Published in final edited form as:
Annu Rev Pharmacol Toxicol. 2014 ; 54: 363–380. doi:10.1146/annurev-pharmtox-010611-134657.

Small-Molecule Allosteric Activators of Sirtuins

David A. Sinclair[1,2] and **Leonard Guarente**[3]

David A. Sinclair: david_sinclair@hms.harvard.edu; Leonard Guarente: leng@mit.edu

[1]Glenn Laboratories for the Biological Mechanisms of Aging, Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115

[2]Department of Pharmacology, School of Medical Sciences, University of New South Wales, Sydney, Australia 2052

[3]Glenn Laboratory for the Science of Aging, Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Abstract

The mammalian sirtuins (SIRT1–7) are NAD^+-dependent lysine deacylases that play central roles in cell survival, inflammation, energy metabolism, and aging. Members of this family of enzymes are considered promising pharmaceutical targets for the treatment of age-related diseases including cancer, type 2 diabetes, inflammatory disorders, and Alzheimer's disease. SIRT1-activating compounds (STACs), which have been identified from a variety of chemical classes, provide health benefits in animal disease models. Recent data point to a common mechanism of allosteric activation by natural and synthetic STACs that involves the binding of STACs to a conserved N-terminal domain in SIRT1. Compared with polyphenols such as resveratrol, the synthetic STACs show greater potency, solubility, and target selectivity. Although considerable progress has been made regarding SIRT1 allosteric activation, key questions remain, including how the molecular contacts facilitate SIRT1 activation, whether other sirtuin family members will be amenable to activation, and whether STACs will ultimately prove safe and efficacious in humans.

Keywords

aging; sirtuin; NAD; allosteric activation; HDAC

INTRODUCTION

Most of the fundamental advances in medicine seen over the past 50 years would not have been possible without a fundamental understanding of enzyme inhibition by small

DISCLOSURE STATEMENT

L.G. consults for GlaxoSmithKline, Chronos, InsideTracker, Elysium Health, and Segterra. D.A.S. consults for GlaxoSmithKline, CohBar, Segterra, Horizon Science, and OvaScience and is an inventor on patents (granted and pending) on sirtuin activation licensed to GlaxoSmithKline, OvaScience, Millipore, and Bayer. D.A.S. is a board member of Horizon Science and Segterra and owns stock in all of the above companies except GlaxoSmithKline. D.A.S. is potentially cofounding a company named MetroBiotech that will develop NAD^+-boosting molecules for diseases. Provisional patents from D.A.S.'s lab will be licensed to MetroBiotech.

molecules. Examples of small-molecule enzyme inhibitors include (*a*) the statins, which inhibit the binding of HMG-CoA to its reductase enzyme, thereby lowering cholesterol synthesis, and (*b*) imatinib (Gleevec®), which binds to the ATP-binding site of ABL kinase and inhibits its activity, thereby mitigating chronic myelogenous leukemia. Other well-known examples of small-molecule enzyme inhibitors include aspirin, penicillin, antiretrovirals, and sildenafil (Viagra®).

Despite the fact that allosteric enzyme activation was first described more than 40 years ago, compared with enzyme inhibition, our understanding of enzyme activation by small molecules is still in its infancy. The dozen known examples of enzymes that can be activated by small molecules include glucokinase (GK), alpha amylase, phosphoinositide-dependent protein kinase 1 (PDK1), AMP-activated protein kinase (AMPK), protein phosphatase 1 (PP1), alcohol dehydrogenase 2 (ALDH2), and ribonuclease L (RNAseL) (1). The reason for the relative paucity of known activators is not clear. It may be that there are a limited number of enzymes that can be activated by small molecules or simply that the limited extent of our knowledge in this area has stifled innovation.

Either way, there are distinct advantages to pursuing activators over inhibitors. For one, they generally do not have to be as potent as inhibitors to induce cellular and physiological effects, in part because their activity is amplified by downstream signaling pathways. Inhibitors, in contrast, can be rendered ineffective by residual enzymatic activity (1). Another advantage is that activators typically bind to interacting proteins or to regulatory regions outside the conserved catalytic domains and can therefore have greater target specificity within an enzyme family. Lastly, activators—especially those that mimic a natural activation mechanism—may also elicit fewer side effects than do inhibitors.

Small-molecule activators of the sirtuin orthologs of Sir2, such as SIRT1, are the subject of this review. These compounds, which were described approximately 10 years ago, have been the subject of intense focus as well as much debate. What is without question is that the number of papers published on these activators and sirtuins has spiked in the past 10–12 years, now approaching a total of ten thousand. In this review, we trace the history of sirtuin activators, starting with the discovery of the polyphenolic activators (2). These are natural products such as resveratrol, piceatannol, and butein. Subsequently, multiple groups (3–5) developed synthetic molecules, which are more potent than polyphenols and certain of which are currently in human clinical trials (6, 7). We consider the biochemical analyses of these various compounds in vitro as well as their antidiabetic, anti-inflammatory, and other salutary effects in experimental animals. Then, we discuss the various controversies that have emerged over the years about the mode of action of sirtuin activators. Finally, we give an update on the most recent findings, which begin to resolve these controversies and suggest a clear mechanism of activation.

SIRTUINS: BACKGROUND

The yeast-silencing gene *SIR2* was shown in genetic studies in the 1990s to be important in regulating replicative life span in budding yeast (8). An additional copy of *SIR2* extended the life span by silencing the rDNA to slow formation of toxic rDNA circles and by

damping rRNA expression (9, 10). A recent genetic study probing the entire yeast genome by quantitative trait locus (QTL) analysis provided confirmation for the earlier work; *SIR2* was the top QTL regulating replicative life span and accounted for most of the differences in life span between highly divergent yeast strains (11). Likewise, homologs of yeast *SIR2* in *Drosophila*, *Caenorhabditis elegans*, and mice also can extend life span in their respective organisms when their expression levels are increased (12–15). The hypothesis that sirtuins control aging is further buttressed by in vivo effects of SIRT1-activating compounds or STACs, as discussed below.

There are seven mammalian homologs of the yeast SIR2: SIRT1, SIRT6, and SIRT7, which are primarily nuclear; SIRT3, SIRT4, and SIRT5, which are mitochondrial; and SIRT2, which is cytosolic (16, 17). Sirtuins such as yeast SIR2 and mammalian homologs SIRT1, SIRT2, and SIRT3 are NAD^+-dependent deacetylases (18). SIRT5 possesses desuccinylase and demalony-lase activities (16, 19), whereas SIRT6 can remove long-chain fatty acids from selected substrate proteins (20). In the deacylation reactions, NAD is cleaved into nicotinamide and *O*-acetyl-ADP-ribose (OAcADPR) (21, 22). The reaction proceeds in two steps. First, NAD is cleaved and ADP-ribose is covalently attached to the acetyl moiety of the substrate (23). Second, hydrolysis of the acetyl-lysine bond liberates OAcADPR, which itself appears to have biological activity (21). Nicotinamide serves as a product inhibitor; thus, high nicotinamide levels in cells can inhibit sirtuins' activity (24).

It was proposed that these NAD^+-dependent activities allow sirtuins to monitor diet and metabolism and regulate the aging process, for example by slowing aging when animals are fed a calorie restriction (CR) diet (24–26). Indeed, many studies now show that sirtuins are a nexus among diet, metabolism, and physiology and can provide many health benefits (13, 27–30). It was initially shown that yeast SIR2 was required for the extended replicative life span when the glucose concentration was reduced from 2% to 0.5% (25). In mammals, there are many examples in which sirtuins are required for physiological adaptations to dietary interventions, including fasting, CR, and a high-fat diet (HFD) (31–37).

Most tellingly, SIRT1 and SIRT3 are induced by CR in many tissues, and their genetic ablation prevents many of the health benefits of the CR diet, including longevity (26, 38–43). Furthermore, the overexpression of SIRT1 is protective in many murine disease models (27, 44–52), and the overexpression of SIRT6 extends mouse life span (53). Among the key functions of sirtuins with regard to diet is inducing the mitochondrial expansion and oxidative metabolism during CR (40, 54, 55), as well as mitigating the effects of reactive oxygen species (ROS) and inflammation during the HFD (48, 56–59).

Over the past 10 years, the abundance and biological importance of acetylation has been found to rival that of phosphorylation. A comparison of mouse embryonic fibroblasts from wild-type and SIRT1 knockout mice identified ~4,623 lysine acetylation sites in 1,800 proteins (60), of which ~10% were significantly increased in SIRT1 knockout cells (60). However, not all of these sites are necessarily direct SIRT1 targets given that acetyltransferases such as Kat5 (Tip60), Kat8 (Myst1), and p300 are also targeted by SIRT1 (60). Among known direct substrates deacetylated and regulated by SIRT1 are PGC-1α (mitochondrial biogenesis and oxidative metabolism); p53 (cell cycle and apoptosis);

FOXOs (metabolism and stress tolerance); SREBP (lipid metabolism); NF-κB (inflammation); and NBS, Ku70, and WRN (DNA repair) (61). In the case of SIRT3, many mitochondrial metabolic enzymes are targeted for deacylation, causing upregulation of β-oxidation, the tricarboxylic acid cycle, and the urea cycle, as well as detoxification of ROS (62). Analysis of SIRT1-mediated changes in the acetylome indicate that, under basal conditions, SIRT1 has a preference for certain substrate sequences, such as a glycine N-terminal to the acetyl-lysine bond or a hydrophobic amino acid on the C-terminal side (60).

In addition, sirtuins can regulate gene expression by directly deacetylating histones H3 and H4 as well as H1 (63). The yeast Sir2 enzyme silences its loci by this mechanism, but this is a dynamic process, and Sir2 can be induced to move from silenced chromatin to sites of DNA breaks (64, 65). Mammalian SIRT1 and SIRT6 can also deacetylate histones (63). As in yeast, DNA breaks recruit SIRT1 from its normal locations (51). Interestingly, there is also evidence that SIRT1 relocates during normal aging, thereby altering global gene expression and potentially driving the aging phenotype itself (51).

SIRTUIN ACTIVATORS: EFFECTS IN VITRO AND IN LOWER ORGANISMS

The identification of the enzymatic activity of yeast Sir2 and mammalian SIRT1 potentiated high-throughput screening for SIRT1-activating compounds or STACs. Indeed, the initial screen of 18,000 compounds identified 21 compounds that activated SIRT1 in vitro by lowering its K_m for the peptide substrate (2). Most of these were in the polyphenol family of natural products, such as resveratrol. A different activating compound, isonicotinamide, increased the V_{max} by competing with nicotinamide and lowering product inhibition (66).

The polyphenol activators are structurally similar in that each has a planar multiphenyl ring structure and several hydroxyls (Figure 1). The most potent activator, resveratrol, increased SIRT1 activity approximately tenfold (2). This compound is found in red wine and had already been associated with health benefits in humans and rodent models (67, 68). Its proposed mechanism of action was that of an antioxidant.

Importantly, administration of resveratrol and other polyphenols in vivo extended life span in yeast (2, 69–71), *C. elegans* (72–74), *Drosophila* (74–77), fish (78), and bees (79), as did gain of function in the SIRT1 gene (although controversies emerged, as discussed below). In yeast, worms, and *Drosophila*, the life-extending effects of the compound depended on the *SIR2* gene.

More intensive high-throughput screening identified novel synthetic compounds as SIRT1 activators, which can be much more potent than resveratrol (80). Like resveratrol, these compounds work by lowering the K_m for the substrate peptide. Importantly, these latter STACs comprised numerous scaffolds, none of which were similar to the polyphenols (Figure 1). These scaffolds include benzimidazoles, thiazolopyridines, and urea-based scaffolds (80–83). Medicinal chemistry has generated thousands of analogs of these scaffolds, which can be up to three orders of magnitude more potent than resveratrol (84). It has been a conundrum that such a diverse set of small molecules can activate SIRT1 but not SIRT2–7, but we believe that this issue has recently been resolved, as described below.

EFFECTS OF SIRTUIN ACTIVATORS IN ANIMALS AND HUMANS

Resveratrol and the newer STACs confer remarkable health benefits in experimental animals. A striking demonstration of the effects of resveratrol was the prevention of the deleterious effects of a HFD in mice, e.g., fatty liver, insulin resistance, and inflammation (80, 85–87). Moreover, HFD mice fed resveratrol or the STAC SRT1720 lived significantly longer than controls (88). The newer STACs showed similar protection against HFD, reduced mortality, and were also protective in genetic models for diabetes in mice and rats (3, 80, 85).

The effects of STACs were associated with an increase in mitochondrial mass in muscle and liver (3, 54, 85, 88, 89). Consistent with the increase in mitochondria, dosed mice showed a large increase in exercise endurance and muscle fiber type shift to red or oxidative fibers (3, 54). These effects of STACs were at least partly associated with SIRT1 activation. Indeed, STACs triggered deacetylation of PGC-1α, and these effects were SIRT1 dependent in cells (3, 85). Resveratrol did not extend the life span of mice fed normal chow ad libitum (89). However, resveratrol did induce many health benefits in these mice, including protection against osteoporosis, cardiovascular disease, cataracts, and metabolic decline. It will be interesting to determine whether the more potent STACs can extend life span, even in mice on a chow diet.

Many of the effects of STACs on mice overlap those triggered by overexpression of SIRT1 or by CR. For example, both SIRT1 overexpression and STACs increase mitochondrial function in skeletal muscle (3, 35, 85, 88, 90). With respect to CR, transcriptional profiling indicates an extensive overlap in the changes induced by STACs and CR (85, 88, 91–93). These include changes to genes involved in mitochondrial function, inflammation, and apoptosis.

An indirect way to assess if STACs work via SIRT1 is to test if their actions are diminished in the absence of the enzyme. There are numerous reports in which STACs have been shown to require SIRT1, mostly using cell culture. These have been extensively reviewed elsewhere (94). In mice, far fewer studies have tested the dependency of STACs on SIRT1, in large part because germline SIRT1 knockout mice often die in utero or are born with developmental abnormalities. In cases in which knockout mice have been tested, using outbred strains or inducible knockouts, the effects of STACs have been partially or completely SIRT1 dependent. Resveratrol's ability to prevent skin tumors, for example, is blunted in the germline knockout (95), whereas its ability to promote mitochondrial function in skeletal muscle is blocked in an inducible SIRT1 knockout mouse (90). Similarly, the ability of SRT1720 to induce mitochondrial function in the liver was abolished when SIRT1 was deleted in adult mice (85), consistent with previous cell culture experiments in cells from the knockout mice (3).

With regard to humans, epidemiological data have long suggested that resveratrol may provide cardiovascular benefits, for example as a possible explanation for the "French paradox." At least three human studies have shown protective effects of resveratrol (96–101). In one double-blind, placebo-controlled study, a 6-month regimen of 100 mg/day

administered to an obese cohort provided significant albeit modest benefits, including reduced systolic blood pressure (101). In a second study, 4 weeks of 1–2 g/day resveratrol improved insulin sensitivity and lowered postpran-dial blood glucose (100). However, a study of 12 weeks of 75 mg/day administered to nonobese postmenopausal women (102) and another study in healthy obese men (103) failed to show an effect of resveratrol. These inconsistencies may derive from the known limitations of resveratrol in bioavailability, pharmacokinetics, and target specificity.

CONTROVERSIES AND COMPLEXITIES ABOUT MECHANISM OF ACTION

Questions about the claims of resveratrol as a SIRT1 activator were soon raised by two groups (104, 105). They found that activation depended on the fluorescent moiety (aminomethylcoumarin, or AMC) attached to the peptide substrate in the assay. The first study proposed that binding of resveratrol to SIRT1 promotes a conformational change that better accommodates the attached coumarin group, which may mimic an endogenous structure, and the second study surmised that sirtuin activation is an artifact. Assays for newer STACs employed a different fluorescent moiety (tetramethylrhodamine, or TAMRA) attached to the peptide substrate. It was later suggested that resveratrol and the synthetic STACs activate by simply binding to the fluorophore (106). By this reckoning, not only was activation physiologically irrelevant, but the activity of the compounds had nothing to do with SIRT1 in vivo.

A second series of studies indicated that resveratrol directly targets proteins other than SIRT1 in vivo but nonetheless upregulates SIRT1 activity to elicit physiological effects. Several papers showed that resveratrol can activate AMPK in vivo (88, 107, 108), leading some researchers to speculate that resveratrol indirectly activates SIRT1 via AMPK. Indeed, AMPK can activate the gene encoding the NAD synthetic enzyme NAMPT (nicotinamide phosphoribosyltransferase) (109), and obese mice lacking the alpha catalytic subunit of AMPK are resistant to the metabolic benefits of resveratrol (4).

A mouse in which SIRT1 can be conditionally knocked out in adults was developed to help disentangle the effects of resveratrol on SIRT1 and AMPK (90). The use of this mouse showed that the mechanism by which resveratrol activates AMPK in mouse skeletal muscle is highly dose dependent. At a low dose of 24 mg/kg/day, the ability of resveratrol to activate AMPK was blocked by acute SIRT1 knockout. At this low dose, resveratrol is thought to target SIRT1, which deacetylates the AMPK kinase LKB1 and triggers activation of AMPK (90, 110). SIRT1 and AMPK then act together to enhance the activity of PGC-1α, a target of both proteins and a key regulator of mitochondrial function (111, 112).

At a dose that was 10 times higher, activation of AMPK occurred in the SIRT1 knockout mouse, but the effects of resveratrol on mitochondrial function were not observed at either dose (90) AMPK activation by resveratrol in these mice may be due to inhibition by resveratrol of Complex I in the electron transport chain and a decline in ATP and the NAD^+:NADH ratio (90, 113). Indeed, high concentrations of resveratrol applied to muscle cells (>25 μM) decrease ATP levels dramatically, compared with lower concentrations that increase ATP (90).

In a different study, Park et al. (114) suggested that resveratrol imparts metabolic benefits by inhibiting phosphodiesterases (PDEs). In this model, PDE inhibition leads to activation of protein kinase A (PKA), then AMPK and NAMPT, increasing NAD^+ levels that activate SIRT1. Consistent with this, a known PDE4 inhibitor, rolipram, produced metabolic effects in mice similar to those produced by resveratrol and the synthetic STACs. In the conditional SIRT1 knockout mouse, however, resveratrol did not increase NAD^+ levels in vivo (90). In addition, Park et al. (114) suggested that the newer synthetic STACs may also activate SIRT1 by directly inhibiting PDEs, although this idea is not consistent with observations that several STACs do not inhibit PDE, even at concentrations 10 times greater than those used to elicit a mitochondrial response (81).

In summary, the weight of evidence has indicated that the effects of resveratrol and other STACs require SIRT1 in vivo, excluding the possibility that the effects of STACs are divorced from SIRT1. But do the activators target SIRT1 directly? The activation of purified enzyme by STACs in vitro is consistent with direct activation, but questions about the fluorescent moieties attached to the peptide substrates remain.

TOWARD A RESOLUTION AND A UNIFIED ACTIVATION MECHANISM TARGETING SIRT1

Another way to view the requirement for the aromatic, fluorescent moieties is that they actually provide important clues about substrate specificity in the activation of SIRT1 by STACs. For example, perhaps the fluorophores reflect a requirement for amino acids near the deacetylation site with aromatic side chains. Indeed, several recent findings have revealed structural and positional requirements: Amino acids must be adjacent to the acetylated lysine for substrate recognition (60) and for activation to occur (81, 82, 115, 116). First, repositioning the AMC group in the substrate peptide from its canonical site immediately C-terminal to the acetylated lysine (+1) to +3, +6, +9, or +12 completely abrogated activation by STACs (81). This finding rules out a drug-fluorophore interaction as the basis for activation. Second, natural peptide substrates with tryptophan or phenylalanine (but not alanine) at +1 instead of AMC were activatable. This finding clearly shows that activation does not require a fluorophore in the substrate, and it was extended by the demonstration that natural substrate sequences, such as PGC-1α (with a tyrosine at +1), were also activatable (81).

In a parallel study, more than 6,000 acetylated nontagged 13-mer peptide substrates were screened for their ability to support SIRT1 activation by resveratrol (116). As seen in the results for the synthetic STACs, there was a preference for large, hydrophobic residues in the C terminus, whereas positively charged residues antagonized activation. Substrates for which resveratrol activated included FOXO4 and the acetyltransferase p300. The sequence-dependent effects explain why STACs show SIRT1-dependent effects in numerous organisms that overlap with, but are not identical to, the effects of SIRT1 overexpression and why STACs failed to stimulate SIRT1 against some substrates.

Interestingly, the assay used to screen for synthetic STACs used a peptide with a TAMRA fluorophore not at +1 but at +6. Why was this substrate activatable? The answer comes from

inspecting the proteome for sequences with aromatic residues at +1 and +6 with respect to lysines; this inspection revealed a high degree of overlap with known SIRT1 substrates, such as FOXO3a and eIF-2α (81). Indeed, the PGC-1α sequence also has hydrophobic amino acids at +1 and +6, and activation is abolished only by substituting these residues with alanine, but not at other positions.

The positional and structural requirements for substrates suggested that there might be sequences within SIRT1 required for activation but not for normal catalytic activity. A genetic screen was thus performed for single amino acid changes in SIRT1 that abolished activation by resveratrol in vitro. Mutating glutamate 230 (E230) to E230K completely prevented activation but did not alter the K_m for the substrate or V_{max} in the absence of activators (81) (Figure 2). Furthermore, this mutation does not alter the physical properties of SIRT1 in exhaustive analyses.

The defect in activation of the SIRT1-E230K mutant is evident by several independent assays (Figure 3). Mutating E230 to alanine exerted similar effects, suggesting that the positive charge of the lysine in the E230K mutant is not important. Amazingly, although the E230K mutant was identified as refractory to resveratrol, activation is blocked or blunted in response to any of the 117 STACs tested, which span three different scaffolds (81) (Figure 4). This finding strongly suggests that all of the STACs identified to date activate SIRT1 by a common mechanism.

But is this mechanism of activation by STACs in vitro relevant to activation in vivo? To address this, endogenous SIRT1 was replaced with wild-type or E230K SIRT1 expressed at comparable levels in primary myocytes and mouse embryo fibroblasts (81). In both cell types, resveratrol and two newer STACs induced increases in mitochondrial mass and gene expression, as well as in ATP, in cells with wild-type but not SIRT1-E230K. It is difficult to explain the properties of the E230K mutant in any model other than a model involving direct activation of SIRT1 by STACs.

MODELS FOR DIRECT ACTIVATION OF SIRT1 BY STACS

All sirtuins have a highly conserved domain for NAD binding and catalysis. Of the seven mammalian sirtuins, SIRT1 has by far the largest amino- and carboxyl-terminal sequences that flank the conserved domain. Importantly, E230 lies outside but immediately N-terminal to the conserved domain. This residue is highly conserved in Sir2 orthologs that range from human to *Drosophila* (Figure 2).

Although there is currently no SIRT1 structure available, it seems likely that the region amino-terminal to the conserved domain containing E230 is the site for activation by STACs. Consistent with this idea, deuterium-exchange measurements reveal that the highest degree of folding across SIRT1 is in the conserved domain and in residues 188–245, the proposed activation site (80, 81). The remainder of the amino terminus (residues 17–187) was found to be unstructured by this method. Further consistent with this idea, deletions through this region in the amino terminus of SIRT1 abolish both binding and activation by STACs (80, 81).

How might the binding of STACs to the amino-terminal activation domain of SIRT1 increase NAD^+-dependent deacetylation of substrates? Important clues to the activation mechanisms of other enzymes have come from studying endogenous activators. Examples include AMPK, PKA, and RNAseL activation (1). Endogenous proteins that activate sirtuins have been discovered in diverse organisms. *Saccharomyces cerevisiae* Sir2 deacetylase, the original sirtuin, is allosterically activated two- to fivefold by its binding partner Sir4 (117), and although there are no known mammalian Sir4 homologs, the activation mechanism is strikingly similar to that of the STACs.

A recently solved Sir2-Sir4 crystal structure shows that Sir2 consists of two independently folded domains: a catalytic domain (Sir2C, amino acids 237–555) and a helical N-terminal domain (Sir2N, amino acids 101–236), arranged in a horseshoe shape (118). Molecular dynamics simulation indicates that the substrate-binding channel of Sir2 can toggle between an open and closed conformation and that Sir4 binding maintains the N-terminal helix in a productive conformation, highly reminiscent of the model for STAC-mediated activation (81). In fact, SIRT1-E230 aligns with a residue in Sir2, D223, that lies within an α-helix that interacts with SIR4 and abolishes SIR4-mediated gene silencing when substituted with a glycine (119).

In mammals, two protein activators of SIRT1 have been described, namely AROS (active regulator of SIRT1) and lamin A (120, 121). Paralleling STACs, both proteins activate by binding in the N terminus of SIRT1. In the case of lamin A, interaction with the SIRT1 N terminus can potentiate further activation by resveratrol (121). It will be interesting to test if the activation mechanism by these proteins is analogous to that of STACs, lowering the K_m for the substrate and requiring the region around E230.

Another clue about the mechanism of allosteric activation by STACs is provided by the finding that STACs increase the binding affinity for the substrate and vice versa. This model of assisted allosteric activation, first proposed by Sauve (122), is similar to the mechanism of GK activation whereby the substrate (glucose) and small-molecule activator act cooperatively to stabilize the active form (1). It is feasible that a concerted allosteric change in SIRT1 occurs between the catalytic domain and the activation domain during binding. This conformational change could, for example, create an interaction between E230 and a positively charged residue in the catalytic domain as the basis of activation.

Finally, we suggest that the extended amino- and carboxyl-terminal domains of SIRT1 evolved to allow regulation of the enzyme by small molecules or by other proteins in cells. As mentioned above, the amino-terminal domain likely serves a regulatory function. In addition, deletion of a 20–amino acid sequence in the carboxyl-terminal domain termed ESA was shown to completely abolish catalytic activity (123, 124). These findings can be rationalized by the idea that endogenous small molecules in cells, perhaps metabolic intermediates, normally bind to both flanking SIRT1 domains to regulate the activity of the central metabolic enzyme. In this regard, the STACs may be viewed as mimics of the endogenous small-molecule activators.

In addition to elucidating how STAC activation occurs at the molecular level, a major challenge in the field is to determine if STACs are efficacious in humans. In this regard, diseases abetted by aging are candidates for clinical studies. Multiple trials are in progress, including those investigating metabolic disorders and proinflammatory diseases (6, 7). Notably, sirtuins SIRT2–7 also mediate physiological effects linked to health benefits (125). For example, SIRT6 was recently shown to be an important tumor suppressor (126). Although it may be a challenge to develop activators for other sirtuins lacking the N-terminal activation domain of SIRT1, the benefits of finding such molecules may be considerable.

In summary, central controversies about STACs have been largely resolved. The most recent studies indicate that STACs, including resveratrol, can directly activate SIRT1 in vivo and in vitro. It is thus likely that many of the physiological and health-related effects of these molecules are due to direct SIRT1 activation.

Acknowledgments

We wish to thank the Glenn Foundation for medical research (D.A.S. and L.G.), the Schulak family (D.A.S.), the Juvenile Diabetes Research Foundation (D.A.S.), the United Mitochonrial Disease Foundation (D.A.S.), and the NIH's National Institute on Aging (D.A.S. and L.G.).

LITERATURE CITED

1. Zorn JA, Wells JA. Turning enzymes ON with small molecules. Nat Chem Biol. 2010; 6:179–88. [PubMed: 20154666]
2. Howitz KT, Bitterman KJ, Cohen HY, Lamming DW, Lavu S, et al. Small molecule activators of sirtuins extend *Saccharomyces cerevisiae* lifespan. Nature. 2003; 425:191–96. [PubMed: 12939617]
3. Feige JN, Lagouge M, Canto C, Strehle A, Houten SM, et al. Specific SIRT1 activation mimics low energy levels and protects against diet-induced metabolic disorders by enhancing fat oxidation. Cell Metab. 2008; 8:347–58. [PubMed: 19046567]
4. Nayagam VM, Wang X, Tan YC, Poulsen A, Goh KC, et al. SIRT1 modulating compounds from high-throughput screening as anti-inflammatory and insulin-sensitizing agents. J Biomol Screen. 2006; 11:959–67. [PubMed: 17099246]
5. Wu J, Zhang D, Chen L, Li J, Wang J, et al. Discovery and mechanism study of SIRT1 activators that promote the deacetylation of fluorophore-labeled substrate. J Med Chem. 2013; 56:761–80. [PubMed: 23316803]
6. Hoffmann E, Wald J, Lavu S, Roberts J, Beaumont C, et al. Pharmacokinetics and tolerability of SRT2104, a first-in-class small molecule activator of SIRT1, after single and repeated oral administration in man. Br J Clin Pharmacol. 2013; 75:186–96. [PubMed: 22616762]
7. Libri V, Brown AP, Gambarota G, Haddad J, Shields GS, et al. A pilot randomized, placebo controlled, double blind phase I trial of the novel SIRT1 activator SRT2104 in elderly volunteers. PLoS ONE. 2012; 7:e51395. [PubMed: 23284689]
8. Kaeberlein M, McVey M, Guarente L. The SIR2/3/4 complex and SIR2 alone promote longevity in *Saccharomyces cerevisiae* by two different mechanisms. Genes Dev. 1999; 13:2570–80. [PubMed: 10521401]
9. Sinclair DA, Guarente L. Extrachromosomal rDNA circles—a cause of aging in yeast. Cell. 1997; 91:1033–42. [PubMed: 9428525]
10. Unal E, Kinde B, Amon A. Gametogenesis eliminates age-induced cellular damage and resets life span in yeast. Science. 2011; 332:1554–57. [PubMed: 21700873]
11. Stumpferl SW, Brand SE, Jiang JC, Korona B, Tiwari A, et al. Natural genetic variation in yeast longevity. Genome Res. 2012; 22:1963–73. [PubMed: 22955140]

12. Tissenbaum HA, Guarente L. Increased dosage of a *sir-2* gene extends lifespan in *Caenorhabditis elegans*. Nature. 2001; 410:227–30. [PubMed: 11242085]
13. Rogina B, Helfand SL. Sir2 mediates longevity in the fly through a pathway related to calorie restriction. Proc Natl Acad Sci USA. 2004; 101:15998–6003. [PubMed: 15520384]
14. Viswanathan M, Guarente L. Regulation of *Caenorhabditis elegans* lifespan by *sir-2.1* transgenes. Nature. 2011; 477:E1–2. [PubMed: 21938026]
15. Banerjee KK, Ayyub C, Ali SZ, Mandot V, Prasad NG, Kolthur-Seetharam U. dSir2 in the adult fat body, but not in muscles, regulates life span in a diet-dependent manner. Cell Rep. 2012; 2:1485–91. [PubMed: 23246004]
16. He W, Newman JC, Wang MZ, Ho L, Verdin E. Mitochondrial sirtuins: regulators of protein acylation and metabolism. Trends Endocrinol Metab. 2012; 23:467–76. [PubMed: 22902903]
17. Houtkooper RH, Pirinen E, Auwerx J. Sirtuins as regulators of metabolism and healthspan. Nat Rev Mol Cell Biol. 2012; 13:225–38. [PubMed: 22395773]
18. Imai S, Armstrong CM, Kaeberlein M, Guarente L. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. Nature. 2000; 403:795–800. [PubMed: 10693811]
19. Peng C, Lu Z, Xie Z, Cheng Z, Chen Y, et al. The first identification of lysine malonylation substrates and its regulatory enzyme. Mol Cell Proteomics. 2011; 10 M111 012658.
20. Jiang H, Khan S, Wang Y, Charron G, He B, et al. SIRT6 regulates TNF-α secretion through hydrolysis of long-chain fatty acyl lysine. Nature. 2013; 496:110–13. [PubMed: 23552949]
21. Borra MT, O'Neill FJ, Jackson MD, Marshall B, Verdin E, et al. Conserved enzymatic production and biological effect of *O*-acetyl-ADP-ribose by silent information regulator 2-like NAD^+-dependent deacetylases. J Biol Chem. 2002; 277:12632–41. [PubMed: 11812793]
22. Tanner KG, Landry J, Sternglanz R, Denu JM. Silent information regulator 2 family of NAD-dependent histone/protein deacetylases generates a unique product, 1-*O*-acetyl-ADP-ribose. Proc Natl Acad Sci USA. 2000; 97:14178–82. [PubMed: 11106374]
23. Sauve AA, Wolberger C, Schramm VL, Boeke JD. The biochemistry of sirtuins. Annu Rev Biochem. 2006; 75:435–65. [PubMed: 16756498]
24. Anderson RM, Bitterman KJ, Wood JG, Medvedik O, Sinclair DA. Nicotinamide and *PNC1* govern lifespan extension by calorie restriction in *Saccharomyces cerevisiae*. Nature. 2003; 423:181–85. [PubMed: 12736687]
25. Lin SJ, Defossez PA, Guarente L. Requirement of NAD and *SIR2* for life-span extension by calorie restriction in *Saccharomyces cerevisiae*. Science. 2000; 289:2126–28. [PubMed: 11000115]
26. Cohen HY, Miller C, Bitterman KJ, Wall NR, Hekking B, et al. Calorie restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase. Science. 2004; 305:390–92. [PubMed: 15205477]
27. Bordone L, Cohen D, Robinson A, Motta MC, van Veen E, et al. SIRT1 transgenic mice show phenotypes resembling calorie restriction. Aging Cell. 2007; 6:759–67. [PubMed: 17877786]
28. Guarente L, Picard F. Calorie restriction—the *SIR2* connection. Cell. 2005; 120:473–82. [PubMed: 15734680]
29. Guarente L. Mitochondria—a nexus for aging, calorie restriction, and sirtuins? Cell. 2008; 132:171–76. [PubMed: 18243090]
30. Sinclair D, Verdin E. The longevity of sirtuins. Cell Rep. 2012; 2:1473–74. [PubMed: 23273894]
31. Xu F, Gao Z, Zhang J, Rivera CA, Yin J, et al. Lack of SIRT1 (mammalian sirtuin 1) activity leads to liver steatosis in the $SIRT1^{+/-}$ mice: a role of lipid mobilization and inflammation. Endocrinology. 2010; 151:2504–14. [PubMed: 20339025]
32. Gillum MP, Erion DM, Shulman GI. Sirtuin-1 regulation of mammalian metabolism. Trends Mol Med. 2011; 17:8–13.
33. Rodgers JT, Puigserver P. Fasting-dependent glucose and lipid metabolic response through hepatic sirtuin 1. Proc Natl Acad Sci USA. 2007; 104:12861–66. [PubMed: 17646659]
34. Jing E, Emanuelli B, Hirschey MD, Boucher J, Lee KY, et al. Sirtuin-3 (Sirt3) regulates skeletal muscle metabolism and insulin signaling via altered mitochondrial oxidation and reactive oxygen species production. Proc Natl Acad Sci USA. 2011; 108:14608–13. [PubMed: 21873205]

35. Verdin E, Hirschey MD, Finley LW, Haigis MC. Sirtuin regulation of mitochondria: energy production, apoptosis, and signaling. Trends Biochem Sci. 2010; 35:669–75. [PubMed: 20863707]
36. Hirschey MD, Shimazu T, Goetzman E, Jing E, Schwer B, et al. SIRT3 regulates mitochondrial fatty-acid oxidation by reversible enzyme deacetylation. Nature. 2010; 464:121–25. [PubMed: 20203611]
37. Picard F, Kurtev M, Chung N, Topark-Ngarm A, Senawong T, et al. Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR-γ. Nature. 2004; 429:771–76. [PubMed: 15175761]
38. Hebert AS, Dittenhafer-Reed KE, Yu W, Bailey DJ, Selen ES, et al. Calorie restriction and SIRT3 trigger global reprogramming of the mitochondrial protein acetylome. Mol Cell. 2013; 49:186–99. [PubMed: 23201123]
39. Qiu X, Brown K, Hirschey MD, Verdin E, Chen D. Calorie restriction reduces oxidative stress by SIRT3-mediated SOD2 activation. Cell Metab. 2010; 12:662–67. [PubMed: 21109198]
40. Someya S, Yu W, Hallows WC, Xu J, Vann JM, et al. Sirt3 mediates reduction of oxidative damage and prevention of age-related hearing loss under caloric restriction. Cell. 2010; 143:802–12. [PubMed: 21094524]
41. Chen D, Steele AD, Lindquist S, Guarente L. Increase in activity during calorie restriction requires Sirt1. Science. 2005; 310:1641. [PubMed: 16339438]
42. Boily G, Seifert EL, Bevilacqua L, He XH, Sabourin G, et al. SirT1 regulates energy metabolism and response to caloric restriction in mice. PLoS ONE. 2008; 3:e1759. [PubMed: 18335035]
43. Kume S, Uzu T, Horiike K, Chin-Kanasaki M, Isshiki K, et al. Calorie restriction enhances cell adaptation to hypoxia through Sirt1-dependent mitochondrial autophagy in mouse aged kidney. J Clin Investig. 2010; 120:1043–55. [PubMed: 20335657]
44. Ramadori G, Fujikawa T, Anderson J, Berglund ED, Frazao R, et al. SIRT1 deacetylase in SF1 neurons protects against metabolic imbalance. Cell Metab. 2011; 14:301–12. [PubMed: 21907137]
45. Satoh A, Brace CS, Ben-Josef G, West T, Wozniak DF, et al. SIRT1 promotes the central adaptive response to diet restriction through activation of the dorsomedial and lateral nuclei of the hypothalamus. J Neurosci. 2010; 30:10220–32. [PubMed: 20668205]
46. Donmez G, Wang D, Cohen DE, Guarente L. SIRT1 suppresses β-amyloid production by activating the α-secretase gene ADAM10. Cell. 2010; 142:320–32. [PubMed: 20655472]
47. Banks AS, Kon N, Knight C, Matsumoto M, Gutierrez-Juarez R, et al. SirT1 gain of function increases energy efficiency and prevents diabetes in mice. Cell Metab. 2008; 8:333–41. [PubMed: 18840364]
48. Pfluger PT, Herranz D, Velasco-Miguel S, Serrano M, Tschop MH. Sirt1 protects against high-fat diet-induced metabolic damage. Proc Natl Acad Sci USA. 2008; 105:9793–98. [PubMed: 18599449]
49. Hsu CP, Odewale I, Alcendor RR, Sadoshima J. Sirt1 protects the heart from aging and stress. Biol Chem. 2008; 389:221–31. [PubMed: 18208353]
50. Qin W, Yang T, Ho L, Zhao Z, Wang J, et al. Neuronal SIRT1 activation as a novel mechanism underlying the prevention of Alzheimer disease amyloid neuropathology by calorie restriction. J Biol Chem. 2006; 281:21745–54. [PubMed: 16751189]
51. Oberdoerffer P, Michan S, McVay M, Mostoslavsky R, Vann J, et al. SIRT1 redistribution on chromatin promotes genomic stability but alters gene expression during aging. Cell. 2008; 135:907–18. [PubMed: 19041753]
52. Firestein R, Blander G, Michan S, Oberdoerffer P, Ogino S, et al. The SIRT1 deacetylase suppresses intestinal tumorigenesis and colon cancer growth. PLoS ONE. 2008; 3:e2020. [PubMed: 18414679]
53. Kanfi Y, Naiman S, Amir G, Peshti V, Zinman G, et al. The sirtuin SIRT6 regulates lifespan in male mice. Nature. 2012; 483:218–21. [PubMed: 22367546]
54. Lagouge M, Argmann C, Gerhart-Hines Z, Meziane H, Lerin C, et al. Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1α. Cell. 2006; 127:1109–22. [PubMed: 17112576]

55. Shi T, Wang F, Stieren E, Tong Q. SIRT3, a mitochondrial sirtuin deacetylase, regulates mitochondrial function and thermogenesis in brown adipocytes. J Biol Chem. 2005; 280:13560–67. [PubMed: 15653680]
56. Hirschey MD, Shimazu T, Jing E, Grueter CA, Collins AM, et al. SIRT3 deficiency and mitochondrial protein hyperacetylation accelerate the development of the metabolic syndrome. Mol Cell. 2011; 44:177–90. [PubMed: 21856199]
57. Chalkiadaki A, Guarente L. High-fat diet triggers inflammation-induced cleavage of SIRT1 in adipose tissue to promote metabolic dysfunction. Cell Metab. 2012; 16:180–88. [PubMed: 22883230]
58. Gillum MP, Kotas ME, Erion DM, Kursawe R, Chatterjee P, et al. SirT1 regulates adipose tissue inflammation. Diabetes. 2011; 60:3235–45. [PubMed: 22110092]
59. Yoshizaki T, Milne JC, Imamura T, Schenk S, Sonoda N, et al. SIRT1 exerts anti-inflammatory effects and improves insulin sensitivity in adipocytes. Mol Cell Biol. 2009; 29:1363–74. [PubMed: 19103747]
60. Chen Y, Zhao W, Yang JS, Cheng Z, Luo H, et al. Quantitative acetylome analysis reveals the roles of SIRT1 in regulating diverse substrates and cellular pathways. Mol Cell Proteomics. 2012; 11:1048–62. [PubMed: 22826441]
61. Li X. SIRT1 and energy metabolism. Acta Biochim Biophys Sin. 2013; 45:51–60. [PubMed: 23257294]
62. Newman JC, He W, Verdin E. Mitochondrial protein acylation and intermediary metabolism: regulation by sirtuins and implications for metabolic disease. J Biol Chem. 2012; 287:42436–43. [PubMed: 23086951]
63. Toiber D, Sebastian C, Mostoslavsky R. Characterization of nuclear sirtuins: molecular mechanisms and physiological relevance. Handb Exp Pharmacol. 2011; 206:189–224. [PubMed: 21879451]
64. Mills KD, Sinclair DA, Guarente L. MEC1-dependent redistribution of the Sir3 silencing protein from telomeres to DNA double-strand breaks. Cell. 1999; 97:609–20. [PubMed: 10367890]
65. McAinsh AD, Scott-Drew S, Murray JA, Jackson SP. DNA damage triggers disruption of telomeric silencing and Mec1p-dependent relocation of Sir3p. Curr Biol. 1999; 9:963–66. [PubMed: 10508591]
66. Sauve AA, Moir RD, Schramm VL, Willis IM. Chemical activation of Sir2-dependent silencing by relief of nicotinamide inhibition. Mol Cell. 2005; 17:595–601. [PubMed: 15721262]
67. Pace-Asciak CR, Hahn S, Diamandis EP, Soleas G, Goldberg DM. The red wine phenolics *trans*-resveratrol and quercetin block human platelet aggregation and eicosanoid synthesis: implications for protection against coronary heart disease. Clin Chim Acta. 1995; 235:207–19. [PubMed: 7554275]
68. Bhat KPL, Kosmeder JW II, Pezzuto JM. Biological effects of resveratrol. Antioxid Redox Signal. 2001; 3:1041–64. [PubMed: 11813979]
69. Morselli E, Galluzzi L, Kepp O, Criollo A, Maiuri MC, et al. Autophagy mediates pharmacological lifespan extension by spermidine and resveratrol. Aging. 2009; 1:961–70. [PubMed: 20157579]
70. Yang H, Baur JA, Chen A, Miller C, Adams JK, et al. Design and synthesis of compounds that extend yeast replicative lifespan. Aging Cell. 2007; 6:35–43. [PubMed: 17156081]
71. Jarolim S, Millen J, Heeren G, Laun P, Goldfarb DS, Breitenbach M. A novel assay for replicative lifespan in *Saccharomyces cerevisiae*. FEMS Yeast Res. 2004; 5:169–77. [PubMed: 15489200]
72. Zarse K, Schmeisser S, Birringer M, Falk E, Schmoll D, Ristow M. Differential effects of resveratrol and SRT1720 on lifespan of adult *Caenorhabditis elegans*. Horm Metab Res. 2010; 42:837–39. [PubMed: 20925017]
73. Viswanathan M, Kim SK, Berdichevsky A, Guarente L. A role for SIR-2.1 regulation of ER stress response genes in determining *C. elegans* life span. Dev Cell. 2005; 9:605–15. [PubMed: 16256736]
74. Wood JG, Rogina B, Lavu S, Howitz K, Helfand SL, et al. Sirtuin activators mimic caloric restriction and delay ageing in metazoans. Nature. 2004; 430:686–89. [PubMed: 15254550]

75. Bauer JH, Goupil S, Garber GB, Helfand SL. An accelerated assay for the identification of lifespan-extending interventions in *Drosophila melanogaster*. Proc Natl Acad Sci USA. 2004; 101:12980–85. [PubMed: 15328413]
76. Bauer JH, Morris SN, Chang C, Flatt T, Wood JG, Helfand SL. dSir2 and Dmp53 interact to mediate aspects of CR-dependent lifespan extension in *D. melanogaster*. Aging. 2009; 1:38–48. [PubMed: 19851477]
77. Wang C, Wheeler CT, Alberico T, Sun X, Seeberger J, et al. The effect of resveratrol on lifespan depends on both gender and dietary nutrient composition in *Drosophila melanogaster*. Age. 2013; 35:69–81. [PubMed: 22083438]
78. Valenzano DR, Terzibasi E, Genade T, Cattaneo A, Domenici L, Cellerino A. Resveratrol prolongs lifespan and retards the onset of age-related markers in a short-lived vertebrate. Curr Biol. 2006; 16:296–300. [PubMed: 16461283]
79. Rascon B, Hubbard BP, Sinclair DA, Amdam GV. The lifespan extension effects of resveratrol are conserved in the honey bee and may be driven by a mechanism related to caloric restriction. Aging. 2012; 4:499–508. [PubMed: 22868943]
80. Milne JC, Lambert PD, Schenk S, Carney DP, Smith JJ, et al. Small molecule activators of SIRT1 as therapeutics for the treatment of type 2 diabetes. Nature. 2007; 450:712–16. [PubMed: 18046409]
81. Hubbard BP, Gomes AP, Dai H, Li J, Case AW, et al. Evidence for a common mechanism of SIRT1 regulation by allosteric activators. Science. 2013; 339:1216–19. [PubMed: 23471411]
82. Dai H, Kustigian L, Carney D, Case A, Considine T, et al. SIRT1 activation by small molecules: kinetic and biophysical evidence for direct interaction of enzyme and activator. J Biol Chem. 2010; 285:32695–703. [PubMed: 20702418]
83. Bemis JE, Vu CB, Xie R, Nunes JJ, Ng PY, et al. Discovery of oxazolo[4,5-*b*]pyridines and related heterocyclic analogs as novel SIRT1 activators. Bioorg Med Chem Lett. 2009; 19:2350–53. [PubMed: 19303289]
84. Szczepankiewicz BG, Ng PY. Sirtuin modulators: targets for metabolic diseases and beyond. Curr Top Med Chem. 2008; 8:1533–44. [PubMed: 19075764]
85. Minor RK, Baur JA, Gomes AP, Ward TM, Csiszar A, et al. SRT1720 improves survival and healthspan of obese mice. Sci Rep. 2011; 1:70. [PubMed: 22355589]
86. Yamazaki Y, Usui I, Kanatani Y, Matsuya Y, Tsuneyama K, et al. Treatment with SRT1720, a SIRT1 activator, ameliorates fatty liver with reduced expression of lipogenic enzymes in MSG mice. Am J Physiol Endocrinol Metab. 2009; 297:E1179–86. [PubMed: 19724016]
87. Liu Y, Dentin R, Chen D, Hedrick S, Ravnskjaer K, et al. A fasting inducible switch modulates gluconeogenesis via activator/coactivator exchange. Nature. 2008; 456:269–73. [PubMed: 18849969]
88. Baur JA, Pearson KJ, Price NL, Jamieson HA, Lerin C, et al. Resveratrol improves health and survival of mice on a high-calorie diet. Nature. 2006; 444:337–42. [PubMed: 17086191]
89. Pearson KJ, Baur JA, Lewis KN, Peshkin L, Price NL, et al. Resveratrol delays age-related deterioration and mimics transcriptional aspects of dietary restriction without extending life span. Cell Metab. 2008; 8:157–68. [PubMed: 18599363]
90. Price NL, Gomes AP, Ling AJ, Duarte FV, Martin-Montalvo A, et al. SIRT1 is required for AMPK activation and the beneficial effects of resveratrol on mitochondrial function. Cell Metab. 2012; 15:675–90. [PubMed: 22560220]
91. Smith JJ, Kenney RD, Gagne DJ, Frushour BP, Ladd W, et al. Small molecule activators of SIRT1 replicate signaling pathways triggered by calorie restriction in vivo. BMC Syst Biol. 2009; 3:31. [PubMed: 19284563]
92. Barger JL, Kayo T, Pugh TD, Prolla TA, Weindruch R. Short-term consumption of a resveratrol-containing nutraceutical mixture mimics gene expression of long-term caloric restriction in mouse heart. Exp Gerontol. 2008; 43:859–66. [PubMed: 18657603]
93. Barger JL, Kayo T, Vann JM, Arias EB, Wang J, et al. A low dose of dietary resveratrol partially mimics caloric restriction and retards aging parameters in mice. PLoS ONE. 2008; 3:e 2264.
94. Lamming DW, Sabatini DM, Baur JA. Pharmacologic means of extending lifespan. J Clin Exp Pathol. 2012; S4:002.

95. Boily G, He XH, Pearce B, Jardine K, McBurney MW. SirT1-null mice develop tumors at normal rates but are poorly protected by resveratrol. Oncogene. 2009; 28:2882–93. [PubMed: 19503100]
96. Magyar K, Halmosi R, Palfi A, Feher G, Czopf L, et al. Cardioprotection by resveratrol: a human clinical trial in patients with stable coronary artery disease. Clin Hemorheol Microcirc. 2012; 50:179–87. [PubMed: 22240353]
97. Tome-Carneiro J, Larrosa M, Gonzalez-Sarrias A, Tomas-Barberan FA, Garcia-Conesa MT, Espin JC. Resveratrol and clinical trials: the crossroad from in vitro studies to human evidence. Curr Pharm Des. 2013; 19:6064–93. [PubMed: 23448440]
98. Bhatt JK, Thomas S, Nanjan MJ. Resveratrol supplementation improves glycemic control in type 2 diabetes mellitus. Nutr Res. 2012; 32:537–41. [PubMed: 22901562]
99. Wong RH, Howe PR, Buckley JD, Coates AM, Kunz I, Berry NM. Acute resveratrol supplementation improves flow-mediated dilatation in overweight/obese individuals with mildly elevated blood pressure. Nutr Metab Cardiovasc Dis. 2011; 21:851–56. [PubMed: 20674311]
100. Crandall JP, Oram V, Trandafirescu G, Reid M, Kishore P, et al. Pilot study of resveratrol in older adults with impaired glucose tolerance. J Gerontol A Biol Sci Med Sci. 2012; 67:1307–12. [PubMed: 22219517]
101. Timmers S, Konings E, Bilet L, Houtkooper RH, van de Weijer T, et al. Calorie restriction-like effects of 30 days of resveratrol supplementation on energy metabolism and metabolic profile in obese humans. Cell Metab. 2011; 14:612–22. [PubMed: 22055504]
102. Yoshino J, Conte C, Fontana L, Mittendorfer B, Imai S, et al. Resveratrol supplementation does not improve metabolic function in nonobese women with normal glucose tolerance. Cell Metab. 2012; 16:658–64. [PubMed: 23102619]
103. Poulsen MM, Vestergaard PF, Clasen BF, Radko Y, Christensen LP, et al. High-dose resveratrol supplementation in obese men: an investigator-initiated, randomized, placebo-controlled clinical trial of substrate metabolism, insulin sensitivity, and body composition. Diabetes. 2012; 62:1186–95. [PubMed: 23193181]
104. Kaeberlein M, McDonagh T, Heltweg B, Hixon J, Westman EA, et al. Substrate-specific activation of sirtuins by resveratrol. J Biol Chem. 2005; 280:17038–45. [PubMed: 15684413]
105. Borra MT, Langer MR, Slama JT, Denu JM. Substrate specificity and kinetic mechanism of the Sir2 family of NAD^+-dependent histone/protein deacetylases. Biochemistry. 2004; 43:9877–87. [PubMed: 15274642]
106. Pacholec M, Bleasdale JE, Chrunyk B, Cunningham D, Flynn D, et al. SRT1720, SRT2183, SRT1460, and resveratrol are not direct activators of SIRT1. J Biol Chem. 2010; 285:8340–51. [PubMed: 20061378]
107. Dasgupta B, Milbrandt J. Resveratrol stimulates AMP kinase activity in neurons. Proc Natl Acad Sci USA. 2007; 104:7217–22. [PubMed: 17438283]
108. Hou X, Xu S, Maitland-Toolan KA, Sato K, Jiang B, et al. SIRT1 regulates hepatocyte lipid metabolism through activating AMP-activated protein kinase. J Biol Chem. 2008; 283:20015–26. [PubMed: 18482975]
109. Fulco M, Cen Y, Zhao P, Hoffman EP, McBurney MW, et al. Glucose restriction inhibits skeletal myoblast differentiation by activating SIRT1 through AMPK-mediated regulation of Nampt. Dev Cell. 2008; 14:661–73. [PubMed: 18477450]
110. Lan F, Cacicedo JM, Ruderman N, Ido Y. SIRT1 modulation of the acetylation status, cytosolic localization, and activity of LKB1: possible role in AMP-activated protein kinase activation. J Biol Chem. 2008; 283:27628–35. [PubMed: 18687677]
111. Suchankova G, Nelson LE, Gerhart-Hines Z, Kelly M, Gauthier MS, et al. Concurrent regulation of AMP-activated protein kinase and SIRT1 in mammalian cells. Biochem Biophys Res Commun. 2009; 378:836–41. [PubMed: 19071085]
112. Gerhart-Hines Z, Dominy JE Jr, Blattler SM, Jedrychowski MP, Banks AS, et al. The cAMP/PKA pathway rapidly activates SIRT1 to promote fatty acid oxidation independently of changes in NAD^+ Mol Cell. 2011; 44:851–63. [PubMed: 22195961]
113. Um JH, Park SJ, Kang H, Yang S, Foretz M, et al. AMP-activated protein kinase-deficient mice are resistant to the metabolic effects of resveratrol. Diabetes. 2010; 59:554–63. [PubMed: 19934007]

114. Park SJ, Ahmad F, Philp A, Baar K, Williams T, et al. Resveratrol ameliorates aging-related metabolic phenotypes by inhibiting cAMP phosphodiesterases. Cell. 2012; 148:421–33. [PubMed: 22304913]
115. Kugel S, Mostoslavsky R. SIRT1 activators: The evidence STACks up. Aging. 2013; 5:142–43. [PubMed: 23474671]
116. Lakshminarasimhan M, Rauh D, Schutkowski M, Steegborn C. Sirt1 activation by resveratrol is substrate sequence-selective. Aging. 2013; 5:1–4. [PubMed: 23474601]
117. Tanny JC, Kirkpatrick DS, Gerber SA, Gygi SP, Moazed D. Budding yeast silencing complexes and regulation of Sir2 activity by protein-protein interactions. Mol Cell Biol. 2004; 16:6931–46. [PubMed: 15282295]
118. Hsu HC, Wang CL, Wang M, Yang N, Chen Z, et al. Structural basis for allosteric stimulation of Sir2 activity by Sir4 binding. Genes Dev. 2013; 27:64–73. [PubMed: 23307867]
119. Cuperus G, Shafaatian R, Shore D. Locus specificity determinants in the multifunctional yeast silencing protein Sir2. EMBO J. 2000; 19:2641–51. [PubMed: 10835361]
120. Kim EJ, Kho JH, Kang MR, Um SJ. Active regulator of SIRT1 cooperates with SIRT1 and facilitates suppression of p53 activity. Mol Cell. 2007; 28:277–90. [PubMed: 17964266]
121. Liu B, Ghosh S, Yang X, Zheng H, Liu X, et al. Resveratrol rescues SIRT1-dependent adult stem cell decline and alleviates progeroid features in laminopathy-based progeria. Cell Metab. 2012; D16:738–50. [PubMed: 23217256]
122. Sauve AA. Pharmaceutical strategies for activating sirtuins. Curr Pharm Des. 2009; 15:45–56. [PubMed: 19149602]
123. Pan M, Yuan H, Brent M, Ding EC, Marmorstein R. SIRT1 contains N- and C-terminal regions that potentiate deacetylase activity. J Biol Chem. 2012; 287:2468–76. [PubMed: 22157016]
124. Kang H, Suh JY, Jung YS, Jung JW, Kim MK, Chung JH. Peptide switch is essential for Sirt1 deacetylase activity. Mol Cell. 2011; 44:203–13. [PubMed: 22017869]
125. Hall JA, Dominy JE, Lee Y, Puigserver P. The sirtuin family's role in aging and age-associated pathologies. J Clin Investig. 2013; 123:973–79. [PubMed: 23454760]
126. Sebastian C, Zwaans BM, Silberman DM, Gymrek M, Goren A, et al. The histone deacetylase SIRT6 is a tumor suppressor that controls cancer metabolism. Cell. 2012; 151:1185–99. [PubMed: 23217706]

SUMMARY POINTS

1. Sirtuins are NAD^+-dependent deacetylases that function as key metabolic sensors and regulate physiological pathways in accord with diet. Sirtuins extend life span in a variety of species and mediate physiological adaptation to CR and many of the health benefits caused by this diet.
2. Small molecules that activate SIRT1 in vitro (STACs) span multiple structural scaffolds, including the natural product resveratrol, benzimidazoles, thiazolopyridines, and urea-based scaffolds.
3. Resveratrol and other STACs directly bind to and allosterically activate the sirtuin SIRT1 via a regulatory domain in the amino terminus of the protein.
4. The STACs appear to function by a common mechanism. A single amino acid change in the activation domain of SIRT1 (E230K) compromises activation by resveratrol and all 117 other STACs tested.
5. The mechanism of SIRT1 activation by STACs appears to be mutually assisted binding between the STACs and the acetylated protein substrate.
6. Substrate specificity around the acetylated lysine indicates that hydrophobic side chains at +1 and +6 are important. Thus, STACs may trigger activation in vivo preferentially toward a subset of SIRT1 targets.
7. Resveratrol and other STACs confer health benefits in rodents and perhaps humans. Many of the physiological changes and health benefits induced by these compounds are consistent with genetic activation of SIRT1. Furthermore, these effects require the SIRT1 gene. Thus, it seems likely that these compounds exert a large degree of their effects via this sirtuin.

FUTURE ISSUES

1. What are the structures of SIRT1 in the presence and absence of STACs, and what further insight do these structures provide about the activation mechanism?
2. If activation were blocked in mice (by knocking in E230K), what phenotypes of resveratrol and other STACs would be blocked?
3. How does the substrate specificity of basal and activated SIRT1 differ across the proteome in vivo?
4. Are there endogenous small molecules that activate SIRT1?
5. Will the more potent STACs extend the life span of mice on the normal chow diet?
6. What human diseases of aging will be treated or prevented by STACs?
7. Will it be possible to develop STACs for SIRT2–7, which lack the activation domain found in SIRT1?

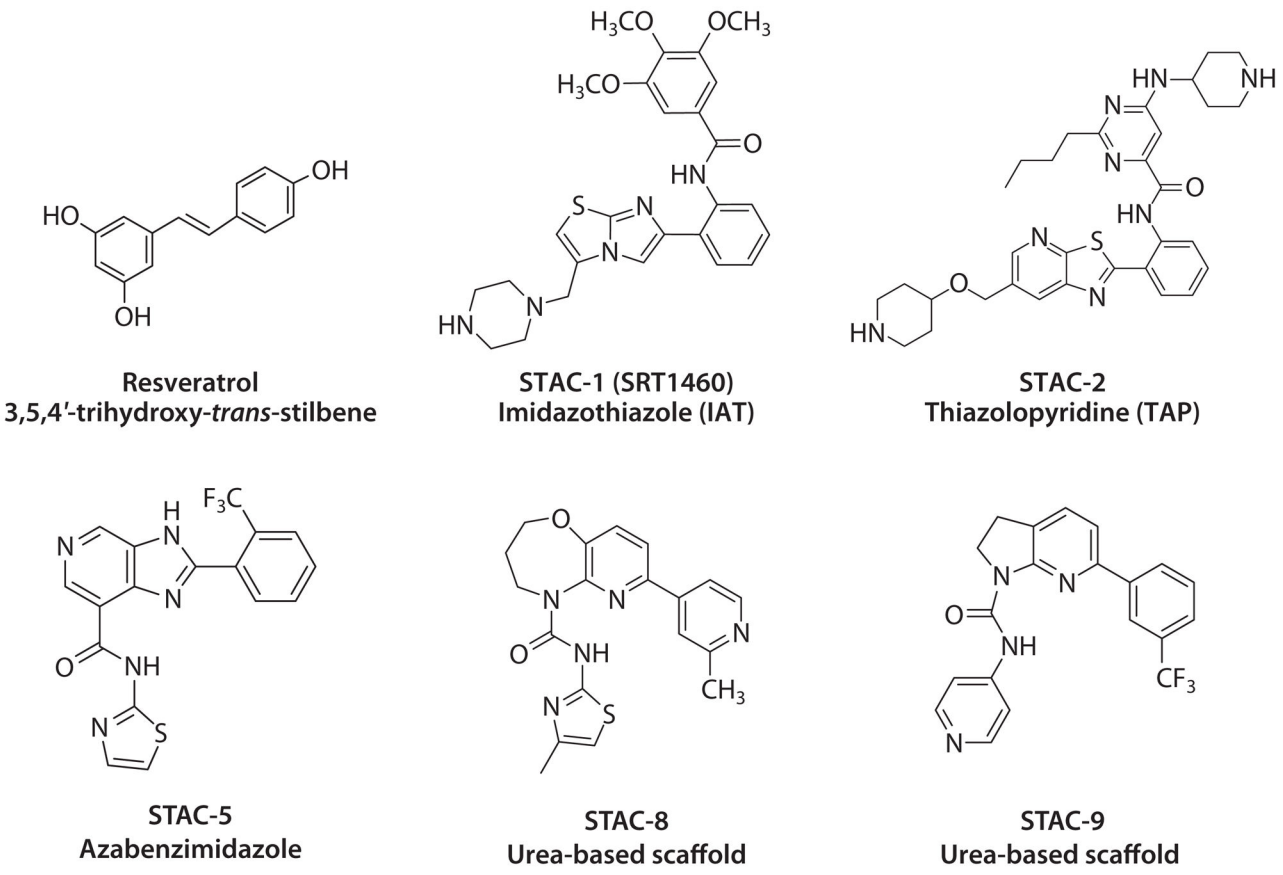

Figure 1.

Structures of SIRT1-activating compounds (STACs). Resveratrol (3,5,4′-trihydroxy-*trans*-stilbene) is a naturally occurring STAC identified in the AMC-based assay that lowers the K_m for the substrate. STACs subsequently identified using the TAMRA-based assay also work by lowering the K_m for substrates but with potency up to three orders of magnitude greater than that of resveratrol. Abbreviations: AMC, aminomethylcoumarin; K_m, Michaelis constant; TAMRA, tetramethylrhodamine.

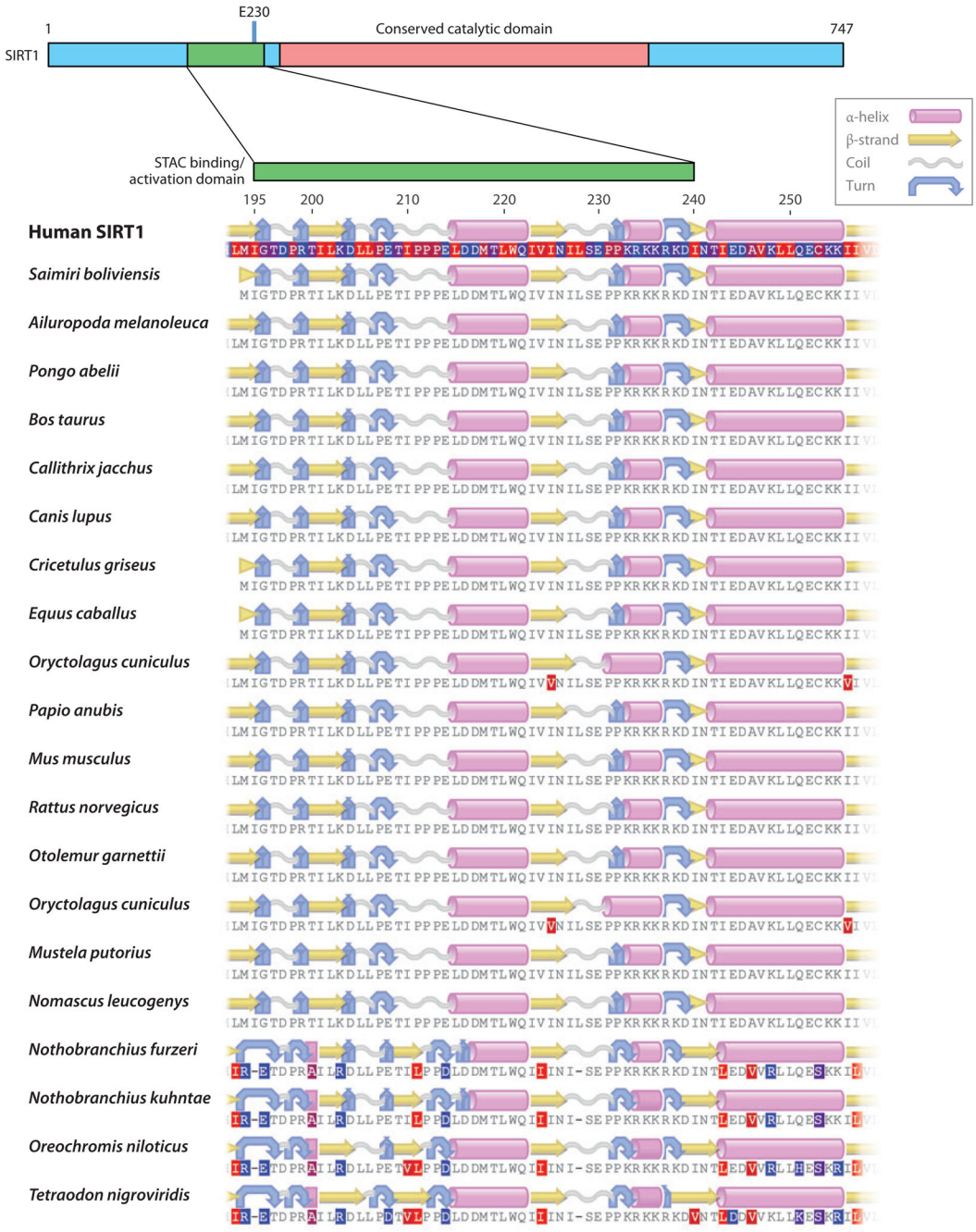

Figure 2.

Conservation of the SIRT1 activation domain. The SIRT1 activation domain encompasses a structured region from amino acids 190 to 240. Deletions through this region abolish SIRT1-activating compound (STAC) binding and activation. A screen for SIRT1 mutations that block activation by resveratrol identified glutamate 230 (E230) as a key residue that mediates activation by STACs. E230 is highly conserved and is predicted to lie within a turn that may allow the N terminus to interact with the catalytic domain to mediate a cooperative or “assisted” allosteric activation that involves binding of the STAC and the substrate.

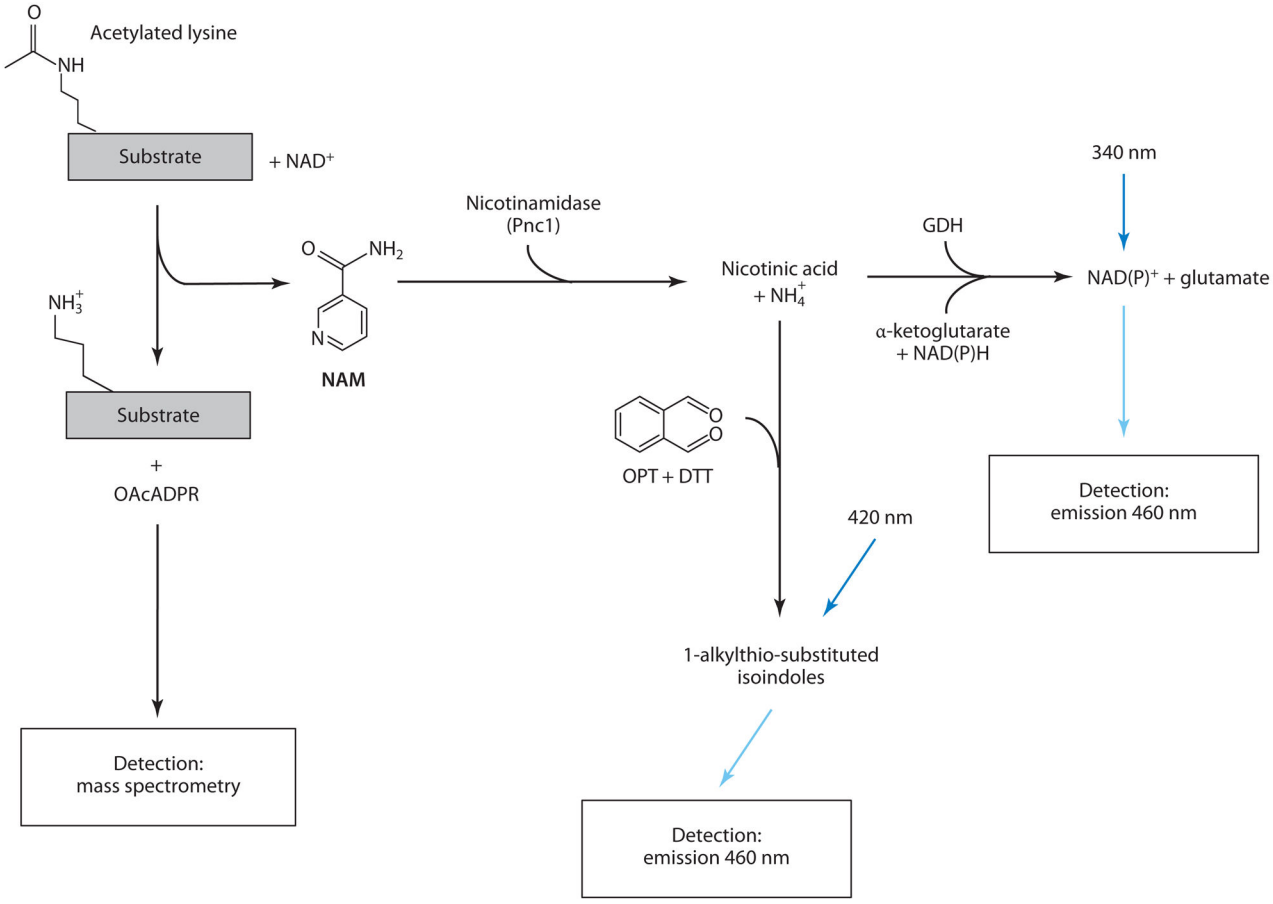

Figure 3.

Assays used to measure activation on native peptide sequences. Deacetylation of the acetylated peptide substrate by SIRT1 generates nicotinamide (NAM) and *O*-acetyl-ADP-ribose (OAcADPR). NAM is detected using a continuous glutamate dehydrogenase (GDH)-coupled assay or by reacting ammonia with o-phthalaldehyde (OPT). OAcADPR is detected using mass spectrometry. Other abbreviation: DTT, dithiothreitol.

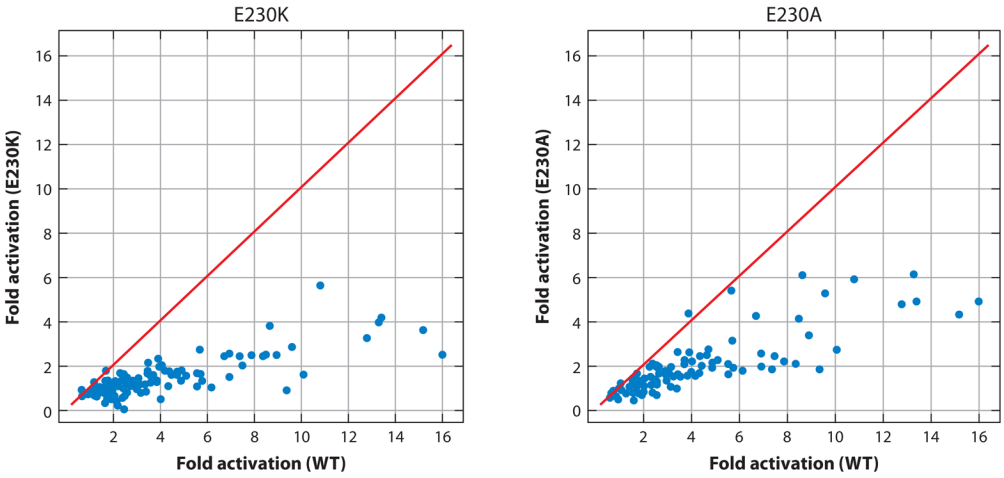

Figure 4.

SIRT1-E230K-mediated activation by chemically diverse SIRT1-activating compounds (STACs). Substitution of glutamate 230 (E230) with lysine (E230K) or with alanine (E230A) blocks or blunts activation by resveratrol and by 117 synthetic STACs with diverse structures including benzimidazoles, thiazolopyridines, and urea-based scaffolds. Primary mouse cells in which SIRT1 has been replaced with SIRT1-E230K lose the ability of STACs to stimulate mitochondrial biogenesis, and function is blocked, indicating that the molecules can act directly on SIRT1 in vivo. Reproduced from Reference 81. Abbreviation: WT, wild type.