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REVIEW

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# Sirtuin deacetylases in neurodegenerative diseases of aging

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**Sirtuin enzymes are a family of highly conserved protein deacetylases that depend on nicotinamide adenine dinucleotide (NAD+) for their activity. There are seven sirtuins in mammals and these proteins have been linked with caloric restriction and aging by modulating energy metabolism, genomic stability and stress resistance. Sirtuin enzymes are potential therapeutic targets in a variety of human diseases including cancer, diabetes, inflammatory disorders and neurodegenerative disease. Modulation of sirtuin activity has been shown to impact the course of several aggregate-forming neurodegenerative disorders including Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis and spinal and bulbar muscular atrophy. Sirtuins can influence the progression of neurodegenerative disorders by modulating transcription factor activity and directly deacetylating proteotoxic species. Here, we describe sirtuin protein targets in several aggregate-forming neurodegenerative diseases and discuss the therapeutic potential of compounds that modulate sirtuin activity in these disorders.**

***Keywords*****:** sirtuin; histone deacetylase; Alzheimer's disease; Parkinson's disease; Huntington's disease; amyotrophic lateral sclerosis; spinal and bulbar muscular atrophy

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## Introduction

Silent Information Regulator 2 (Sir2) proteins, also known as sirtuins, were originally identified as genetic silencing factors [1, 2] and were later found to prolong lifespan in yeast [3, 4]. Sir2 is a deacetylase that acts on histones and other proteins in the presence of nicotinamide adenine dinucleotide (NAD+) [5] and it also possesses mono-ADP-ribosyltransferase activity [6], functions that are conserved in eukaryotic organisms [7]. NAD-dependent deacetylation by sirtuins was later linked with caloric restriction and aging in several organisms [4, 8]. These findings have launched a new field within the discipline of biology with an increasing number of laboratories devoted to studying the role of mammalian sirtuins during normal cellular senescence and in aging-related diseases [9].

In mammals, there are seven members of the sirtuin family and they have been associated with protection against diseases of aging by a variety of mechanisms such as regulation of stress response, apoptosis and DNA repair [10-14]. Sirtuins are categorized as class III histone deacetylases (HDACs); however, it is worth noting that not all sirtuin substrates are histones and several members of this protein family do not have deacetylase activity [15, 16]. Phylogenetic analysis suggests that HDACs evolved before histones and a major function of these enzymes is the deacetylation of non-histone substrates [15, 16]. Some researchers have proposed that sirtuins should be renamed NAD-dependent deacylases to reflect the repertoire of enzymatic activities performed by these proteins [17].

Based on sequence similarity, the sirtuins from eubacteria, archaea and eukaryotes are categorized into five groups that have varied enzymatic activities [18]. Class I sirtuins (SIRT1, SIRT2, and SIRT3) have robust deacetylase activity in the presence of NAD+ [19], whereas Class II sirtuins (SIRT4) have ADP-ribosyltransferase activity [20]. Class III sirtuins (SIRT5) have NAD-dependent demalonylase and desuccinylase activities in addition to deacetylase activity [19, 21, 22]. The class IV sirtuins (SIRT6 and SIRT7) are deacetylase enzymes that may be weaker and more substrate-specific than the class I deacetylases *in vitro* [19, 23-27], and SIRT6 has also been shown to have ADP-ribosyltransferase activity [23-

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25]. A fifth group of sirtuins, class U, has been identified in bacteria and is phylogenetically intermediate between class I and class IV sirtuins [18].

The seven mammalian sirtuins have different subcellular localizations, a significant consideration when evaluating their *in vivo* substrates. SIRT1 is generally thought to have a nuclear localization, although cytoplasmic SIRT1 has also been reported and may be associated with apoptosis, differentiation and oncogenic transformation [28-32]. SIRT2 is a predominantly cytoplasmic protein that has been shown to deacetylate tubulin, but may also shuttle to the nucleus where it functions as a mitotic checkpoint protein [33, 34]. SIRT3, SIRT4 and SIRT5 are localized to the mitochondria, but have different enzymatic activities [19, 20, 22, 35]. SIRT6 is a chromatin-associated nuclear protein [24, 25], and SIRT7 is localized to nucleoli [36]. The subcellular localizations and enzymatic activities for the mammalian sirtuins are diagrammed in Figure 1.

Over the past few years, sirtuins have been explored in Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis and spinal and bulbar muscular atrophy by a variety of techniques including *in vitro* assays, cell culture, animal models of neurodegenerative disease and studies of human tissue. In this review, we will summarize recent findings in sirtuin neurobiology, highlight the mechanism of action for sirtuins in these neurodegenerative disorders, and discuss the therapeutic potential of compounds that modulate sirtuin activity.

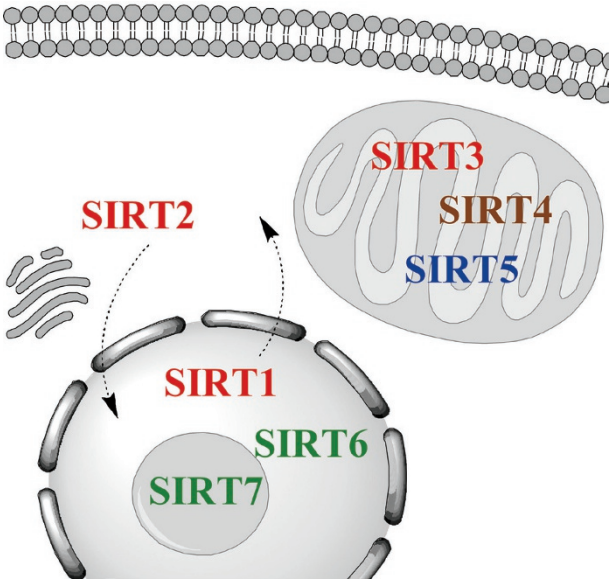


**Figure 1** Subcellular localization and function of the mammalian sirtuins. The seven sirtuins are categorized into four groups based on their sequence homology, and these proteins have varied enzymatic activities and subcellular localizations [18]. Class I sirtuins (SIRT1, SIRT2, and SIRT3) are depicted with red text. These proteins have robust deacetylase activity in the presence of NAD+ [19]. SIRT1 is generally thought to have a nuclear localization, although cytoplasmic SIRT1 has also been reported in neurons and other cell types [28-32], SIRT2 is a predominantly cytoplasmic protein that may shuttle to the nucleus [33, 34] and SIRT3 is a mitochondrial protein [35]. The class II sirtuin (SIRT4) is highlighted in brown. This protein has ADP-ribosyltransferase activity and is also a mitochondrial protein [20]. The class III sirtuin (SIRT5) is depicted in blue and this mitochondrial enzyme has NAD-dependent demalonylase and desuccinylase activities in addition to a weaker deacetylase activity [19, 21, 22]. The class IV sirtuins (SIRT6 and SIRT7) are in green text. SIRT6 is a nuclear protein with weak deacetylase activity and ADP-ribosyltransferase activity [23-25], and SIRT7 is localized to nucleoli and it has deacetylase activity [19, 26, 27].

## Alzheimer's disease

Alzheimer disease (AD) is the most common neurodegenerative disorder, affecting nearly half of all people over the age of eighty five [37]. AD is genetically heterogeneous and has been linked with mutations in genes encoding amyloid precursor protein (APP), presenilin 1 (PS1) and presenilin 2 (PS2) as well as the ε4 allele of apolipoprotein E. Amyloid precursor protein cleavage by the β- and γ-secretase complexes leads to the formation of amyloid-β (Aβ) peptides that can aggregate and form amyloid plaques. Amyloid plaques and neurofibrillary tangles comprising hyperphosphorylated tau protein are the pathologic hallmarks of the human disease. Current treatments are not curative; therefore, the validation of new therapeutic targets is crucial [38].

The initial clues that modulation of sirtuin activity might affect AD pathology came from studies reporting that a sirtuin agonist, resveratrol, was able to attenuate cell death induced by Aβ and oxidized lipoproteins in cell culture models [39-41]. These cell culture findings were confirmed in several subsequent studies [42, 43] and resveratrol was found to enhance proteasome-mediated clearance of Aβ [44]. Several studies examining the effect of resveratrol *in vivo* found that this compound reduced the plaque burden in the brains of transgenic mice overexpressing APP [45, 46]. The topic of whether resveratrol specifically modulates sirtuin activity via a direct or indirect mechanism that also involves AMP kinase (AMPK), phosphoinositide 3-kinase (PI3K) or other targets is controversial [47-53]. Several studies have also reported that resveratrol only potentiates SIRT1 activity in the presence of a fluorescent moiety that is used for *in vitro* pharmacologic screens [49, 54, 55]. A more recent

paper suggests that resveratrol activates SIRT1 activity via an allosteric mechanism and the fluorescent tag used in *in vitro* assays mimics large hydrophobic residues present at positions -1 and -6 of substrates including PGC-1α and Foxo3a [56]. Therefore, in addition to drug studies, it is important to also use genetic strategies with concomitant evaluation of downstream targets to evaluate whether modulation of sirtuin pathways can account for a specific biological phenotype.

In 2005, a study directly linked neuroprotection with SIRT1 expression by using lentiviral-mediated overexpression, and protection from Aβ-induced neurotoxicity was observed in mixed cortical culture models [57]. The proposed mechanism was that SIRT1 and resveratrol reduced Aβ-stimulated NF-κB signaling in microglia. The following year, a second group confirmed that SIRT1 is neuroprotective in Aβ models of AD using a combination of cell culture systems and murine models. They found that neuronal SIRT1 expression decreased levels of ROCK1, a serine/threonine Rho kinase previously shown to regulate Aβ metabolism, and this effect enhanced α-secretase activity, thus promoting a non-amyloidogenic pathway for processing APP [14, 58].

Additional *in vivo* evidence that SIRT1 may ameliorate Aβ pathology was established in the APP/PS1 model of AD. In this model, overexpression of SIRT1 decreased plaque burden, improved behavioral phenotypes and potentiated α-secretase-mediated processing by deacetylating retinoic acid receptor β, a transcriptional activator of ADAM10. ADAM10 is a component of the α-secretase, which processes APP along an anti-amyloidogenic pathway that decreases formation of toxic Aβ42 species [59].

Tau pathology is a major aspect of Alzheimer's disease pathology and neurofibrillary tangles distribution correlates with cognitive impairment in patients [60]. The first study exploring the effect of SIRT1 on neurodegenerative changes in a mouse model that exhibits tau pathology was published in 2007 [13]. This study used resveratrol and lentiviral-mediated SIRT1 overexpression in cell culture models and the p25 mouse model of AD, a transgenic line that recapitulates additional aspects of AD including hyperphosphorylated tau protein, neurofibrillary pathology and neuronal loss [61]. This paper reported behavioral effects including reduced learning impairment and molecular changes such as deacetylation of PGC-1α and p53 in the presence of active SIRT1, but the effect on tau and amyloid processing was not explored [13].

A subsequent study using the sirtuin inhibitor nicotinamide found that tau phosphorylation is ameliorated in a triple transgenic model of AD. Neuroprotection by a sirtuin inhibitor may have been due to the use of a compound that affects multiple sirtuins in a mouse model that displays both tau and amyloid pathologies simultaneously [12]. Levels of acetylated α-tubulin increased in this study; therefore, the protective effect of nicotinamide may have been partially due to inhibition of SIRT2 [12].

Subsequent work by a third group of researchers has found that SIRT1 deletion causes increased tau acetylation, phosphorylation, cognitive defects and early mortality in the P301L tau mouse model [62, 63]. These observations are supported by *in vitro* and neuronal culture data indicating that SIRT1 can directly deacetylate tau protein at multiple residues. Studies on human tissue also indicate that tau is acetylated at an early stage during the disease process in patients. The mechanism proposed by these investigators is that removal of acetyl groups may expose lysine residues to ubiquitin ligases so that tau protein could be marked for proteasomal degradation [62, 64]. Major mechanisms that have been proposed for SIRT1 in Alzheimer's disease pathogenesis are summarized in Figure 2.

Although these preclinical studies provide compelling evidence that SIRT1 and resveratrol may influence both Aβ and neurofibrillary tau pathology in cell culture and animal models, the crucial test will be whether there is a clinical benefit for patients with AD. There are several ongoing or recently completed clinical trials that may address this issue by testing various formulations of resveratrol in AD patients. A phase II double blind, placebo-controlled trial sponsored by the Alzheimer's Disease Cooperative Study is currently recruiting patients with mild-to-moderate dementia who will be treated for 12 months with resveratrol or placebo. Evaluation using brain imaging and cerebrospinal fluid biomarkers are primary outcomes [65]. In addition, the Department of Veteran's Affairs is sponsoring a phase III trial to investigate the effects of resveratrol in combination with glucose and malate over 12 months in AD patients using cognitive testing as a primary outcome [66]. A third study evaluates the use of Etanercept, an anti-inflammatory agent that inhibits TNF-α, in combination with nutritional supplements including resveratrol, versus the nutritional supplements alone over a period of 6 weeks using cognitive testing as the primary outcome [67]. These clinical trials are an important first step in evaluating the safety and efficacy of targeting this pathway in human populations.

## Parkinson's disease

Parkinson's disease (PD) is the second most common neurodegenerative disorder, affecting 1% of the population over 60 years of age in industrialized countries [68].

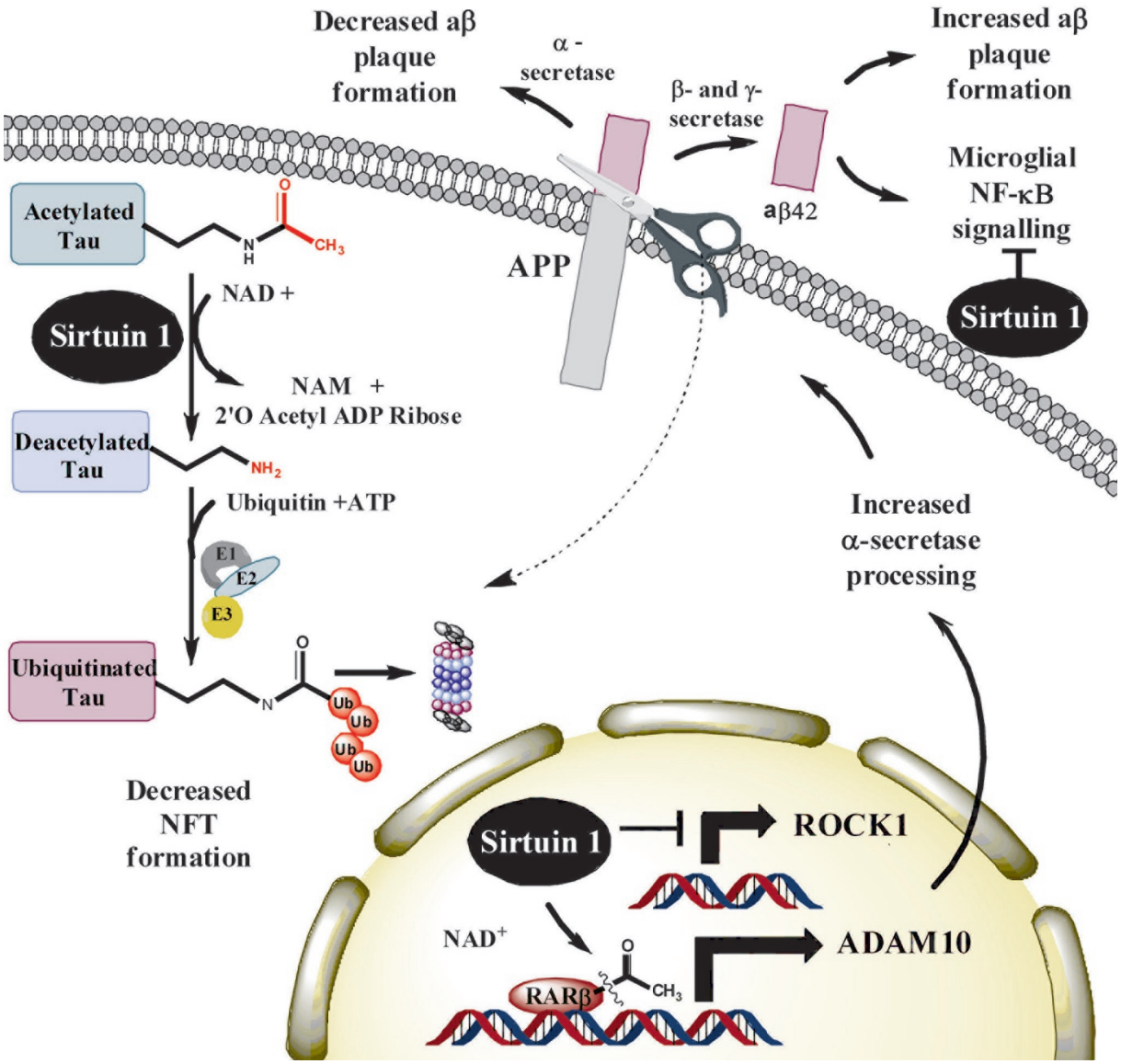


**Figure 2** Mechanisms of activity for sirtuins in Alzheimer's disease. *In vitro* and neuronal culture data show that SIRT1 can directly deacetylate tau protein at multiple residues [62-64]. The mechanism proposed by these investigators is that removal of acetyl groups may expose lysine residues to ubiquitin ligases so that tau protein is marked for proteasomal degradation [62, 63]. This process decreases accumulation of hyperphosphorylated PHF tau, cognitive defects and early mortality in the P301L tau mouse model [62, 63]. Overexpression of SIRT1 has also been shown to decrease plaque burden and improve behavioral phenotypes by deacetylating retinoic acid receptor β, a transcriptional activator of ADAM10. ADAM10 is a component of the α-secretase, which processes APP along an anti-amyloidogenic pathway that decreases formation of toxic Aβ42 species [59]. An independent study has also shown that SIRT1 expression may decrease levels of ROCK1, a serine/threonine Rho kinase previously shown to regulate Aβ metabolism, and this effect also promotes the non-amyloidogenic α-secretase pathway [14, 58]. The SIRT1 agonist resveratrol was also found to enhance proteasome-mediated clearance of Aβ [44] and reduce the plaque burden in the brains of transgenic mice overexpressing APP [45, 46]. Experiments using mixed cortical culture models have also shown that SIRT1 acts to reduce Aβ-stimulated NF-κB signaling in microglia [57].

PD is a movement disorder causing tremor, rigidity, bradykinesia and postural instability; however, cognitive and behavioral changes including sleep impairments, olfactory deficits and neuropsychiatric disorders can also manifest [69]. Neuropathologic analysis shows a substantial loss of dopaminergic neurons in the substantia nigra and accumulation of intracytoplasmic Lewy bodies, inclusions that contain α-synuclein and ubiquitin [69].

While there is no cure for PD, medications and surgery can improve some of the symptoms.

Activation or overexpression of SIRT1 and its homologues by genetic means or resveratrol treatment has been shown to be protective in cell culture, worm and mouse models of PD [11, 70-73]. The mechanism for SIRT1 activity in this disease has been supported in multiple studies that have shown a role of SIRT1 in the activation of heat shock factor 1 (HSF1), which affects transcription of molecular chaperones including heat shock protein 70 that regulate homeostasis of cellular proteins [11, 74, 75].

SIRT1 is not the only class III HDAC linked to a neuroprotective phenotype in PD. In 2007, researchers identified a compound that increases the inclusion size of α-synuclein aggregates and examined this molecule's *in vitro* activity. This small molecule was a SIRT2 inhibitor, and a secondary screen of structural analogues to identify more potent molecules was performed. The SIRT2 inhibitors showed dose-dependent rescue of α-synuclein-mediated toxicity in cell culture systems and were also able to protect dopaminergic neurons from cell death in a *Drosophila* model of PD [76]. While the exact mechanism for reducing α-synuclein-A53T-mediated cell death is not known, these compounds decreased the number but increased the size of α-synuclein aggregates in a cellular model. A recent study validated these observations showing that genetic deletion of SIRT2 is protective in a chemically induced mouse model of PD using 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [77]. The proposed mechanism is that SIRT2 becomes active as a response to MPTP-induced stress causing Foxo3a deacetylation, which leads to increased levels of the pro-apoptotic factor Bim and neuronal death [77]. Major mechanisms that have been proposed for SIRT1 and SIRT2 in Parkinson's disease pathogenesis are diagrammed in Figure 3.

The experimental evidence that SIRT1 and SIRT2 have opposing effects on PD models of neurodegeneration *in vivo* means that target specificity within this class of histone deacetylases is important. The identification of multiple compounds that affect sirtuin activity and improve PD pathogenesis is encouraging. With sufficient evidence from preclinical studies and more information about the safety of using these compounds, it will be possible to test whether these molecules will help patients suffering from these neurodegenerative disorders in the upcoming years.

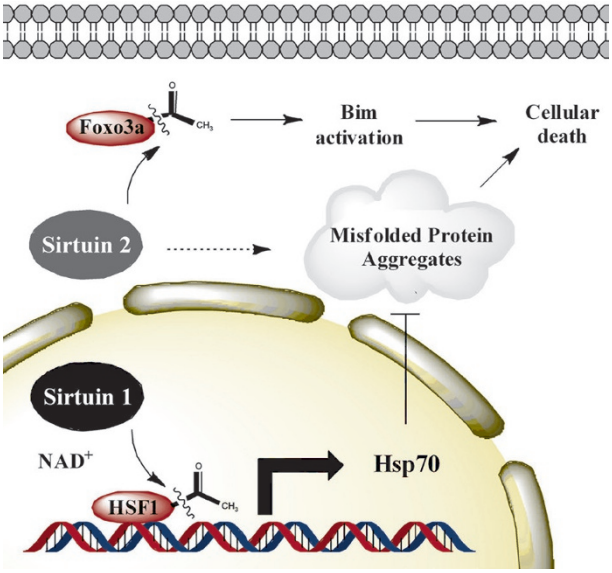


**Figure 3** Sirtuin-mediated deacetylation in Parkinson's disease. In PD, SIRT1 can deacetylate heat shock factor 1 (HSF1), which increases hsp70 transcription and decreases the formation of abnormal protein aggregates [11, 74, 75]. Genetic deletion of SIRT2 is protective in a chemically induced MPTP-model of PD [77]. It is proposed that SIRT2-mediated Foxo3a deacetylation leads to increased levels of the pro-apoptotic factor Bim and neuronal death [77]. Studies using small molecule inhibitors have shown that SIRT2 modulates the size and number of α-synuclein aggregates [76].

## Huntington's disease

Huntington's disease (HD) is an autosomal dominant neurological disorder characterized by cognitive dysfunction, personality changes, and loss of coordination and motor functions. It is caused by the expansion of a CAG repeat that codes for a stretch of glutamine residues, affecting the conformation and aggregation propensity of the huntingtin protein [78]. This disease causes increasing disability over many years and current treatments alleviate symptoms but are not curative.

Sirtuin deacetylases have been investigated as pharmacologic targets to slow the progression of HD in cell culture and animal models that recapitulate elements of the human disease. In nematode models of polyglutamine cytotoxicity, both resveratrol treatment and genetic overexpression of SIRT1 were shown to be neuroprotective [79, 80]. This finding was confirmed in primary neuronal culture derived from knock-in mice carrying a mutant huntingtin protein [79]. However, the converse is true in *Drosophila* models where genetic or pharmacological reduction of either SIRT1 or SIRT2 homologues was shown to be neuroprotective [81]. It is not clear why sirtuins have different effects on HD pathogenesis in these animal systems.

Studies using pharmacologic approaches to altering

sirtuin activity have also reported widely differing effects in mouse models of HD. In a chemically-induced model of HD, cognitive and motor deficits caused by 3-nitropropionic acid administration were improved by pretreating the animals with resveratrol [82]. However, treatment with resveratrol did not improve survival in the N171-82Q mouse model of HD, a transgenic line that overexpresses a truncated huntingtin protein, although there was some improvement in peripheral tissues [83]. A third study used the HDR6/1mouse model, which overexpresses a moderately sized fragment of N-terminal huntingtin. These mice were treated with nicotinamide, a chemical inhibitor that may affect multiple sirtuin proteins. This treatment increased BDNF and PGC-1α gene expression and improved HD-associated motor deficits, but did not reduce huntingtin aggregation or weight loss in these mice. All three studies taken together suggest that resveratrol and nicotinamide may be useful therapeutic compounds; however, pharmacokinetic and pharmacodynamics studies should be performed to confirm that active metabolites indeed penetrate disease-relevant tissues and cause changes in HD-relevant molecular targets.

Genetic models of SIRT1 overexpression have provided more clarity with multiple mouse models showing that SIRT1 is protective against mutant huntingtin neurotoxicity. Using the R6/2 model, a transgenic mouse that overexpresses truncated N-terminal huntingtin, Jeong *et al*. [84] showed that deleting the catalytic exon of SIRT1 exacerbated the disease, while overexpressing SIRT1 by 2-3-fold improved survival and reduced protein aggregation in HD. A second study used a truncated huntingtin N171-82Q model in addition to a full-length huntingtin protein model and found that SIRT1 overexpression improved motor function and attenuated brain atrophy in these transgenic mouse lines [10].

Although both studies showed a clear improvement in HD symptoms, several mechanisms for the role of SIRT1 were proposed. In the disease state, mutant huntingtin interacts directly with SIRT1, inhibiting its enzymatic activity. One possible mechanism is that SIRT1 deacetylates TORC1, facilitating its interaction with CREB and transcription of BDNF, a neurotrophic factor that is protective in HD [84]. Other possible consequences of the inhibition of SIRT1 activity by mutant huntingtin include decreased activation of TrkB, the receptor for BDNF, and Foxo3a, a transcription factor linked to BDNF and DARPP32 expression in neurons [10, 78, 84]. While there are several plausible explanations, the basic observation that SIRT1 is protective has been described in three genetic mouse models of HD. Further validation studies will examine the regulation of transcriptional networks by SIRT1 and its use as a therapeutic target in HD.

SIRT2 has also been investigated in the context of HD and pharmacologic inhibition has been shown to decrease neurodegeneration in *Drosophila*, cell culture and mouse models [85, 86]. The mechanism for this phenomenon has been linked with SIRT2 inhibitor-mediated decrease in sterol biosynthesis, a pathway that has been shown to be dysfunctional in HD models [85]. Cholesterol metabolism may affect myelination and synapse maintenance, however, the mechanism by which cholesterol homeostasis affects HD pathophysiology has not been fully elucidated [87]. Genetic deletion of SIRT2 did not affect disease progression, α-tubulin acetylation, or biosynthesis of cholesterol in the R6/2 mouse model of HD [88]. It is plausible that compensatory changes in other proteins may occur when SIRT2 is deleted in the mouse germline, whereas SIRT2 inhibitors produce an acute reduction in the protein's activity. It is also possible that changes in sterol biosynthetic pathways induced by SIRT2 inhibitors may be due to off-target effects from these compounds. More recently, viniferin, a naturally occurring resveratrol derivative, was found to be protective in cell culture models of HD. This compound increases SIRT3 levels, causing deacetylation of MnSOD and LKB [89]. As a result, activation of AMPK by its upstream kinase LKB promotes mitochondrial biogenesis and increases cell survival in HD models [89]. The major mechanisms that have been proposed for sirtuins in HD pathogenesis are diagrammed in Figure 4. While the mechanism for neuroprotection in HD is still unclear, multiple research groups have identified compounds that target three distinct sirtuins and appear to be protective in mouse models of HD. Additional preclinical studies will need to explore their safety profiles and whether they may also be of benefit in patients.

## Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is the most common form of motor neuron disease, a rapidly progressive condition that affects muscle strength and coordination. Over the past 20 years, causal mutations in a functionally diverse set of genes including SOD1 [90], TDP43 [91, 92], FUS [93], UBQLN2 [14] and C9ORF72 [94-97] have been identified, and there are also many proposed etiologies that focus on distinct aspects of disease pathogenesis [98]. In addition to playing a role in familial ALS, recent studies have also shown that these proteins may be altered in sporadic forms of the disease [14, 95, 96, 99-105]. People on an average live 2-5 years following diagnosis, and there are no known cures for the disease; therefore, the investigation of new therapeutic

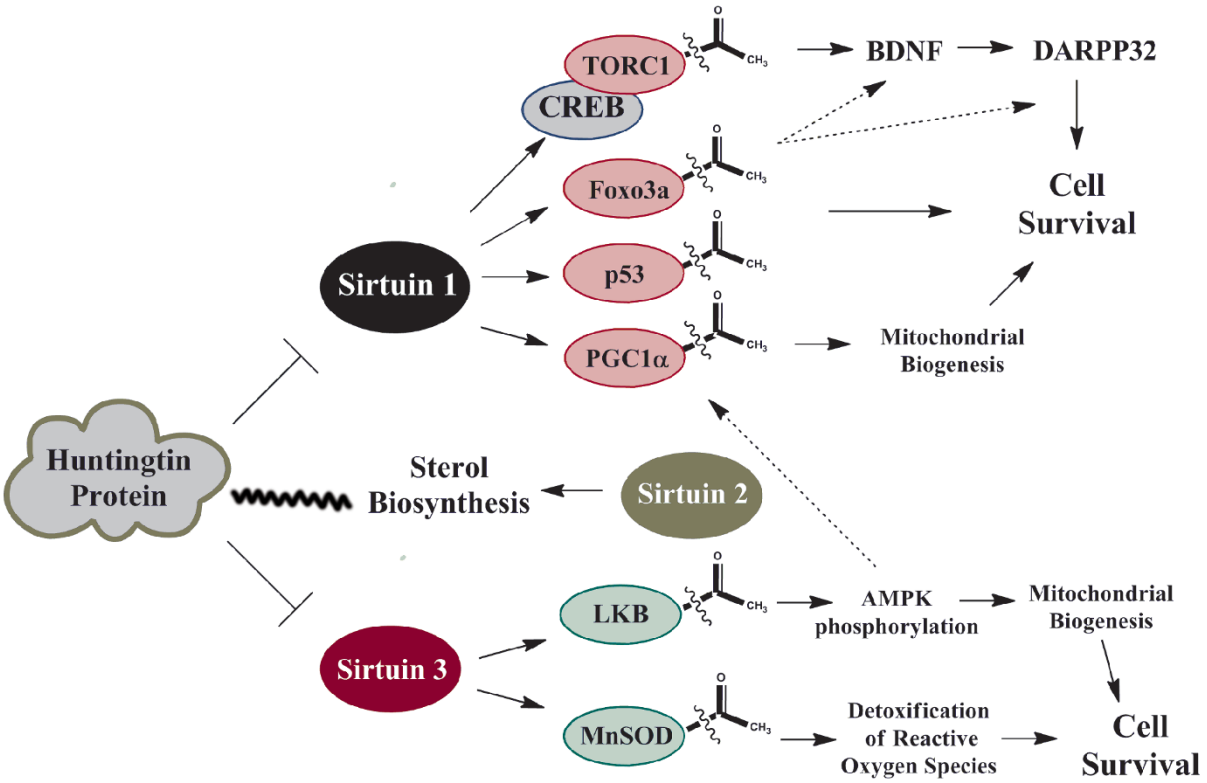


**Figure 4** Sirtuin targets in Huntington's disease. Mutant huntingtin protein can directly inhibit SIRT1 activity, affecting multiple downstream targets. According to one model, SIRT1 deacetylates TORC1, facilitating its interaction with CREB, which is linked to BDNF and DARPP32 expression in neurons [10, 78, 84]. Other possible consequences of the inhibition of SIRT1 activity by mutant huntingtin include the decreased deacetylation and altered activities of Foxo3a, p53 and PGC-1α [10]. In addition, sterol biosynthesis is dysregulated in HD (indicated by the wavy line) and studies using SIRT2 inhibitors have shown decreased neurodegeneration accompanied with decreased sterol biosynthesis in HD models [85-87, 127]. It has been proposed that SIRT2 affects cholesterol biosynthetic pathways, which may affect myelination and synapse maintenance in HD mouse models [85-87]. More recently, viniferin, a naturally occurring resveratrol derivative, was shown to be protective in cell culture models of HD. This compound increases SIRT3 levels, affecting LKB and MnSOD acetylation [89].

targets is crucial.

The first study linking SIRT1 with ALS pathogenesis used both resveratrol and lentiviral injection and found that these interventions provided short term neuroprotection, although clinical outcomes documenting an improvement in motor function or lifespan of the whole animal were not assessed [13]. Several subsequent studies used tissue culture to demonstrate that resveratrol protects neurons in a cell-based model of ALS [106, 107]. Further analysis found that resveratrol elicits protection from neurotoxic factors in patient cerebrospinal fluid (CSF) using rat brain cortical motor neurons. This study found that incubating neuronal cultures with CSF from ALS patients was more damaging than exposing neuronal cells to CSF from control subjects. Furthermore, resveratrol protected cultured neurons from cytotoxic factors present in CSF whereas riluzole, the only FDA approved medication for the treatment of ALS, was not beneficial in this cellular model [107]. These findings suggest that resveratrol may act via a different mechanism from riluzole, a medication that is believed to work by reducing glutamate excitotoxicity or blocking voltage-gated sodium channels [108].

Two papers have investigated the effect of resveratrol in the G93A mouse model of ALS, the most commonly used transgenic line that develops ALS pathology due to overexpression of a mutant SOD1 [109]. While dietary resveratrol treatment was not sufficient to effect disease outcomes [110], intraperitoneal injection of resveratrol was sufficient to produce a significant improvement in both symptoms and survival of G93A mice [111]. The proposed mechanism was that SIRT1 can deacetylate HSF1, inducing the transcription of molecular chaperones such as hsp70 and hsp25, and decreasing motor neuron death [11, 70, 75, 111]. Resveratrol is reported to have a short half-life *in vivo* [112], and the different ad-

ministration routes used in these studies may account for their different findings. SIRT1 levels have been shown to change both in G93A mouse models [113] as well as in patient tissue [114], suggesting that this protein may be a relevant disease target both in mouse models and human disease.

A recent paper identified SIRT3 as a relevant player in ALS pathogenesis using a cell-based model. SIRT3 was shown to protect against mitochondrial fragmentation and neuronal cell death induced by SOD1 G93A overexpression [115]. The major mechanisms that have been proposed for sirtuins in ALS pathogenesis are diagrammed in Figure 5A. Additional research confirming the effect of pharmacologic intervention in animal models of ALS will be important in evaluating whether sirtuin modulatory compounds should enter the human clinical trial pipeline.

## Spinal and bulbar muscular atrophy

Spinal and bulbar muscular atrophy (SBMA), also known as Kennedy's disease, is a slowly progressive

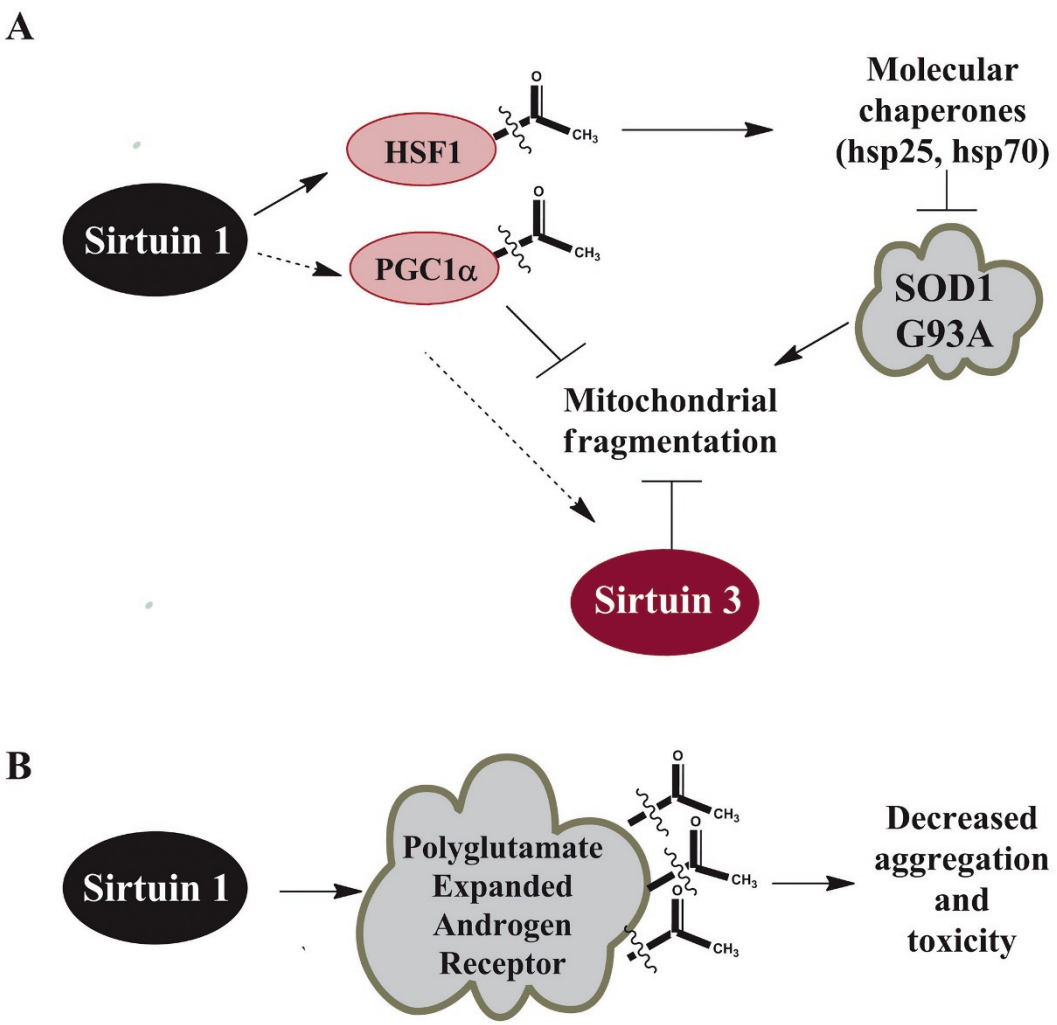


**Figure 5** Sirtuin targets in motor neuron diseases. **(A)** In the SOD1 G93A mouse model of amyotrophic lateral sclerosis, intraperitoneal injection of resveratrol is protective. The proposed mechanism is that SIRT1 can deacetylate HSF1, inducing transcription of hsp70 and hsp25. Induction of these heat shock proteins decrease proteotoxic stress from misfolded protein aggregates [11, 70, 75, 111]. SIRT3 and PGC-1α were also shown to protect against mitochondrial fragmentation and neuronal cell death induced by SOD1 G93A overexpression in cell culture [115]. **(B)** In spinal and bulbar muscular atrophy (SBMA), the polyglutamine-expanded androgen receptor can be directly deacetylated by SIRT1 at lysines 630, 632, and 633. It has been shown that deacetylation of the androgen receptor at these residues can decrease aggregation and toxicity in cellular models of SBMA [124].

neurodegenerative disease affecting motor and sensory neurons [116, 117]. SBMA is a polyglutamine repeat disorder caused by the expansion of a CAG trinucleotide repeat in the N-terminal region of the androgen receptor (AR) [116-118]. This disorder causes muscle weakness in males between the age of 30 and 50 and is also associated with endocrine problems including testicular atrophy, infertility and gynecomastia due to androgen insensitivity [117]. Surgical or pharmacological reduction of testosterone has been shown to ameliorate the SBMA disease phenotype in animal models [119, 120]; however, this therapy has not yet shown a clear benefit in human clinical trials [121, 122].

A recent publication has shown that the polyglutamine-expanded androgen receptor can be directly deacetylated by SIRT1. Although SIRT1 was previously shown to repress androgen receptor activity via deacetylation [123], the role of this process in SBMA had not previously been explored. SIRT1 was found to protect motor neurons with polyglutamine-expanded androgen receptors by deacetylating lysines 630, 632, and 633 [124]. The role of SIRT1 in SBMA is illustrated in Figure 5B. This work is an important finding with great therapeutic potential, because targeting AR acetylation via SIRT1 activation is a treatment strategy that may be safer and have fewer side effects than other avenues that are being explored such as long-term androgen withdrawal.

## Conclusions

Numerous sirtuin targets are altered in these neurodegenerative disorders, and compounds modulating the activity of sirtuins are promising therapeutic strategies. There are three major mechanisms through which SIRT1 has been shown to affect neurodegenerative disorders. SIRT1 has been shown to deacetylate transcription factors and coactivators, whose downstream targets influence aggregate formation. One example of this mechanism is the deacetylation of HSF1, which induces the transcription of molecular chaperones that may be relevant to the pathogenesis of both PD and ALS [11, 70, 75, 111]. In AD, overexpression of SIRT1 affects the acetylation of retinoic acid receptor β, a transcriptional activator of ADAM10, which influences the processing of APP along an anti-amyloidogenic pathway [59].

A second mechanism through which SIRT1 has been shown to influence neurodegenerative disease is by modulating the protein turnover of proteotoxic species by direct deacetylation. An example of this mechanism is observed in AD where direct deacetylation of tau protein by SIRT1 may promote protein clearance by removing acetyl groups from lysine residues that are subsequently marked by ubiquitination to signal for protein degradation by the proteasome system [62]. This mechanism has also been employed to explain the effect of SIRT1 on the stability of some of its other targets [125, 126].

The enzymatic activity of SIRT1 can also be reduced by direct interaction with proteotoxic species. An example of this phenomenon is observed in HD where mutant huntingtin protein directly interacts with SIRT1, interfering with its ability to activate downstream targets such as TORC1, Foxo3a and PGC-1α [10, 78, 84]. Overexpression of SIRT1 by genetic means is protective and compounds that either increase SIRT1 activity or disrupt the interaction between SIRT1 and mutant huntingtin may be of clinical benefit.

In the past few years, it has become clear that modulation of mammalian sirtuins may be a valuable therapeutic strategy in several neurodegenerative diseases. However, many research questions remain. Given the large number of biological substrates affected by sirtuins, the identification of precise and disease-relevant molecular targets is a challenging goal. Multiple HDACs have been shown to influence the course of diseases; however, the degree to which their molecular activities can be coordinated has not been fully characterized. Evaluating the safety and efficacy of small molecules that can modulate sirtuin activity will be important elements in the development of new therapies to treat neurodegenerative diseases.

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