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Biochemical Effects of SIRT1 Activators

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SIRT1 is the closest mammalian homologue of enzymes that extend life in lower organisms. Its role in mammals is incompletely understood, but includes modulation of at least 34 distinct targets through its nicotinamide adenine dinucleotide (NAD^+)-dependent deacetylase activity. Recent experiments using small molecule activators and genetically engineered mice have provided new insight into the role of this enzyme in mammalian biology and helped to highlight some of the potentially relevant targets. The most widely employed activator is resveratrol, a small polyphenol that improves insulin sensitivity and vascular function, boosts endurance, inhibits tumor formation, and ameliorates the early mortality associated with obesity in mice. Many of these effects are consistent with modulation of SIRT1 targets, such as PGC1α and NFκB, however, resveratrol can also activate AMPK, inhibit cyclooxygenases, and influence a variety of other enzymes. A novel activator, SRT1720, as well as various methods to manipulate NAD^+ metabolism, are emerging as alternative methods to increase SIRT1 activity, and in many cases recapitulate effects of resveratrol. At present, further studies are needed to more directly test the role of SIRT1 in mediating beneficial effects of resveratrol, to evaluate other strategies for SIRT1 activation, and to confirm the specific targets of SIRT1 that are relevant *in vivo*. These efforts are especially important in light of the fact that SIRT1 activators are entering clinical trials in humans, and "nutraceutical" formulations containing resveratrol are already widely available.

Sirtuins

SIRT1 came to the attention of the pharmaceutical industry via an unlikely route. A screen for particularly stress-resistant strains of budding yeast turned up a mutation in a gene called *SIR4* (Silent Information Regulator 4) [1] that, as the name implies, had previously been shown to mediate transcriptional silencing at specific loci [2]. Further experimentation revealed that in addition to being stress-resistant, yeast carrying the mutant *sir4-42* allele are able to produce more buds before entering a terminal senescent state – the yeast equivalent of an extension of lifespan. The authors then went on to show that two additional SIR genes, *SIR2* and *SIR3* are required to mediate the effect, and subsequently, that increasing the gene dosage of wild type *SIR2* in the absence of other modifications is sufficient to extend yeast lifespan [3]. Intriguingly, *SIR2* is conserved all the way up to the level of mammals, where seven homologues have been identified (SIRT1-7), with SIRT1 being the closest based on sequence homology [4]. In fact, closer inspection has revealed that almost all organisms, including yeast, contain multiple *SIR2* homologues (termed "sirtuins"), but it is *SIR2* itself, and the closest corresponding gene

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in each organism that have thus far received the most attention, and been most firmly linked to longevity. Extra copies of these genes extend lifespan in worms [5] and flies [6], and the relevance of the mammalian homologue, SIRT1, to human health has been a subject of much discussion in recent years.

The first enzymatic activity described for Sir2 was a weak ability to transfer the ADP-ribose moiety of nicotinamide adenine dinucleotide (NAD^+) to other proteins and itself [7]. However the major physiologically relevant activity of this enzyme, and its mammalian counterpart, SIRT1, is the NAD^+-dependent deacetylation of acetylated lysine residues on histone and non-histone substrates [8,9], which proceeds through a novel mechanism involving an ADP-ribosylated intermediate [10]. In fact, the description of ADP-ribosyltransferase activity was prescient, since other sirtuins, such as SIRT4 in mammals, appear to function exclusively as ADP-ribosyltransferases [11]. The use of NAD^+ as a cofactor for deacetylation is unique to sirtuins and may provide a way to couple their activity to its metabolic state of the cell and/or allow their activity to be sensed by other enzymes. Whereas class I and II histone deacetylases simply release acetate, sirtuins (also called class III deacetylases) release nicotinamide and a novel metabolite, *O*-acetyl-ADP-ribose (AAR), that has been speculated to act as a second messenger (Figure 1). Although its lack of commercial availability and inherent instability have been a barrier to probing its function, experiments in yeast have shown that AAR can contribute to changes in the stoichiometry and structure of the Sir2/3/4 complex, and may influence heterochromatin spreading [12]. AAR is readily hydrolyzed to generate ADP-ribose in mammalian cells [13], and both bind to an inactive Nudix hydrolase domain in transient receptor melastatin-related ion channel 2 (TRPM2), potentiating its ability to induce cell death in response to oxidative and other insults [14,15]. The normal function of TRMP2 is poorly understood, however cell death is accompanied by an influx of Na^+ and Ca^{2+} ions, and it has been speculated that milder stimulation may play a role in Ca^{2+} signaling [14]. The quantitative contribution of SIRT1 to the cellular AAR pool has not been assessed, although there is evidence that disruption of other sirtuins is sufficient to decrease the concentration in yeast [16] and mammalian cells [14]. Moreover, some effects of AAR, such as its contribution to heterochromatin spreading in yeast [12], may be dependent on local production. Recently, evidence has been presented that both AAR and ADP-ribose can reduce free radical production in yeast and divert glucose into the pentose phosphate pathway, thereby increasing cellular NADPH levels [17]. The former occurs via inhibition of electron transport at complex I, and the latter through inhibition of glycolysis at the glyceraldehyde-3-phosphate dehydrogenase step. These observations highlight the potential importance of AAR as a product of SIRT1 activity, even though to date much more progress has been made on the characterization of its deacetylation substrates.

Histones are a major class of substrates for sirtuins. In yeast, Sir2 deacetylates histone H4 at lysine 16 to maintain heterochromatin at the mating type loci, telomeres, and the rDNA [8]. The inherent instability of tandem repeats at the rDNA leads to formation of extrachromosomal rDNA circles (ERCs) that are thought to limit yeast lifespan [18]. Loss of Sir2 dramatically accelerates ERC formation and shortens lifespan, while increasing Sir2 activity reduces ERCs and extends yeast lifespan [3]. Interestingly, Sir2 appears to also promote longevity in an ERC-independent manner through its effects on telomeric heterochromatin [19]. In human cells, SIRT1 can deacetylate histones H4 at lysine 16, H3 at lysine 9, and H1 at lysine 26 [20], and appears to act at discrete sites, to which it is directed by binding partners such as Peroxisome proliferator activated receptor gamma (PPARγ) [21] and Clock [22]. Through these interactions, SIRT1 is able to transcriptionally repress genes involved in adipogenesis, and to contribute to circadian oscillations, respectively. There is no evidence for ERC formation in mammalian cells, although the rDNA appears to be one of the regions in which SIRT1 mediates transcriptional silencing [23]. At telomeres, a conserved role in chromatin maintenance may have been picked up by another Sir2 homologue, SIRT6, which deacetylates histone H3 lysine

9 in that region [24]. Even in the absence of ERCs, there is some evidence for a conserved role of SIRT1 in maintaining global genomic stability in mammals [25], however, non-histone substrates have thus far been linked to most of its reported effects.

The first non-histone substrate identified for SIRT1 was p53 [26,27]. Deacetylation of human p53 at lysine 382 suppresses its transcriptional activity and renders cells resistant to DNA damage and oxidative stress-induced apoptosis. Surprisingly, p53-dependent gene transcription and apoptosis were found to be roughly equivalent in mice lacking SIRT1 [28]. Although evidence continues to accumulate that the interplay between SIRT1 and p53 is important in cancer development, the mouse results clearly suggest that other targets of SIRT1 mediate many of its effects *in vivo*. Consistent with this, and with the view that sirtuins might use their deacetylase activity to fine-tune cellular metabolism based on NAD^+ availability, a wide range of SIRT1 targets have since been identified (at least 34 distinct proteins), with roles in processes ranging from differentiation to circadian rhythms (Figure 2). Some of the best-characterized effects of SIRT1 include increasing stress-resistance and influencing metabolism through class O Forkhead box (FOXO) transcription factors [29], suppressing Nuclear factor kappa B (NFκB)-dependent inflammatory responses [30], and promotion of gluconeogenesis, fatty acid oxidation, and mitochondrial biogenesis through Peroxisome proliferator activated receptor gamma coactivator 1α (PGC1α) [31,32]. However, nearly every target that has been identified is a potential mediator of crucial effects *in vivo*, and there is no doubt that more remain to be discovered. Resolving the relative importance, or net effect, of SIRT1's deacetylation targets will be an enormous task, but may prove well worth the effort.

Resveratrol

A major advance in the effort to understand the role of SIRT1 *in vivo* was the discovery that resveratrol (3,5,4′-trihydroxystilbene), a small polyphenol found at low doses in wine (Figure 3), can activate the enzyme in an *in vitro* assay and extend yeast lifespan [33]. Resveratrol has since been reported to extend lifespan in worms and flies, and in all three organisms, the effect is dependent on their respective SIRT1 homologues [34]. These invertebrate results have become somewhat controversial, since they have been alternately disputed [35,36] and reproduced [37-41] in all three organisms. (Fly results were reproduced by the original lab using a different assay.) More recently, resveratrol has been shown to extend lifespan in a short-lived species of fish, although it has not been possible to test the involvement of sirtuins in that organism. Beneficial effects of resveratrol have also been reported in mammalian cells, and, as discussed in more detail below, in mice. A subset of these effects have been shown to depend on SIRT1 [42-44] including the prevention of skin cancer *in vivo* [45]. However, resveratrol influences many mammalian enzymes, and possesses intrinsic antioxidant capacity, greatly complicating the interpretation of its effects. Moreover, it has come to light that the *in vitro* activation of SIRT1 by resveratrol is substrate-dependent. Specifically, resveratrol activates the enzyme against an acetylated peptide containing a covalently attached fluorophore, but fails to affect deacetylation of the same peptide when the fluorophore is removed [36,46]. This result raises questions about the validity of the original screen, and suggests that it will be important to test the effect of resveratrol using full-length endogenous substrates of SIRT1. Despite this controversy, many of resveratrol's effects in mice are consistent with activation of SIRT1 and modulation of its targets. The following section highlights some of the salient effects of resveratrol treatment in rodents, along with SIRT1-dependent and independent pathways that may contribute to the observations.

Cancer

Resveratrol potently inhibits carcinogenesis at multiple stages in rodent models [44,47]. Direct inhibition of cyclooxygenases [47], as well as the aryl hydrocarbon receptor [48,49] and

cytochrome P450 enzymes [50,51], likely account for much of this protection. However, SIRT1-dependent mechanisms may also play a role, since SIRT1 overexpression is sufficient to blunt intestinal tumorigenesis [52], and mice lacking SIRT1 exhibit a markedly reduced protective effect when given resveratrol as a preventive agent for skin cancer [45]. Moreover, SIRT1-dependent inhibition of NFκB could contribute to a decrease in cyclooxygenase activity through transcriptional silencing of the Cox2 isoform, and a decrease in Cox2 message has been reported following resveratrol treatment [53]. *In vitro* mechanistic studies have implicated a large number of downstream pathways in resveratrol's antiproliferative, pro-apoptotic, and other tumor-suppressive effects, and a complete discussion is beyond the scope of this review. For a more comprehensive analysis, the reader is directed to recent reviews dedicated exclusively to this topic [54,55].

Cardiovascular Disease

Resveratrol has at least three distinct properties that confer protection against cardiovascular disease. It induces a "preconditioning" effect that limits the damage during acute ischemia/reperfusion injuries [56], it improves vascular function [57], and it blocks platelet aggregation [58].

The protective effect of resveratrol against ischemic injuries is incompletely understood, but can be blocked by antagonists of nitric oxide synthase or adenosine in isolated hearts [59], and is absent in hearts from inducible nitric oxide synthase (iNOS)-null mice [60]. The protective effect of resveratrol against ischemic injury in brain is lost in PPARα-null mice [61], and again appears to require nitric oxide, although an endothelial nitric oxide synthase (eNOS)-specific inhibitor is sufficient to completely block the effect in this tissue [62]. At the time when most of these observations were made, no relationship was known between SIRT1 and PPARα or adenosine, and nitric oxide had been shown to function upstream of SIRT1, by increasing expression [63]. However, SIRT1 has since been shown to positively regulate PPARα [64], and to create a potential positive feedback loop by deacetylating and activating eNOS [65], raising the possibility that direct activation of SIRT1 could have a role in preconditioning. Moreover, inhibition of sirtuins by sirtinol or nicotinamide blocks the protective effect of resveratrol against ischemia in brain [66], and in cultured cardiomyocytes [67], respectively.

Resveratrol induces vasorelaxation *in vitro* [68] and lowers blood pressure in obese Zucker rats [69], as well as several experimentally-induced models of hypertension [44]. In aortas from obese or aged animals, resveratrol restores acetylcholine-dependent relaxation [70], which is dependent on nitric oxide signaling. Resveratrol's protective effect appears to be mediated by suppression of age- and obesity-induced increases in NADPH oxidase expression. This enzyme is a major contributor to the production of superoxide radicals, which cause local oxidative stress and impair nitric oxide signaling by reacting to form peroxynitrate. Resveratrol also modestly induces eNOS expression in vascular tissue, which may help overcome the inactivation of nitric oxide by oxidative stress. The mechanism by which resveratrol suppresses NADPH oxidase in vasculature has not been firmly established, however SIRT1 can inhibit production of an upstream signal, tumor necrosis factor α (tnfα), by macrophages, most likely through inhibition of NFκB [71]. In addition, overexpression the SIRT1 target PGC-1α in vascular endothelial cells is sufficient to suppress NADPH oxidase expression [72], providing a second potentially SIRT1-dependent mechanism. Resveratrol may further stimulate PGC-1α through activation of AMP-activated protein kinase (AMPK). AMPK is a particularly attractive hypothesis to explain resveratrol's effects, since there is evidence that the PPARγ ligand rosiglitazone reduces oxidative stress through an AMPK-dependent mechanism involving suppression of NADPH oxidase [73]. AMPK activation by resveratrol can be independent of SIRT1 [74], but can also be mediated by SIRT1-dependent deacetylation of the

upstream kinase LKB1 [75]. Whether any or all of these mechanisms are relevant to the suppression of NADPH oxidase by resveratrol *in vivo* remains to be seen.

Resveratrol is able to inhibit platelet aggregation *in vitro* [76] and *in vivo* in hypercholesterolemic rabbits [58] and normal mice [77]. Its activity may be partially related to disruption of Mitogen-activated protein kinase (MAPK) signaling [78] and phosphoinositide metabolism [79], as well as enhanced nitric oxide signaling to guanylate cyclase [77]. It is also tempting to speculate that a major contribution to the *in vivo* effects might be mediated by direct and irreversible inhibition of cyclooxygenase I activity [80], resulting in decreased production of thromboxane A2, a potent inducer of clotting and vasoconstriction. One attractive aspect of this model, given the short half-life of resveratrol *in vivo*, is that it does not require a sustained dose. Inhibition of cyclooxygenase I is the same mechanism proposed to account for the cardioprotective effects of low-dose aspirin [81], and intriguingly, resveratrol remains effective even in platelets from aspirin-resistant individuals [82].

Insulin Sensitivity

Resveratrol has consistently been found to ameliorate insulin resistance in obese animals [32,83]. This effect does not appear to be directly related to overall body weight, but is accompanied by a dramatic reduction of ectopic fat deposits in non-adipose tissues, particularly the liver [83]. In human studies, such ectopic fat deposits have been shown to precede clinical disease in subjects at risk for type II diabetes, and to associate with reduced respiratory capacity and tissue mitochondrial content [84]. In rodents, the protective effects of resveratrol are accompanied by an increase in mitochondrial biogenesis and respiratory capacity that offset effects of obesity [32,83], and could thereby explain the restoration of insulin sensitivity. PGC-1α, the “master regulator” of mitochondrial biogenesis is activated upon deacetylation by SIRT1 [31], and this has been shown to occur in tissues of resveratrol-treated animals, ostensibly accounting for the increase in mitochondrial content [32,83]. In addition, a SIRT1-dependent decrease in the expression of protein tyrosine phosphatase 1B (PTP1B) has been described in resveratrol-treated animals [42]. Since PTP1B inactivates the insulin receptor, this provides a second mechanism by which resveratrol might act through SIRT1 to improve insulin sensitivity. Furthermore, overexpression of SIRT1 is sufficient to eliminate ectopic fat deposits in liver and to improve insulin sensitivity in obese mice [85]. Although the mechanism is far from completely elucidated, these results provide significant support for the idea that resveratrol might protect against the metabolic syndrome and type II diabetes by activating SIRT1.

Energy Expenditure

Resveratrol has a biphasic effect on energy expenditure. Without any significant effect on food intake, resveratrol causes a modest, but significant increase in the body weight of mice at low doses [70], and a loss of body weight and reduction of adiposity at high doses [32]. Notably, the elimination of ectopic fat deposits described above occurs even at doses too low to decrease total body weight. Although the decrease in body weight at high doses of resveratrol is accompanied by an increase in oxygen consumption, cold tolerance, and endurance, resting body temperature is unchanged and spontaneous locomotion is decreased, raising interesting questions about where the excess energy actually goes [32]. Overexpression of SIRT1 from the β-actin locus, which results in high expression in adipose and brain, decreases body weight and adiposity, similar to the effect of a high dose of resveratrol [86]. However overexpression under the endogenous promoter from a bacterial artifical chromosome leads to a different expression profile, and can actually decrease energy expenditure in some cases, and possibly increase body weight on a leptin-deficient background [85,87]. Therefore it is conceivable that the disparate effects of resveratrol at high and low doses reflect effects on SIRT1 in different

tissues. Indeed, there is evidence that SIRT1 is differentially regulated across tissues by diet, suggesting that whole-body activation may be too simplistic an approach [88]. Of course, many other targets of resveratrol could contribute to its effects on body weight. One such target is the cannabinoid receptor CB1 [89], which can regulate body weight through food intake and appetite-independent mechanisms.

Learning and Memory

Resveratrol improves cognitive function in models of neurodegenerative diseases, and following neuronal injury, but has not been clearly demonstrated to improve cognition in normal, healthy rodents [90,91]. Normal age-related cognitive impairment is ameliorated by resveratrol in a short-lived species of fish, in parallel with an increase in lifespan [92], but similar data have not been reported for any mammalian species. In mice, resveratrol improves rotarod performance [32,83], which can have a cognitive component, but may also be explained by changes in endurance. Further research in this area is needed to clarify the involvement of SIRT1 in neuroprotective effects of resveratrol, and whether activation of SIRT1 could be detrimental, rather than protective, in neurons under certain conditions [93,94]. Intriguingly, a SIRT1 allele has been linked to cognition in a human association study, although is not yet possible to know what consequence the allele has on SIRT1 function [95].

Survival

Placing mice on a high fat diet (60% by energy content) at one year of age results in an approximately 25% decrease in remaining lifespan, and this effect is completely blocked by resveratrol administration, independent of any effect on body weight [83]. This effect appears to represent amelioration of the detrimental effects of obesity, rather than slowing the rate of aging, since no significant change in longevity has been detected in mice fed a standard diet plus resveratrol at similar or higher doses [70]. These results are in accordance with the well-known correlation between insulin sensitivity and longevity, since resveratrol dramatically improves glucose tolerance only in the context of obesity. Similarly, whole-body overexpression of SIRT1 improves glucose tolerance only in obese mice [85,87], although overexpression in a more limited subset of tissues from the β-actin promoter is effective even in lean animals [86]. To date, lifespans have not been published for SIRT1 overexpressing or heterozygous animals. SIRT1 null animals are short-lived, and do not respond favorably when placed on a calorie-restricted diet [94], however these mice manifest a number of developmental defects and altered metabolism [96,97], that make it difficult to interpret their phenotypes with respect to aging. Two critical questions remaining to be answered are whether SIRT1 overexpression can recapitulate the effect of resveratrol on survival in obese mice, and whether it will influence longevity in lean animals.

“Off-Target” Effects of Resveratrol

Perhaps the biggest liability of resveratrol as a tool to probe the function of SIRT1 is its lack of specificity. Resveratrol has other direct targets in mammalian cells, some of which were identified prior to SIRT1, and many of which have their own complex and potentially beneficial consequences. For example, some of the cardioprotective and anti-inflammatory effects or resveratrol may be due to direct inhibition of cyclooxygenases [47], and an alternate explanation for many of the effects attributed to direct SIRT1 activation, such as increases in mitochondrial biogenesis and fatty acid oxidation, could be provided by indirect activation of AMPK [83,98]. In fact, the relationship between SIRT1 and AMPK is more complex, since each has been reported to influence the other [75,99], however, activation of AMPK by resveratrol is at least partially independent of SIRT1 [74,100]. Additional direct effects of resveratrol that could have important consequences *in vivo* include inhibition of kinases

[101,102], modulation of the estrogen receptor [103], the aryl hydrocarbon receptor [49], and a cannabinoid receptor [89], and inhibition of quinone reductase 2 [104], to name a few.

Another important caveat is that the bioavailability of resveratrol in mammals is low enough that the doses required to activate SIRT1 *in vitro* and in cells are only briefly or never achieved in serum *in vivo* [105]. This has even led to the suggestion that metabolites of resveratrol might be the active forms, rendering most published work on the molecule irrelevant [106]. Notably, concentrations insufficient to activate SIRT1 would also be insufficient to affect most of the other targets, or stimulate AMPK, however a minority of higher affinity targets might still be affected. These include the aryl hydrocarbon receptor (AhR), quinone reductase 2 (NQO2, QR2), and the cannabinoid receptor CB1, each of which is affected by nanomolar concentrations of resveratrol. The CB1 receptor in particular is intriguing, since it is already considered a promising drug target for the treatment of obesity and related comorbidities. In fact, the antiobesity drug rimonabant, like resveratrol, is an antagonist of this receptor [107]. Although rimonabant was taken off the market due to a high incidence of psychiatric disorders, it has been shown to increase energy expenditure, improve insulin sensitivity, suppress inflammation, and prevent cancer, similar to resveratrol. One discrepancy is that rimonabant also suppresses food intake, which does not occur with resveratrol treatment, at least in mice. Suppression of AhR-dependent gene transcription by resveratrol could account for some of its anti-carcinogenic and anti-inflammatory effects, and it has been suggested that this mechanism could account for cardioprotective effects [108]. The function of NQO2, and consequences of its inhibition are less clear, but it has been suggested that blocking its activity could lead to induction of phase II antioxidant enzymes [104], which has been observed following resveratrol treatment [109]. The abundance of cellular targets and questions about bioavailability raise the possibility that SIRT1 activation might occur through an indirect mechanism, downstream of one of the higher affinity targets. This requires an enormous fluke, since resveratrol was selected through an *in vitro* assay, however it is almost impossible to distinguish experimentally from direct activation unless the upstream pathway is known. Ultimately, reconciling the pharmacokinetics of resveratrol with its biological consequences will require a better understanding of its distribution and accumulation within specific tissues or compartments, and a thorough consideration of many potentially relevant targets. These complications highlight the need to verify effects that have been attributed to SIRT1 using knockout animals and more specific methods of SIRT1 activation *in vivo*.

SRT1720

Besides resveratrol, a number of other naturally occurring polyphenols, such as quercetin, fisetin, and butein activate SIRT1 and extend lifespan in lower organisms [33,34]. However, all are structurally related and share the same caveats. Recently, a more potent synthetic SIRT1 activator that is structurally unrelated to resveratrol was described and designated SRT1720 (Figure 3) [110]. Although its full spectrum of effects remains to be determined, SRT1720 would ostensibly not share “off-target” effects of resveratrol, and is therefore a useful tool for verifying putative SIRT1-dependent effects *in vivo*. Importantly, SRT1720 does not acutely activate AMPK [111]. Similar to both resveratrol treatment and SIRT1 overexpression, SRT1720 improves insulin sensitivity and glucose tolerance in obese mice. Moreover, a longer treatment course lowers body weight, induces mitochondrial biogenesis, eliminates ectopic fat deposits, and increases endurance [111]. These phenotypes are associated with deacetylation of the SIRT1 target PGC-1α, suggesting a direct mechanism, and providing further correlation with the effects of resveratrol. Deacetylation of the SIRT1 substrates FOXO1 and p53 were also observed following SRT1720 treatment, and modulation of their activities might contribute the observed phenotypes as well. One important caveat to the long-term studies with SRT1720 is that activation of AMPK eventually occurs. However, this is most likely a downstream consequence of the metabolic changes induced by SIRT1 activation, since unlike

resveratrol, SRT1720 does not stimulate AMPK in cells. Furthermore, a microarray based study has shown a striking degree of overlap in the transcriptional effects of resveratrol and SRT1720 following a relatively short treatment, suggesting that activation of SIRT1 is responsible for many of the observed changes [112]. Experiments are currently underway to test whether SRT1720 recapitulates other salient effects of resveratrol, such as preconditioning, reduction of vascular oxidative stress, prevention of platelet aggregation, neuroprotection, cancer prevention, and delaying mortality in obese mice.

Isonicotinamide

Nicotinamide, which is produced by sirtuin enzymes, is a potent inhibitor of their activity. It has been estimated that physiological concentrations of nicotinamide are sufficient to reduce basal Sir2 activity in yeast by 2.5-6 fold, and SIRT1 activity in mouse cells by up to 20-fold [113]. The mechanism of inhibition involves re-entry of nicotinamide into the catalytic site of the enzyme after its release, where it can combine with a relatively stable reaction intermediate, resulting in regeneration of the original acetylated lysine and NAD^+. Based on this observation, Sauve et al [113] were able to show that isonicotinamide can relieve inhibition of Sir2 activity by competing with nicotinamide for binding in the same pocket within the enzyme. Since isonicotinamide cannot participate in the reverse reaction, the intermediate is stabilized and more likely to complete the deacetylation step. Isonicotinamide is therefore expected to be an activator of sirtuins under normal physiological conditions (i.e when their activity is partly held in check by nicotinamide). Consistent with this, effects of isonicotinamide have been correlated with those of resveratrol in cell culture models, although in most cases the SIRT1-dependence was not thoroughly tested [114,115]. Recently, it was shown that isonicotinamide represses expression of cytosolic phophoenolpyruvate carboxykinase (PEPCK) and that the effect depends on the presence of both SIRT1 and its substrate, hepatocyte nuclear factor 4α (HNF4α) [116]. Activation of SIRT1 with resveratrol or other polyphenols, or overexpression of the enzyme also repressed PEPCK transcription, suggesting that a reduction in gluconeogenesis might contribute to the long-term improvements in insulin sensitivity in mice treated with resveratrol or overexpressing SIRT1. The high concentrations of isonicotinamide required for efficacy likely limit any *in vivo* applications, although it appears to be well tolerated in mice, even at 1% in the drinking water over the entire lifespan [117].

NAD^+ Metabolism

Another strategy for increasing sirtuin activity is by increasing the availability of the cofactor NAD^+. There is an unresolved debate in the field about whether NAD^+:NADH ratio or some combination of absolute NAD^+ and nicotinamide concentrations best predicts the activity of sirtuins *in vivo* [118,119]. However, it is widely agreed that increasing flux through the NAD^+ salvage pathway, which generates NAD^+ from nicotinamide, is sufficient to cause activation. In yeast, Pnc1 catalyzes the rate-limiting step in this pathway, and its overexpression is sufficient to extend lifespan in a Sir2-dependent manner [120]. In mammals, Nicotinamide phosphoribosyltransferase (Nampt) plays an analogous role and its overexpression is sufficient to increase the catalytic activity of SIRT1 [121], as well as promote cell survival in a SIRT3 and SIRT4-dependent manner [122]. *In vivo*, the product of its reaction, nicotinamide mononucleotide, appears to be limiting for SIRT1 activity in pancreatic beta cells [123], and circadian oscillations in Nampt expression cause SIRT1 activity to fluctuate in liver and white adipose tissue [22,124]. Overexpression of Nampt in the heart is sufficient to promote survival of cardiac myocytes under a number of stresses, although the involvement of sirtuins in this process is not known [125]. These results underscore the fact that much additional work will be required to fully elucidate the consequences of increasing cellular NAD^+, which serves as a cofactor in many crucial processes. Another potential route to increased NAD^+ synthesis is through the administration of nicotinamide riboside, a precursor that is enriched in milk and

may be involved in normal NAD^+ metabolism [126]. Much like Pnc1 overexpression, supplementing yeast media with nicotinamide riboside is sufficient to confer Sir2-dependent lifespan extension, although the latter would presumably increase NAD^+ without lowering nicotinamide levels.

It is important to keep in mind that resveratrol and SRT1720 are generally considered to target SIRT1 (albeit that resveratrol may also affect SIRT7 [127], and data on the specificity of SRT1720 are not widely available), while isonicotinamide and NAD^+ availability are likely to affect all seven mammalian sirtuins. As has been discussed, each of these strategies to increase SIRT1 activity also comes with a large number of caveats and probable off-target effects. However, by combining mechanistically distinct approaches, and incorporating data from overexpression and loss of function studies, it will be possible to move forward and improve our understanding of the role of SIRT1 *in vivo*. Based on rodent studies, there is good reason to be optimistic that this could lead to the development of therapies that will benefit human health.

Human Trials

Nicotinamide and nicotinic acid are forms of vitamin B3 (niacin), and their biological functions, including the ability to act as NAD^+ precursors, have been adequately reviewed elsewhere [128]. There is also significant interest in the potential use of nicotinamide riboside to drive NAD^+ synthesis, particularly in nervous tissue, where it may be more effective than niacin [129]. However, it is resveratrol that has thus far received the most attention as a potential strategy to activate SIRT1 therapeutically. Humans have historically been exposed to low doses of resveratrol primarily through consumption of red wine, and traditional Chinese and Japanese medicines, and it is attractive to speculate that the health benefits associated with these substances might be at least partially attributed to their resveratrol content. Intriguingly, a recent study showed that light wine consumption is associated with a ~5 year increase in life expectancy [130], however alcohol alone has a significant protective effect (~2 years in the aforementioned study), and many other wine components are thought to improve health. Moreover, the resveratrol doses employed in animal studies significantly exceed those that could be obtained from wine. With the more recent availability of higher doses resveratrol in “nutraceutical” formulations, claims of benefits based on anecdotal evidence have become widespread. In many cases, studies are poorly controlled or uncontrolled, and in almost all cases, the formulations administered contain multiple compounds or complex extracts, making it even more difficult to assess the effect of resveratrol per se [131]. Based on peer-reviewed literature, it seems that resveratrol is well tolerated in humans over short periods of time, but further trials are needed to establish its ultimate effects on health and disease. The first measurements of resveratrol and its metabolites in human plasma and urine following an oral dose were reported in 2001 [132], and since then, and at least 9 additional human trials have been conducted to assess pharmacokinetic parameters [133,134]. Safety and potential side effects have been investigated in two phase I clinical trials. The first employed a single dose of up to five grams, with a 14-day follow-up [135]. The second employed doses of up to 150 mg every four hours for two days, followed by an additional 24 hours of observation [136]. Notably, the doses from both studies greatly exceed the ~5-22 mg/kg that increased survival in obese mice when the preferred method of allometric scaling is employed [137], and the five gram dose remains well in excess even by direct extrapolation from body weight. In both studies, the effects reported were minor, and not clearly linked to the treatment, including symptoms such as headache, dizziness, and anomalous biochemical or hematological measurements that were not consistent across treatment groups. It has also been reported in company press releases that SRT501, a proprietary formulation of resveratrol developed by Sirtris Pharmaceuticals, has undergone phase I trials employing up to five grams daily for 28 days (www.sirtrispharma.com/news-press.html). This was reported to result in improved

glucose tolerance, although neither these data, nor any other beneficial effect of a SIRT1 activator in humans, has yet been published in a peer-reviewed journal. This may soon change, since a number of more advanced clinical trials have been announced to test resveratrol against cancer, type II diabetes, Alzheimer's disease, and viral infections. In addition, at least one controlled study of resveratrol has been initiated in monkeys [138], and Sirtris has announced its intention to proceed with phase I clinicals trials of another proprietary SIRT1 activator, designated SRT2104. The entry of SIRT1 activators into human trials is exciting, but also highlights the need to better define the pathways that mediate their effects *in vivo*.

Summary

As the homologue of enzymes that promote longevity in lower organisms, SIRT1 provides a tantalizing drug target. The ever-growing list of proteins whose activities are influenced by SIRT1-dependent deacetylation supports its potential importance in mammalian biology, but also add to the difficulty in understanding its function. Overexpression studies and treatment of rodents with small molecule activators have led to significant improvements in physiology, many of which are consistent with effects on specific SIRT1 targets. However, many caveats and uncertainties remain. Much of the strongest evidence for beneficial effects has been obtained with resveratrol, the strategy with perhaps the least certain mechanism of action. Moreover, disparate phenotypes have been reported even between independent lines of SIRT1-overexpressing mice, suggesting that the consequences SIRT1 activation will be complex and context-dependent. Data from three independent transgenic lines, as well as resveratrol and SRT1720 treatment, support a role for SIRT1 in promoting insulin sensitivity and glucose tolerance in obese animals, highlighting the therapeutic potential of modulating this pathway. Establishing a clear mechanistic explanation for these benefits should be a high priority, given the need for tools to combat obesity and the metabolic syndrome, and the number of humans already entering controlled or self-administered clinical trials with SIRT1 activators.

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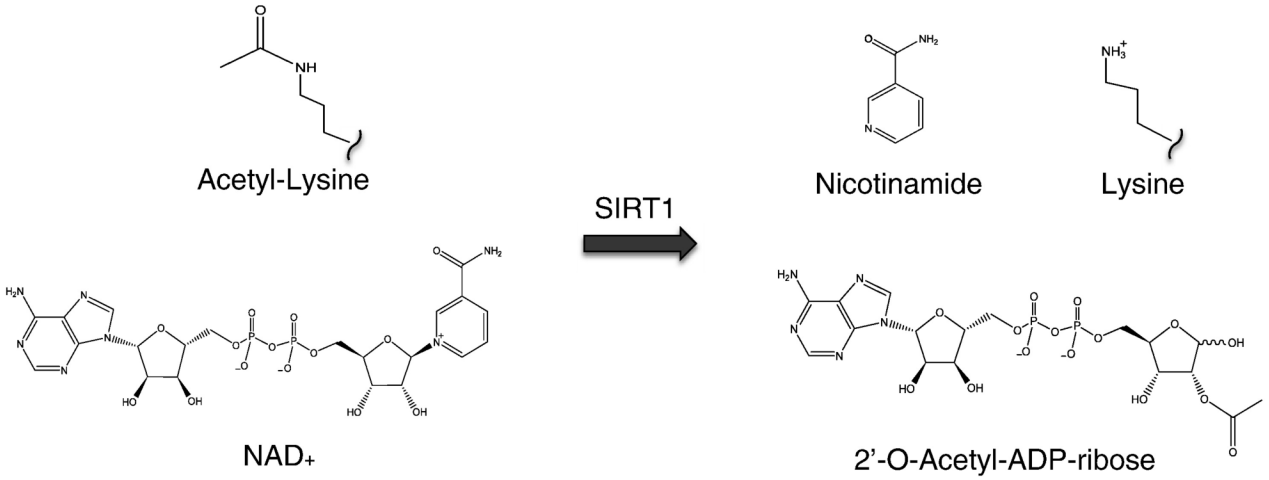

Figure 1.
Substrates and products of the reaction catalyzed by SIRT1. Note that only the side chain is shown for lysine, since SIRT1 does not have a conventional consensus sequence [139], although preference for specific peptides can be demonstrated [140].

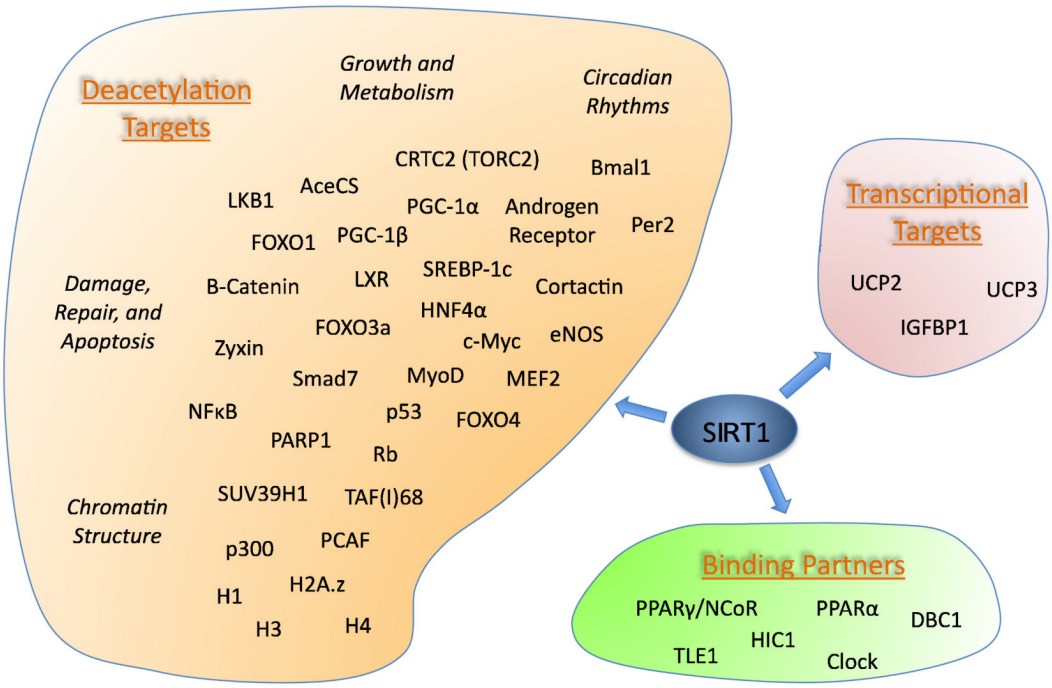

Figure 2.

Substrates, transcriptional targets, and binding partners of SIRT1. At least 34 direct deacetylation targets are known, with activities that impact almost every aspect of cellular physiology. For simplicity, only a few of the major themes are indicated. Several transcriptional targets of SIRT1 have been indicated because they are likely to be physiologically important and are not obvious results of the deacetylation targets and binding partners listed (although silencing of IGFBP1 is probably related to deacetylation of FOXOs). Note that the interaction of SIRT1 with binding partners can have varying results. SIRT1 overexpression silences PPARγ targets [21], but enhances expression of PPARα targets [64]. DBC1 directly inhibits SIRT1 activity [141,142], while HIC1 directs it to its own promoter, silencing SIRT1 expression [143]. TLE1 mediates deacetylation of NFκB [144], and Clock directs SIRT1 to the promoters of genes involved in circadian rhythms [22,145].

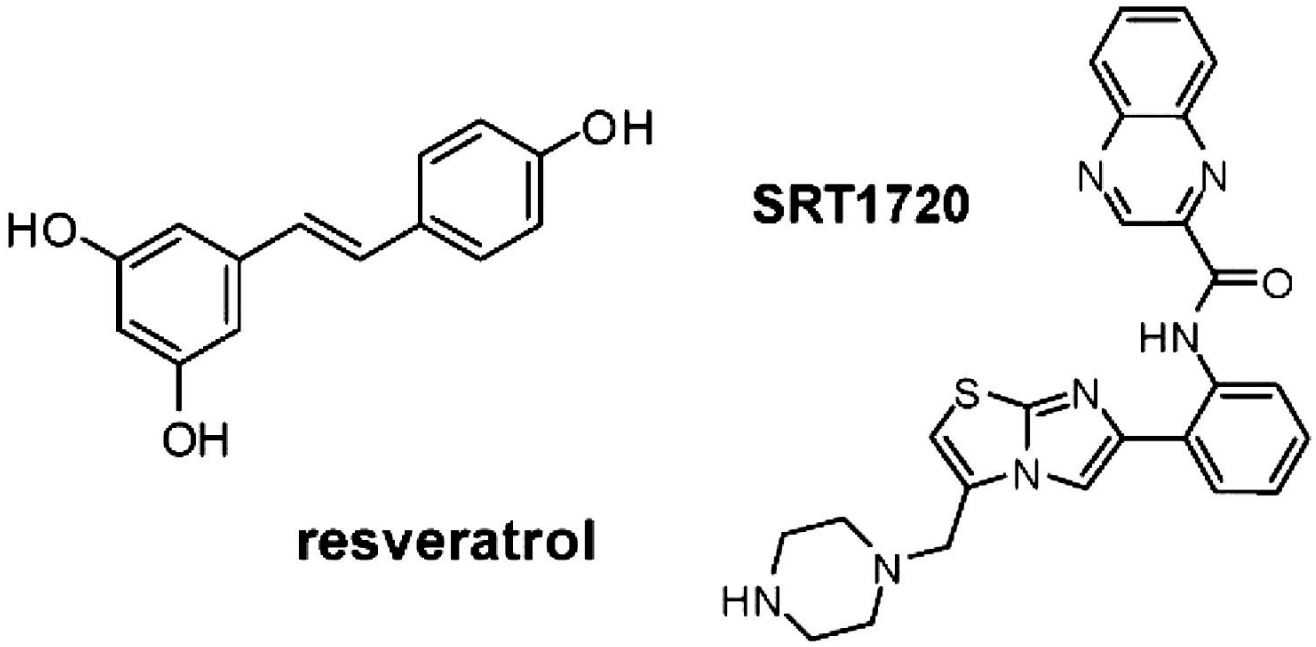

Figure 3.
Structures of SIRT1 activators. Resveratrol is a naturally occurring polyphenol, and SRT1720 was synthesized by Sirtris Pharmaceuticals.