aging. The potential therefore exists for development of drugs that target one or other of the seven sirtuins, either directly or indirectly, for treatment of the seven deadly chronic conditions of aging that plague human society in the modern era.

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