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A therapeutic role for sirtuins in diseases of aging?

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The sirtuins are a group of proteins linked to aging, metabolism and stress tolerance in several organisms. Among the many genes that have been shown to affect aging in model organisms, sirtuin genes are unique in that their activity level is positively correlated with lifespan (i.e. they are anti-aging genes). Sirtuins are a druggable class of enzymes (i.e. amenable to intervention by small molecules) that could have beneficial effects on a variety of human diseases. In view of the many functions of Sirtuin 1 (SIRT1) in cells, this review focuses on its role in regulating important aspects of mitochondrial biology. Mitochondria have been linked to aging, and also to diseases of aging. Thus, sirtuins might provide a key link between mitochondrial dysfunction, aging and metabolic disease.

Therapeutic applications of sirtuins for metabolic diseases

Sirtuins are a family of related proteins that were first linked to longevity and stress tolerance in budding yeast and other lower eukaryotic organisms [1,2]. Genes encoding sirtuins are distinguished from many other genes that affect aging because they are anti-aging genes (i.e. increasing their activity extends life span). As such, in *Saccharomyces cerevisiae* (yeast), *Caenorhabditis elegans* (worms) and *Drosophila melanogaster (*flies*)*, Sirtuin 2 (SIRT2) orthologs retard aging as a function of their gene dosage [3–5].

The biochemical activity of SIR2 orthologs is unique. They are NAD-dependent protein deacetylases [Class 3 histone deacetylases (HDACs)] [6,7]. NAD and NADH are involved in hundreds of metabolic (usually catabolic) reactions in cells. Thus, the biochemical activity of SIR2 led to the idea that sirtuins connected diet and metabolism to aging. It has long been known that low calorie diets, known as 'calorie restriction' (CR) extend the lifespan of a wide variety of organisms, including yeast, *C. elegans, Drosophila* and rodents [8] and it has been proposed that sirtuins might, in part, mediate this effect [9].

Sirtuins apparently mediate their life-extending effects in different organisms by targeting different pathways. For example, in yeast Sir2p deacetylates histones at several loci, including the rDNA (ribosomal DNA) locus, thereby stabilizing the rDNA from recombination and reducing the rate of formation of toxic rDNA circles [10]. These circles limit the replicative lifespan of mother cells in most yeast strains. However, in *C. elegans*, SIR2.1 functions as a coactivator of the forkhead transcription factor DAF-16 in a complex induced by heat or oxidative stress and mediated by 14–3-3 proteins [11]. DAF-16 can be activated to turn on target genes (such as that for super-oxide dismutase 3) by low insulin signaling, as well as by stress. SIR2.1 is required only for the stress-responsive pathway of DAF-16 activation.

In mammals, SIRT1 deacetylates many key transcription factors and co-factors, such as p53 [12], FOXO (forkhead) proteins [13,14], peroxisome proliferation activating receptor (PPAR)-gamma co-activator-1α (PGC-1α) [15] and nuclear factor-κB (NF-κB) [16] thereby affecting crucial cellular pathways involved in stress resistance and metabolism. There are several factors supporting the action of sirtuins in mediating salutary physiological effects of CR in mammals:

(i) The known effects of SIRT1 on the transcription factors and co-factors mentioned above trigger stress tolerance (p53, forkhead, Ku70/BAX [17] and metabolic changes (PGC-1α, FOXO 1, FOXO3, FOXO4) reminiscent of CR.
(ii) CR upregulates the levels of SIRT1 in several metabolic tissues, including liver, muscle and brain [18,19].
(iii) SIRT1 knockout mice fail to display a prominent phenotype of CR (i.e. increased physical activity) [20].

In addition to SIRT1, there are six other mammalian sirtuins, SIRT2–7 [21]. Broad mechanisms of action and potential therapeutic applications are summarized in Table 1. SIRT1, 2, 3, 6 and possibly 5 are NAD-dependent deacetylases, SIRT4 and 6 are ADP-ribosyltransferases, and an activity for SIRT7 has not yet been described. Sirtuin-mediated deacetylation and ADP-ribosylation are related in that both cleave NAD as the initial chemical step in the reaction cycle, as shown in Figure 1. In deacetylation, the ADP-ribosyl transfer directly participates in the removal of the acetyl group from the protein substrate to generate 2,3-O-acetyl-ADP-ribose, whereas in ribosylation, the ADP ribosyl moiety is transferred to the protein substrate [1,22–24]. Deacetylation of sirtuin substrates can inhibit or induce their activities, whereas ADP-ribosylation has only been shown to be inhibitory to date (see following section).

The sirtuins are a druggable class of enzymes (i.e. amenable to intervention by small molecules) that could have beneficial effects on a variety of human diseases. Here

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Table 1. Mechanism of action and therapeutic potential of sirtuins

Sirtuin	Mechanism of action	Therapeutic area
SIRT1	NAD-dependent deacetylase, deacetylates proteins (e.g.PGC-1α)	Metabolic, mitochondrial, cardiovascular
SIRT2	NAD-dependent deacetylase	Cancer, neurological
SIRT3	NAD-dependent deacetylase, deacteylates and activates mitochondrial acteylCoA synthase	Metabolic, mitochondrial, cardiovascular
SIRT4	ADP-ribosyltransferase ADP-ribosylates and inhibits mitochondrial glutamate dehydrogenase	Metabolic, mitochondrial, cardiovascular
SIRT5	NAD-dependent deacetylase	To be determined
SIRT6	NAD-dependent deacetylase ADP-ribosyltransferase	Cancer
SIRT7	Unknown	To be determined

we focus on the role of SIRT1 in regulating important aspects of mitochondrial biology. Mitochondria have been linked with aging and also diseases of aging, so sirtuins might provide a key link between mitochondrial dysfunction, aging and metabolic disease. An elegant study showed that elderly subjects, matched appropriately for lean and fat mass against their young counterparts, were more likely to be insulin resistant and also had a 40% reduction in oxidative phosphorylation [25]. Mitochondrial energy coupling did not change (i.e. reactive oxygen species (ROS) levels were not affected) so the drop in oxidative phosphorylation was probably owing to a reduction in either the number or function of mitochondria in the elderly. Furthermore, another study by the same group showed that the otherwise healthy offspring of patients with type 2 diabetes had impaired mitochondrial function potentially because of an inherited defect in oxidative phosphorylation [26]. Thus, any regulator of mitochondrial function might be particularly relevant to metabolic disease.

Current treatments for type 2 diabetes address only the symptoms of diabetes through lifestyle changes or drugs such as metformin, which do not directly address contributions to the disease from any underlying mitochondrial defects. Therapeutics that can increase mitochondrial biogenesis or alter the function of mitochondria might represent a novel mechanism of action to address the causes of type 2 diabetes, and other diseases of aging. In this review, we focus on connections between sirtuins and mitochondria, which might present opportunities for new strategies for treating indications that are dramatically influenced by the function of this key cellular organelle.

SIRT1

SIRT1 seems to have a significant role in mammalian metabolism. It has been shown to deacetylate and thereby

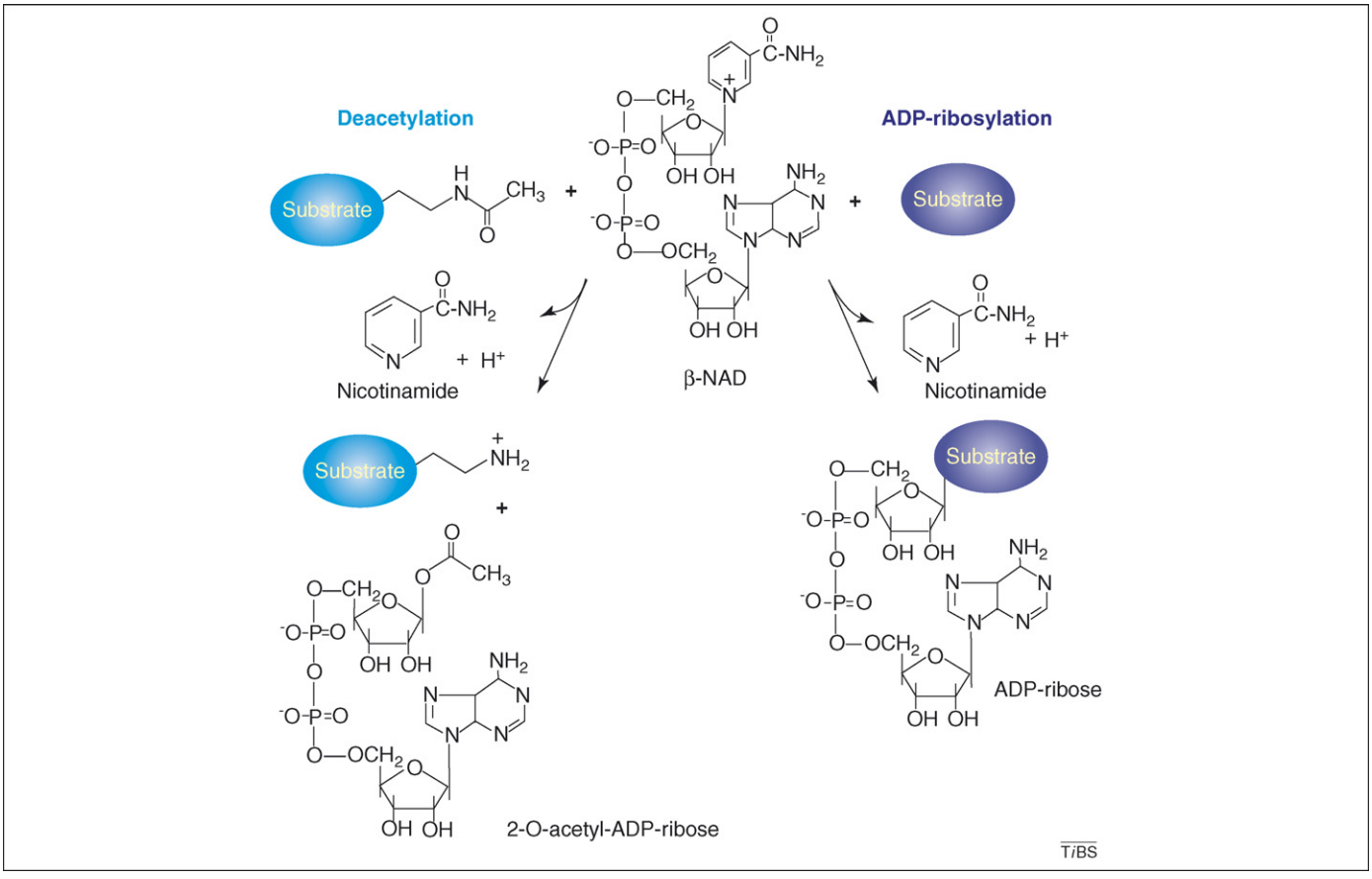

Figure 1. Sirtuin biochemistry. Sirtuins catalyze either ADP-dependent deacetylation or ADP-ribosylation reactions. Both mechanisms cleave NAD to release nicotinamide (NAM).

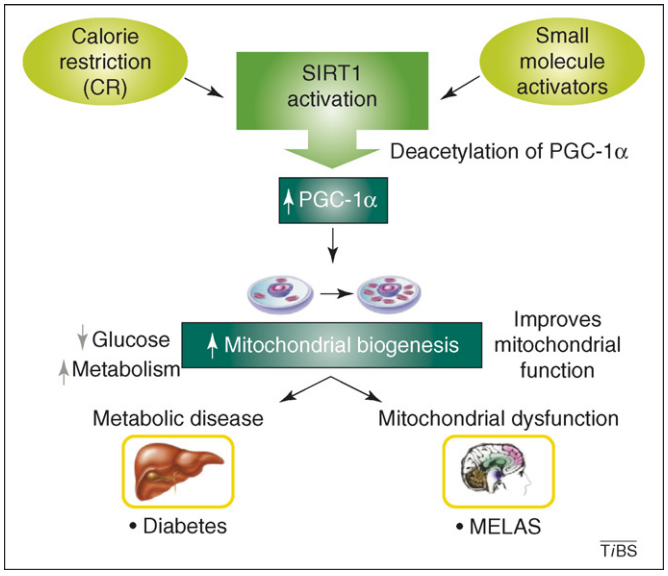

Figure 2. SIRT1 activation: mechanism and phenotype. CR or small molecule activators of SIRT1 deacetylate PPAR-γ coactivator-1 α (PGC-1α) leading to mitochondrial biogenesis, which might be therapeutic for various diseases of aging such as metabolic disorders (e.g. type 2 diabetes) or mitochondrial disorders [e.g. mitochondrial myopathy, encephalopathy, lactic acidosis and mitochondrial encephalopathy lactic acidosis and stroke-like symptoms (MELAS)].

activate a crucial cofactor in mitochondrial biogenesis: PGC-1α [15]. Why is mitochondrial biogenesis beneficial? Mitochondrial activity in metabolically active tissues, such as muscle, will increase metabolic rate, drive glucose metabolism, and thereby improve insulin sensitivity. Thus SIRT1 activation is a promising strategy for treating type 2 diabetes, obesity, and metabolic syndrome (Figure 2). In addition, the number of functional mitochondria is known to decrease with aging, so an increase in mitochondrial biogenesis could exert an anti-aging effect by buffering this decline.

A second, more subtle, aspect of mitochondrial biogenesis might also have a role in anti-aging. A leading hypothesis of a cause of aging is the oxidation of macromolecules in cells owing to the generation of ROS by mitochondria [27]. One mechanism for generating ROS is the stalling of electrons along the electron transport chain. Electron transport can become stalled when protons are pumped to the cytoplasm across a hyper-polarized mitochondrial membrane, that is, when the demand for ATP synthesis is high and the charge gradient (charge difference per surface area) is steep (Figure 3). An increase in mitochondrial biogenesis will increase mitochondrial surface area, thereby reducing hyper-polarization of the membrane and leading to the production of ROS. By this reckoning, mitochondrial biogenesis could be beneficial even if it did not result in a measurable increase in metabolic rate (because of some other limiting component), and it has been found that humans on CR induce mitochondrial biogenesis without an observed increase in metabolic rate [19,28,29].

More tangible evidence that SIRT1 activation might have benefit via mitochondrial function comes from studies of the polyphenol, resveratrol, in mice. Resveratrol and other polyphenolic compounds are made by plants in response to stress. Resveratrol was recently shown to affect the activity of SIRT1 *in vitro* [30] although its effects seem to depend on the nature of the substrate for deacetylation [22,31]. However, *in vivo*, resveratrol has been shown to exert effects dependent on sirtuin orthologs – extension of lifespan in yeast, *C. elegans* and *Drosophila*, and metabolic effects on mammalian cells [30,5,24]. Two recent studies show that deleterious effects of high fat, high caloric diets in mice were mitigated by resveratrol feeding [32,33]. In one study, the shortening of lifespan by the high fat diet was reversed. In a second study, resveratrol increased SIRT1 activation, PGC-1α deacetylation, and mitochondrial biogenesis in muscle. These studies provide a powerful indication that SIRT1 activation offers a promising approach for treating metabolic disorders. However, one must be aware of possible targets other than SIRT1 for resveratrol, for example, AMP-dependent protein kinase [34].

There is also evidence to suggest neuroprotective effects of SIRT1 activation. The Wallerian mouse, which is protected against neuronal degeneration, harbors a mutation increasing levels of an NAD biosynthetic enzyme, Nmnat-1. In addition, NAD itself protects dorsal root ganglions (DRGs) against chemical or physical assaults in culture, and this effect was shown to require SIRT1 [35]. These findings have been confounded by a report showing that NAD also protects DRGs in SIRT1$^{-/-}$ mice [36]. We surmise that NAD might have multiple protective effects on neurons, at least some of which are mediated by SIRT1. Several other studies have shown neuroprotective effects of SIRT1 or resveratrol or NAD in cultured neurons [35,37,38].

Neurons are the most metabolically active cells in the body and as such might depend most crucially on functional mitochondria. Many neurodegenerative diseases, such as Huntington's disease and Parkinson's disease might be marked by mitochondrial dysfunction, and therefore activation of mitochondrial biogenesis via SIRT1 and PGC-1α might hold promise in treating these diseases [37,39].

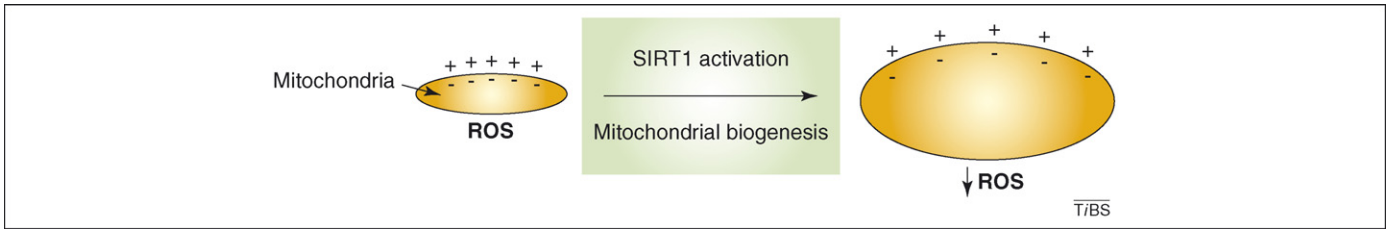

Figure 3. Mitochondrial biogenesis reduces ROS. Mitochondrial biogenesis triggered by SIRT1 activation and PGC-1α deacetylation leads to an increase in the size of mitochondria. Increased mitochondrial surface area should mitigate hyperpolarization of the mitochondrial membrane and stalling of electrons in the electron transport chain, thereby reducing production of ROS.

SIRT3 and SIRT4

SIRT3 is produced in brown adipose tissue in response to cold and its expression levels seem to be directly related to metabolism. Specifically, overexpression of SIRT3 leads to increased respiration and also to a reduction in ROS [40]. In addition, SIRT3 was recently shown to deacetylate and activate mitochondrial acetyl-CoA synthetase (ACS) [41,42] to enable metabolism of acetate sourced from the diet or produced endogenously. SIRT4 ADP ribosylates and inhibits the mitochondrial enzyme glutamate dehydrogenase (GDH) [43] thereby inhibiting the conversion of glutamate (and glutamine) to the TCA cycle intermediate, α ketoglutarate. SIRT4 provides perhaps the clearest example of a physiological response to CR that is mediated by a sirtuin. Under CR, SIRT4-mediated inhibition of GDH is alleviated thereby facilitating the use of glutamate and glutamine as a source of carbon and energy for gluconeogenesis in the liver. In addition, the down-regulation of SIRT4 by CR enables these amino acids to function as insulin secretagogues in the β cells of the pancreas. Because this regulation enables the animal to use amino acids as energy sources during the energy limitation imposed by CR, it makes sense that it also sensitizes their β-cells to respond to amino acids by secreting insulin.

SIRT6 and 7 might also have metabolic functions. SIRT6 has been linked to base excision repair and glucose homeostasis [44]. SIRT6 knockout mice show increased DNA damage, have low levels of IGF1, and have a catastrophic decline in blood glucose beginning two weeks after birth. SIRT7 is the only mammalian sirtuin localized to nucleoli and has been shown to be a positive regulator of ribosomal RNA (rRNA) synthesis, and therefore of ribosome biogenesis [45].

Both SIRT3 and SIRT4 are attractive candidates as potential therapeutics for metabolic disease, given their location in the mitochondria and their links to metabolism described above [46–49,24]. SIRT4 is particularly interesting because it presents a case in which a sirtuin inhibitor might have efficacy for individuals in a pre-diabetic state, in this case by stimulating insulin secretion in individuals with glucose intolerance. It remains to be seen whether the mitochondrial sirtuins SIRT3, SIRT4 and SIRT5 will also regulate electron transport and ATP synthesis directly. This function could enable these sirtuins to exert direct effects on mitochondrial activities related to aging – ROS production by the electron transport chain, and apoptosis (which can be driven by release of mitochondrial components to the cytoplasm). It would be interesting, for example, if these mitochondrial sirtuins held the potential to limit ROS production, (as was suggested for SIRT3 [46]) and to reduce apoptosis during CR.

Sirtuin drug discovery

At present, several inhibitors and activators of SIRT1 have been described [50–52]. Inhibitors such as nicotinamide (NAM), sirtinol and splitomycin have been useful in helping to dissect the function of SIRT1 in the laboratory. Sirtinol and splitomycin function as competitive inhibitors by blocking the active site, whereas high concentrations of NAM drive reversal of the first step in deacetylation – the cleavage of NAD to an ADP-ribosyl intermediate and NAM. However, the therapeutic use of these inhibitors is limited, first because they have low potency, and second because they are not specific. In addition, use of NAM is complicated by the fact that it can be converted to NAD *in vivo* and NAM can inhibit other classes of enzymes (e.g. poly-ADP ribosyl polymerases). Inhibitors of other sirtuins might prove useful in diabetes, for example (for reasons described above) a SIRT4 inhibitor might increase insulin secretion by β cells [43].

There are two potential conceptual modes for activating sirtuins: direct and allosteric (Figure 4). An example of direct activation involves the NAM analog, iso-nicotinamide (INAM), which binds directly to SIRT1 and works by competing with the inhibitor NAM for binding to the catalytic site [53]. INAM is thought to prevent the reversal of the first step of deacetylation by inhibiting binding of endogenous NAM to the enzyme intermediate.

In the allosteric model, a protein region flanking the catalytic domain of SIRT1 might bind to it and inhibit catalysis (Figure 4a). It is also possible that inhibition of the catalytic domain by the flanking domain would occur by intermolecular interactions during oligomerization, [54]. This inhibition could be alleviated by the binding of a small molecule to SIRT1 to interfere with the inhibitory protein–protein interaction. An example of allosteric

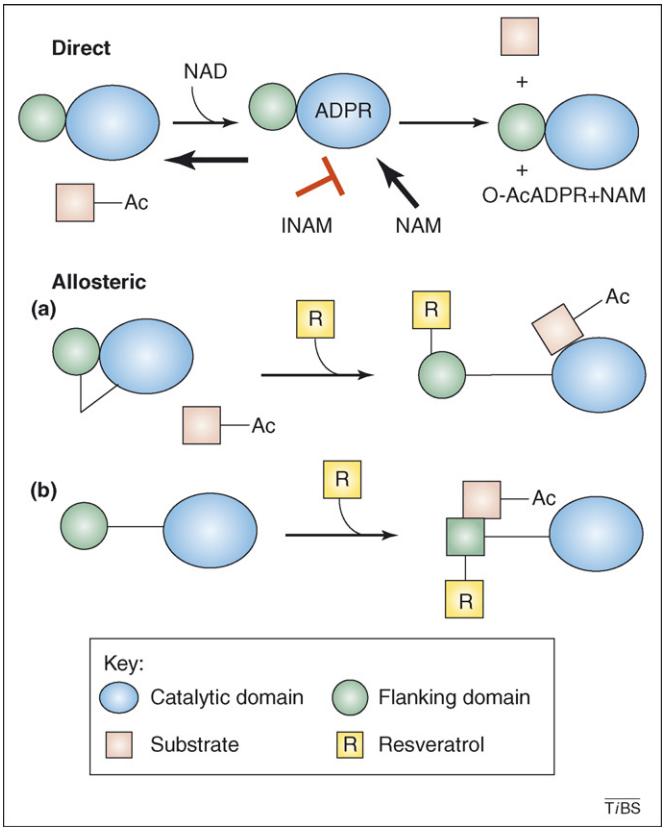

Figure 4. Mechanisms of activation of SIRT1: direct and allosteric. Direct activation: INAM binds directly to the catalytic domain (e.g. SIRT1) to competitively block inhibition by NAM at the catalytic site. ADPR indicates the reaction intermediate with ADP-ribose bound to the catalytic domain, O-AcADPR is the O-acetylated ADP-ribose product, Ac denotes an acetyl group. Allosteric activation: (**a**) A protein region flanking the catalytic domain of SIRT1 might bind to it and inhibit catalysis. (**b**) Flanking domain binds to protein substrate. Inhibition of the catalytic domain by the flanking domain would occur by intermolecular interactions during oligomerization. This inhibition could be alleviated by the binding of resveratrol, which could interfere with the inhibitory protein–protein interaction.

activation of SIRT1 might be provided by the action of resveratrol, other polyphenolic compounds and other small molecules such as quinoxalines [55]. Evidence for an allosteric mechanism for resveratrol awaits the demonstration that this compound directly binds to SIRT1 and can alter its conformation. Moreover, the generation and analysis of resveratrol-refractory SIRT1 mutants would provide important evidence for the direct action of this compound *in vivo*.

In living cells, it is likely that SIRT1 binds to its protein substrates via its long N- and C-terminal flanking domains. Therefore, one can imagine a class of activators that would function by binding to SIRT1 and increasing the affinity of the sirtuin for one or more substrates *in vivo* (Figure 4b). Again, it is possible that resveratrol works by this mechanism, and, moreover, it should be possible to screen for novel activators in cell-based assays that record the activity of a specific SIRT1 substrate (e.g. PGC-1α). Such an approach could have the benefit of yielding small molecules or drugs that specifically target one SIRT1-mediated pathway or another.

Conclusion

Sirtuins are anti-aging proteins that have therapeutic potential for a range of diseases of aging, including metabolic disorders, neurodegenerative disorders, cancer and cardiovascular disease. The link between sirtuin activation and mitochondrial biogenesis by the SIRT1-mediated activation of PGC-1α provides a novel mechanism of action for the treatment of diseases for which therapy is currently limited. We expect that the many other physiological pathways regulated by SIRT1, as well as the other six mammalian sirtuins, will offer further opportunities for novel therapeutic interventions.

References

1 Sauve, A.A. *et al.* (2006) The biochemistry of sirtuins. *Annu. Rev. Biochem.* 75, 435–465
2 Blander, G. and Guarente, L. (2004) The Sir2 family of protein deacetylases. *Annu. Rev. Biochem.* 73, 417–435
3 Kaeberlein, M. *et al.* (1999) The SIR2/3/4 complex and SIR2 alone promote longevity in Saccharomyces cerevisiae by two different mechanisms. *Genes Dev.* 13, 2570–2580
4 Tissenbaum, H.A. and Guarente, L. (2001) Increased dosage of a sir-2 gene extends lifespan in *Caenorhabditis elegans*. *Nature* 410, 227–230
5 Wood, J.G. *et al.* (2004) Sirtuin activators mimic caloric restriction and delay ageing in metazoans. *Nature* 430, 686–689
6 Imai, S. *et al.* (2000) Transcriptional silencing and longevity protein Sir2 is an NAD- dependent histone deacetylase. *Nature* 403, 795–800
7 Landry, J. *et al.* (2000) The silencing protein SIR2 and its homologs are NAD-dependent protein deacetylases. *Proc. Natl. Acad. Sci. U. S. A.* 97, 5807–5811
8 Weindruch, R., and Walford, R.L. (1988) *The Retardation of Aging and Disease by Dietary Restriction*, Charles C. Thomas
9 Guarente, L. (2000) Sir2 links chromatin silencing, metabolism, and aging. *Genes Dev.* 14, 1021–1026
10 Sinclair, D.A. and Guarente, L. (1997) Extrachromosomal rDNA circles–a cause of aging in yeast. *Cell* 91, 1033–1042
11 Berdichevsky, A. *et al.* (2006) *C. elegans* SIR-2.1 interacts with 14-3-3 proteins to activate DAF-16 and extend life span. *Cell* 125, 1165–1177
12 Vaziri, H. *et al.* (2001) hSIR2(SIRT1) Functions as an NAD-Dependent p53 Deacetylase. *Cell* 107, 149–159
13 Brunet, A. *et al.* (2004) Stress-Dependent Regulation of FOXO Transcription Factors by the SIRT1 Deacetylase. *Science* 303, 2011–2015
14 Motta, M.C. *et al.* (2004) Mammalian SIRT1 represses forkhead transcription factors. *Cell* 116, 551–563
15 Rodgers, J.T. *et al.* (2005) Nutrient control of glucose homeostasis through a complex of PGC-1alpha and SIRT1. *Nature* 434, 113–118
16 Yeung, F. *et al.* (2004) Modulation of NF-kappaB-dependent transcription and cell survival by the SIRT1 deacetylase. *EMBO J.* 23, 2369–2380
17 Cohen, H.Y. *et al.* (2004) Acetylation of the C Terminus of Ku70 by CBP and PCAF Controls Bax-Mediated Apoptosis. *Mol. Cell* 13, 627–638
18 Cohen, H.Y. *et al.* (2004) Calorie restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase. *Science* 305, 390–392
19 Civitarese, A.E. *et al.* (2007) Calorie Restriction Increases Muscle Mitochondrial Biogenesis in Healthy Humans. *PLoS Med.* 4, e76
20 Chen, D. *et al.* (2005) Increase in activity during calorie restriction requires Sirt1. *Science* 310, 1641
21 Frye, R.A. (1999) Characterization of five human cDNAs with homology to the yeast SIR2 gene: Sir2-like proteins (sirtuins) metabolize NAD and might have protein ADP-ribosyltransferase activity. *Biochem. Biophys. Res. Commun.* 260, 273–279
22 Denu, J.M. (2005) The Sir2 family of protein deacetylases. *Curr. Opin. Chem. Biol.* 9, 431–440
23 Sauve, A.A. and Schramm, V.L. (2004) SIR2: the biochemical mechanism of NAD(+)-dependent protein deacetylation and ADP-ribosyl enzyme intermediates. *Curr. Med. Chem.* 11, 807–826
24 Haigis, M.C. and Guarente, L.P. (2006) Mammalian sirtuins–emerging roles in physiology, aging, and calorie restriction. *Genes Dev.* 20, 2913–2921
25 Petersen, K.F. *et al.* (2003) Mitochondrial dysfunction in the elderly: possible role in insulin resistance. *Science* 300, 1140–1142
26 Petersen, K.F. *et al.* (2004) Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. *N. Engl. J. Med.* 350, 664–671
27 Harmon, D. (1956) Aging: a theory based on free radical and radiation chemistry. *J. Gerontol.* 11, 298
28 Wallace, D.C. (1999) Mitochondrial diseases in man and mouse. *Science* 283, 1482–1488
29 Heilbronn, L.K. *et al.* (2006) Effect of 6-month calorie restriction on biomarkers of longevity, metabolic adaptation, and oxidative stress in overweight individuals: a randomized controlled trial. *J. Am. Med. Assoc.* 295, 1539–1548
30 Howitz, K.T. *et al.* (2003) Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan. *Nature* 425, 191–196
31 Kaeberlein, M. *et al.* (2005) Substrate-specific activation of sirtuins by resveratrol. *J. Biol. Chem.* 280, 17038–17045
32 Baur, J.A. *et al.* (2006) Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* 444, 337–342
33 Lagouge, M. *et al.* (2006) Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. *Cell* 127, 1109–1122
34 Dasgupta, B. and Milbrandt, J. (2007) Resveratrol stimulates AMP kinase activity in neurons. *Proc. Natl. Acad. Sci. U. S. A.* 104, 7217–7222
35 Araki, T. *et al.* (2004) Increased nuclear NAD biosynthesis and SIRT1 activation prevent axonal degeneration. *Science* 305, 1010–1013
36 Wang, J. *et al.* (2005) A local mechanism mediates NAD-dependent protection of axon degeneration. *J. Cell Biol.* 170, 349–355
37 Qin, W. *et al.* (2006) Neuronal SIRT1 activation as a novel mechanism underlying the prevention of Alzheimer's disease amyloid neuropathology by calorie restriction. *J. Biol. Chem.* 281, 21745–21754
38 Tang, B.L. and Chua, C.E. (2007) SIRT1 and neuronal diseases. *Mol. Aspects Med.*, DOI: 10.1016/j.mam.2007.02.001
39 Mancuso, M. *et al.* (2006) Mitochondrial dysfunction, oxidative stress and neurodegeneration. *J. Alzheimers Dis.* 10, 59–73
40 Shi, T. *et al.* (2005) SIRT3, a mitochondrial sirtuin deacetylase, regulates mitochondrial function and thermogenesis in brown adipocytes. *J. Biol. Chem.* 280, 13560–13567
41 Schwer, B. *et al.* (2006) Reversible lysine acetylation controls the activity of the mitochondrial enzyme acetyl-CoA synthetase 2. *Proc. Natl. Acad. Sci. U. S. A.* 103, 10224–10229
42 Hallows, W.C. *et al.* (2006) Sirtuins deacetylate and activate mammalian acetyl-CoA synthetases. *Proc. Natl. Acad. Sci. U. S. A.* 103, 10230–10235

43 Haigis, M.C. *et al.* (2006) SIRT4 inhibits glutamate dehydrogenase and opposes the effects of calorie restriction in pancreatic beta cells. *Cell* 126, 941–954
44 Mostoslavsky, R. *et al.* (2006) Genomic instability and aging-like phenotype in the absence of mammalian SIRT6. *Cell* 124, 315–329
45 Ford, E. *et al.* (2006) Mammalian Sir2 homolog SIRT7 is an activator of RNA polymerase I transcription. *Genes Dev.* 20, 1075–1080
46 Scher, M.B. *et al.* (2007) SirT3 is a nuclear NAD+-dependent histone deacetylase that translocates to the mitochondria upon cellular stress. *Genes Dev.* 21, 920–928
47 Onyango, P. *et al.* (2002) SIRT3, a human SIR2 homologue, is an NAD-dependent deacetylase localized to mitochondria. *Proc. Natl. Acad. Sci. U. S. A.* 99, 13653–13658
48 Schwer, B. *et al.* (2002) The human silent information regulator (Sir)2 homologue hSIRT3 is a mitochondrial nicotinamide adenine dinucleotide-dependent deacetylase. *J. Cell Biol.* 158, 647–657
49 Michishita, E. *et al.* (2005) Evolutionarily conserved and nonconserved cellular localizations and functions of human SIRT proteins. *Mol. Biol. Cell* 16, 4623–4635
50 Bedalov, A. *et al.* (2001) Identification of a small molecule inhibitor of Sir2p. *Proc. Natl. Acad. Sci. U. S. A.* 98, 15113–15118
51 Grozinger, C.C.E. *et al.* (2001) Identification of a class of small molecule inhibitors of the sirtuin family of NAD-dependent deacetylases by phenotypic screening. *J. Biol. Chem.* 276, 38837–38843
52 Bitterman, K.J. *et al.* (2002) Inhibition of silencing and accelerated aging by nicotinamide, a putative negative regulator of yeast sir2 and human SIRT1. *J. Biol. Chem.* 277, 45099–45107
53 Sauve, A.A. *et al.* (2005) Chemical activation of Sir2-dependent silencing by relief of nicotinamide inhibition. *Mol. Cell* 17, 595–601
54 Zhao, K. *et al.* (2004) Structure and substrate binding properties of cobB, a Sir2 homolog protein deacetylase from *Escherichia coli*. *J. Mol. Biol.* 337, 731–741
55 Nayagam, V.M. *et al.* (2006) SIRT1 modulating compounds from high-throughput screening as anti-inflammatory and insulin-sensitizing agents. *J. Biomol. Screen.* 11, 959–967