THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 276, NO. 51, pp. 42419–42427, December 14, 2012

Sirtuin Catalysis and Regulation*[S]

Published, JBC Papers in Press, October 18, 2012, DOI 10.1074/jbc.R112.378877

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Sirtuins are a family of NAD$^+$-dependent protein deacetylases/deacylases that dynamically regulate transcription, metabolism, and cellular stress response. Their general positive link with improved health span in mammals, potential regulation of pathways mediated by caloric restriction, and growing links to human disease have spurred interest in therapeutics that target their functions. Here, we review the current understanding of the chemistry of catalysis, biological targets, and endogenous regulation of sirtuin activity. We discuss recent efforts to generate small-molecule regulators of sirtuin activity.

Accumulating data indicate that lysine acetylation is a prevalent regulatory mechanism of protein function, with thousands of acetylated proteins identified by mass spectrometry (1–3). Sir2 (silent information regulator 2 or sirtuin) protein deacetylases are a class of evolutionarily conserved enzymes that function in critical cellular processes such as transcription, DNA repair, metabolism, and stress resistance (4). Among the major classes of lysine deacetylases, the sirtuins utilize a unique catalytic mechanism that consumes NAD$^+$, providing a direct connection between protein deacetylation and central metabolic pathways. There are seven human sirtuins (SIRT1–7), each with diverse subcellular localization and protein substrates (5). SIRT1–3 display robust deacetylation activity, whereas recent reports implicate SIRT5 as a protein desuccinylase and demalonylase (6). Thus, sirtuins can be considered deacylases. The activities of several other human sirtuins are unsettled. SIRT6 and SIRT7 display weak deacetylase activity *in vitro*, and SIRT4 was reported to harbor ADP-ribosyltransferase activity (7, 8). Structural analysis of the sirtuin family members reveals a conserved catalytic core composed of two subdomains, a Rossmann fold domain at one end and a smaller, more variable zinc-binding domain at the opposite end (Fig. 1). The two domains are connected by several loops that form a binding cleft for the nicotinamide and ribose moieties of NAD$^+$ and the acyllysine substrate. Several invariant amino acids are located in the cleft and are responsible for substrate binding and catalysis. The varying hydrophobicity and charge distribution of the acyl-substrate binding cleft allow for varied substrate selectivity among the different human sirtuins (6, 9). Given their regulatory role in transcription, metabolism, and genome maintenance, sirtuins are desirable targets for therapeutic development. In this minireview, we highlight the current molecular understanding of the chemical mechanism, regulation, and substrate selectivity of sirtuins.

Unique Chemistry

Sirtuins catalyze NAD$^+$-dependent deacetylation of acetyllysine, resulting in the production of deacetylated lysine, nicotinamide, and 2′-*O*-acetyl-ADP-ribose (*O*AADPr)[3] (Fig. 2) (10). Kinetic and biochemical studies revealed that the enzyme binds the acetyllysine substrate prior to NAD$^+$. Nicotinamide is cleaved from NAD$^+$ and is the first product released, followed by deacetylated lysine and *O*AADPr (11). In aqueous solution, non-enzymatic intramolecular transesterification yields the predominant mixture of 2′-*O*AADPr and 3′-*O*AADPr. The use of NAD$^+$ as a co-substrate distinguishes sirtuins from other classes of protein deacetylases. Curiously, SIRT6 is the only human sirtuin capable of tightly binding NAD$^+$ in the absence of an acetylated substrate, suggesting that SIRT6 might also function as an NAD$^+$ sensor, possibly without active deacylation (8). Great interest lies in understanding the coupling of NAD$^+$ consumption to the production of *O*AADPr, a metabolite that exhibits signaling functions but has been less studied (12). *O*AADPr was linked with decreased reactive oxygen species levels, gene silencing, and ion channel activation and was shown to block starfish oocyte maturation (reviewed in Ref. 12). Several *O*AADPr-metabolizing enzymes have been reported, including the NUDIX (nucleoside diphosphate linked to moiety *x*) hydrolases, ARH3 (ADP-ribosylhydrolase 3), and macrodomain proteins (12). NUDIX hydrolases cleave the pyrophosphate bond of *O*AADPr, forming AMP and 2- and 3-*O*-acetyl 5-phosphate (Fig. 2). Human macrodomain proteins are capable of hydrolyzing *O*AADPr, affording free acetate and ADP-ribose (13). In lower organisms, some sirtuins and macrodomain proteins are genetically coupled within the same operon or physically connected as fusion proteins, providing evidence for an as-yet-unknown pathway that involves sirtuins, macrodomain enzymes, and *O*AADPr (13).

The initial chemical step of the sirtuin reaction involves nucleophilic addition of the acetyl oxygen to C1′ of the nicotinamide ribose, forming a C1′-*O*-alkylamidate intermediate (supplemental Fig. 1). The mechanism of nucleophilic attack has been subject to discussion, with S_N1, concerted S_N2, and dissociative S_N2-like mechanisms proposed (reviewed in Ref. 14). A detailed study using kinetic isotope effects and computational approaches suggested that the first step of the reaction proceeds via a concerted yet highly asynchronous substitution

* J. M. D. is a consultant for Sirtris Pharmaceuticals, Inc., a GSK company. This is the first article in the Thematic Minireview Series on Sirtuins: From Biochemistry to Health and Disease.

[S] This article contains supplemental Fig. 1.

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[3] The abbreviations used are: *O*AADPr, 2′-*O*-acetyl-ADP-ribose; PGC-1α, peroxisome proliferator-activated receptor-γ coactivator 1α; NAMPT, nicotinamide phosphoribosyltransferase; AMPK, AMP-activated kinase.

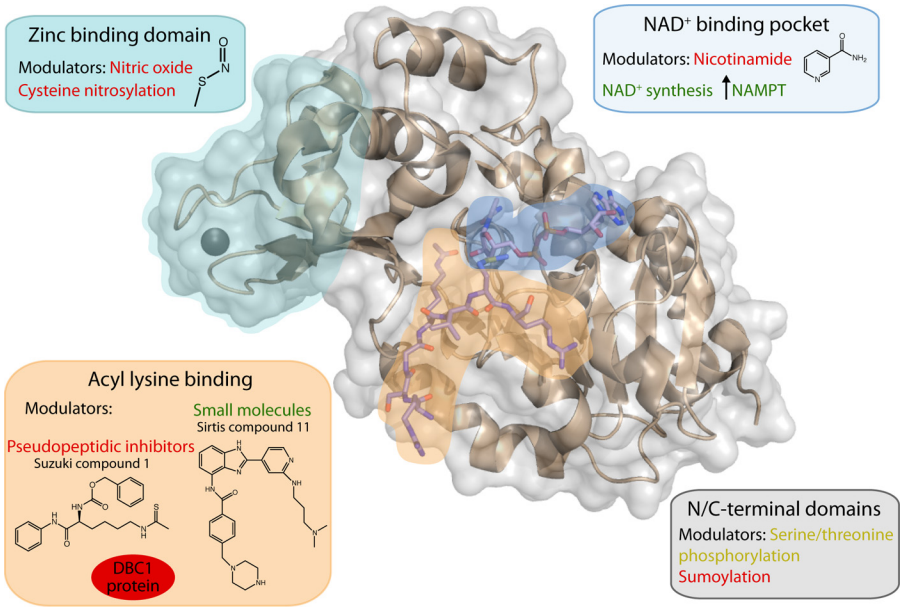

FIGURE 1. **Representative structure of a human sirtuin (Protein Data Bank code 3GLR) bound to acetylated peptide and NAD$^+$.** Key locations for sirtuin modulation are highlighted. Positive regulators of sirtuin activity are indicated in *green*, negative regulators are indicated in *red*, and regulators that can activate or inhibit depending on the sirtuin are in *yellow*. Proposed activators include NAD$^+$ synthesis, Sirtris compound 11 (90) and other reported activators, and phosphorylation of SIRT1. Inhibitors include cysteine nitrosylation, DBC1 binding SIRT1, pseudopeptidic inhibitors (95) and other small molecules, nicotinamide, and sumoylation of SIRT1.

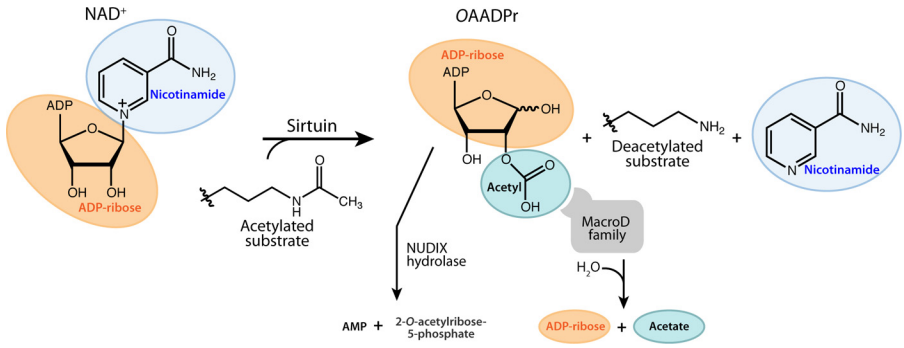

FIGURE 2. **Substrates and products of the sirtuin-catalyzed reaction and potential fate of the product *O*AADPr.** The unique use of NAD$^+$ as a co-substrate distinguishes sirtuins from other deacetylase classes and provides a direct link to energy metabolism.

mechanism (15). Several biochemical studies support the formation of the alkylamidate intermediate. These include nicotinamide base-exchange reactions (16) and ^{18}O labeling studies that provide evidence for the direct transfer of the acetyl oxygen to the 1′-hydroxyl of *O*AADPr (17, 18). Utilization of acetyllysine analogs further demonstrated the existence of the alkylamidate intermediate. Thioacetyllysine- and acetylazalysine-containing peptides form stalled alkylamidate intermediates when used as sirtuin substrates (19, 20). Upon formation of the alkylamidate intermediate, the 2′-hydroxyl group of the NAD$^+$ ribose is activated by a conserved active-site histidine (supplemental Fig. 1). The activated hydroxyl attacks the *O*-alkylamidate carbon to afford a 1′,2′-cyclic intermediate (20). Recently, the bicyclic intermediate was trapped and structurally resolved by incubating co-crystals of SIRT5 and an H3K9 thiosuccinyl-peptide with NAD$^+$, providing direct evidence for the proposed catalytic mechanism (21). A base-activated water molecule then attacks the cyclic intermediate, affording deacetylated lysine and *O*AADPr (supplemental Fig. 1) (10, 17).

Although deacylation is thought to be the primary function of human sirtuins, yeast Sir2 was first implicated as having the capability to transfer ADP-ribose from NAD$^+$ to nucleophilic amino acids on protein substrates (22). SIRT4 and SIRT6 have also been reported to catalyze ADP-ribosyl transfer to glutamate dehydrogenase and poly(ADP-ribose) polymerase 1, respectively (7, 23). This activity is not robust and has been subject to debate. Detailed kinetic characterization of the ADP-ribosyltransferase activity of a yeast and bacterial sirtuin indicated that ADP-ribosylation may be a low efficiency side reaction (~0.1% of the deacetylation reaction) of sirtuins due to the susceptibility of active site-bound ADP-ribose to nucleophilic attack (24). Understanding the mechanistic details of sirtuin-catalyzed reactions is an important step toward a complete understanding of sirtuin function and in the development of chemical tools to probe their biology.

Substrate Recognition and Acyl Group Specificity

A number of proteomics studies have greatly enhanced our understanding of lysine acetylation as a global post-transla-

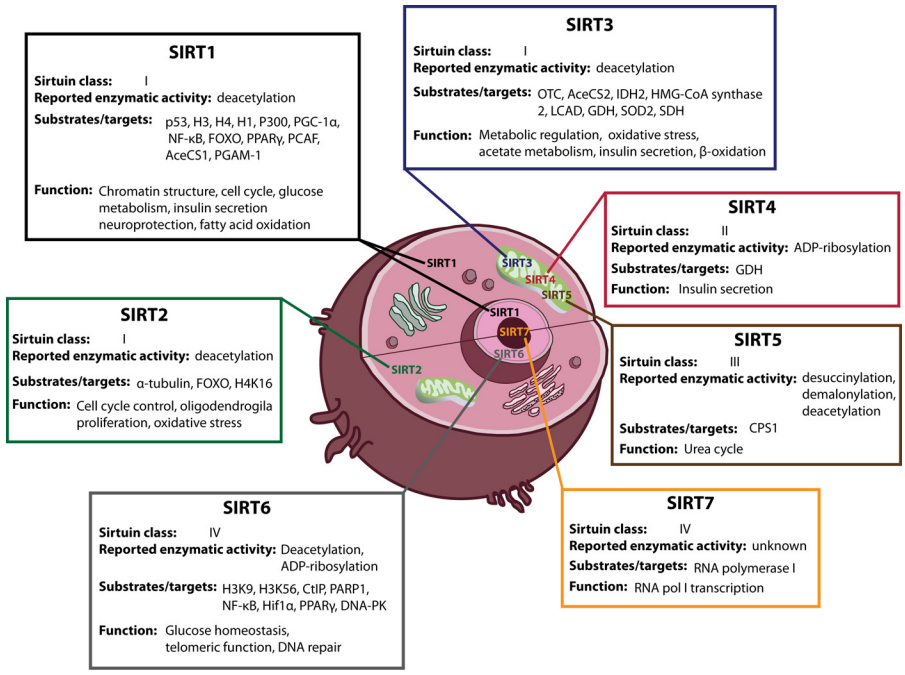

FIGURE 3. **Subcellular localization of mammalian sirtuins and reported enzymatic activities, substrates/targets, and cellular functions.** *PPARγ*, peroxisome proliferator-activated receptor-γ; *PCAF*, p300/CBP-associated factor; *OTC*, ornithine transcarbamylase; *LCAD*, long chain acyl-CoA dehydrogenase; *GDH*, glutamate dehydrogenase; *SDH*, succinate dehydrogenase; *pol*, polymerase; *CtIP*, CTBP-interacting protein; *DNA-PK*, DNA-dependent protein kinase.

tional modification regulating diverse cellular processes (2, 3, 25). The number of reported sirtuin targets is continually increasing (Fig. 3). SIRT1 deacetylates a number of histone and non-histone proteins, including, but not limited to, histones H3 and H4 (26), p53 (27), NF-κB (28), phosphoglycerate mutase 1 (29), and peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α) (30). SIRT3 is reported to deacetylate and modulate the activity of several metabolic enzymes, including ornithine transcarbamylase (31), long chain acyl-CoA dehydrogenase (32), manganese superoxide dismutase (33), acetyl-CoA synthetase 2 (34), and isocitrate dehydrogenase 2 (35, 36). With the recent expansion of the acetylome comes the challenge of identifying the physiologically relevant sites and the enzymes responsible for the addition and removal of these modifications.

Protein Recognition—Although a full understanding of protein substrate selection is lacking, a number of reports have addressed the amino acid sequence specificity of sirtuins. Initial structural studies suggested that acetyllysine-peptide binding is largely dominated by peptide backbone hydrogen bonds, rather than through side chain interactions (37). Consistent with this idea, Blander *et al.* (38) used an acetyllysine-peptide library and concluded that SIRT1 displayed no substrate specificity *in vitro*. Different conclusions were reached when the specificity of SIRT1 was probed by a combinatorial, one-bead, one-peptide acetyl-peptide library (39) and by a mass spectrometry-based deacetylation assay of peptide substrates immobilized on self-assembled monolayers on gold slides (40). These results suggested that SIRT1 specificity is largely context-dependent, in which preference for an amino acid at a given position depends on the presence or absence of a specific amino acid at an adjacent position. Smith *et al.* (9) used SPOT-peptide array analysis and machine learning approaches to determine that SIRT3 displays a preference for aromatic and basic residues surrounding the acetyllysine while disfavoring negatively charged residues. Additional crystallographic studies of *Thermotoga maritima* Sir2 suggested that the first residue N-terminal to the acetyllysine and the second residue C-terminal to the acetyllysine play significant roles in substrate binding (41). Such unbiased library methods will be important to determine the substrate specificity for other sirtuins, including SIRT4–7, which have few known targets and possess extremely low deacetylase activity on commonly used substrates.

Sirtuin-catalyzed Protein Deacylation—In addition to acetyl-CoA, other abundant acyl-CoAs might serve as acyl donor molecules for the post-translational modification of lysine residues. Recent studies identified a series of acyl groups (propionyl, butyryl, succinyl, malonyl, and crotonyl) as post-translational modifications of lysine residues (Fig. 4) in histone and non-histone proteins (6, 42–46). Mass spectrometric and biochemical analyses identified propionyllysine and butyryllysine residues within histone H4 and on lysine 23 of histone H3 (42, 47). Several acetyltransferases, including human p300 and CBP (CREB-binding protein), *Saccharomyces cerevisiae* EsaI, and some bacterial acetyltransferases, can catalyze lysine propionylation and butyrylation (42, 43, 48). SIRT1–3 can catalyze depropionylation and debutyrylation, but with varying efficiencies compared with deacetylation (43, 49). Mass spectrometry-based proteomics studies recently identified succinyllysine, malonyllysine, and crotonyllysine as previously unidentified modifications of histone proteins in several eukaryotic cell types (46, 50). Crotonyllysine was shown by chromatin immunoprecipitation analysis to be associated with active promoters or enhancers in human somatic and mouse germ cell genomes, suggesting a possible role in transcriptional control (50).

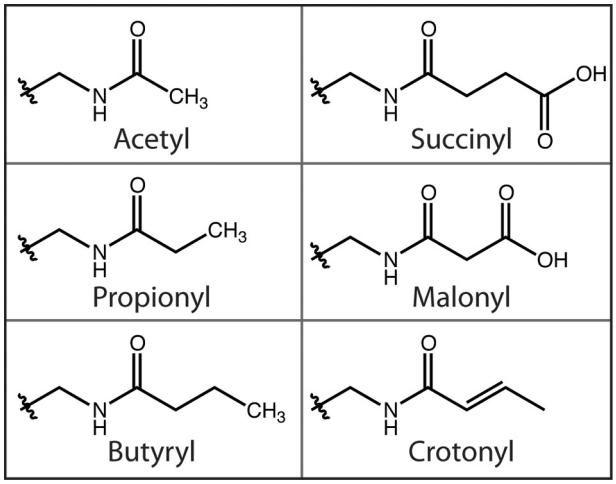

FIGURE 4. **Structures of known acyl modifications found on lysine residues.**

Although many of these newly described modifications were reported for histone proteins, post-translational succinylation and malonylation were identified and verified in several metabolic enzymes from mammalian cells (6, 45). Furthermore, these studies found that mitochondrially localized SIRT5 could catalyze desuccinylation and demalonylation *in vitro* (6, 45). Utilizing an HPLC-based assay, Du *et al.* (6) reported that the catalytic efficiency for demalonylation and desuccinylation for three separate peptide sequences was 29- to >1000-fold higher than that for deacetylation, suggesting that SIRT5 functions as an NAD^+-dependent desuccinylase and demalonylase rather than as a deacetylase. Isolation of *O*-succinyl- and *O*-malonyl-ADPr confirmed that deacylation proceeds by the known sirtuin deacetylation mechanism. Deletion of SIRT5 appeared to increase the level of succinylation on CPS1 (carbamoyl phosphate synthase 1) (6), which was previously reported to be a target of SIRT5 (51). A crystal structure of SIRT5 bound to a succinylated peptide revealed the structural basis for this acyl group preference. The carboxyl group of the succinyllysine interacts via hydrogen bonds to Tyr-102 and Arg-105 in the active site (6). These residues are conserved among many members of the class III sirtuins, suggesting other class III sirtuins might also function as desuccinylases and demalonylases.

Cellular Regulation of Sirtuin Activity

Depending on any one particular report, sirtuins can act as either positive or negative regulators of pathways involved in disease development. For example, among published studies, *SIRT1* and *SIRT3* are implicated as tumor promoters or suppressors (52, 53), although the vast majority of evidence suggests that they improve health span in adult animals when their expression is induced appropriately. Because sirtuins are involved in a number of central physiological processes, endogenous signaling pathways likely control their activity in a tissue-specific, signal-dependent, and temporally programmed manner. The apparent duality of sirtuin function in disease might simply stem from an incomplete understanding of sirtuin regulation and cellular context of function. Quite surprisingly, there is relatively sparse detailed knowledge of endogenous regulatory mechanisms for sirtuins. A summary of the current understanding is discussed below.

Transcriptional Regulation—The seven sirtuins are nuclear-encoded and ubiquitously expressed in human tissues but display unique subcellular localization (5, 54). SIRT1, SIRT6, and SIRT7 localize to the nucleus; SIRT3–5 localize to the mitochondria; and SIRT2 is found primarily in the cytoplasm (Fig. 3) (5). Some evidence suggests the presence of full-length SIRT3 in the nucleus during cellular stress (55). Caloric restriction, the only confirmed treatment to extend mammalian life span (56), is known to enhance the transcription of *SIRT1* and *SIRT3*, a result that continues to spur exploration into the role of these sirtuins in mediating the effects of caloric restriction (31, 57).

Two recent studies highlight the interplay between nutrient availability and sirtuin transcription. In response to fasting, SIRT1 expression is increased by cAMP response element-binding protein, a known inducer of gluconeogenic genes. Increased SIRT1 protein levels result in deacetylation and activation of PGC-1α, a known regulator of genes involved in mitochondrial biogenesis, thermogenesis, reactive oxygen detoxification, and gluconeogenesis. Activation of PGC-1α by SIRT1 turns on the expression of a number of catabolic proteins in metabolism. In response to refeeding, carbohydrate response element-binding protein binds to the promoter of *SIRT1* and decreases its transcription, serving as a molecular switch to the anabolic state (58). Other recent studies show that the transcription of *SIRT3* is induced by PGC-1α in muscle cells, brown adipose, and hepatocytes through binding to an estrogen-related receptor-binding element in the *SIRT3* promoter region (59, 60). The mitochondrial metabolic reprogramming activities of PGC-1α may be mediated through increased SIRT3 protein levels. A unique cross-talk among sirtuins is suggested, as nutrient status leads to increased SIRT1 expression, which deacetylates and activates PGC-1α, ultimately leading to the induction of *SIRT3* transcription.

Post-translational Modifications and Complex Formation—The highly conserved catalytic core of human sirtuins is surrounded by variable N- and C-terminal extensions, which appear to act as regulatory regions that harbor sites for post-translational modification and act as docking regions for protein complex formation.

Phosphorylation sites have been identified on all human sirtuins, but the functional impact has been investigated only for SIRT1 and SIRT2. Independent studies report multiple phosphorylation sites located in the N- and C-terminal domains of SIRT1 and implicate different kinases in regulating SIRT1 activity, including DYRK (dual specificity tyrosine phosphorylation-regulated kinase), JNK1 (c-Jun N-terminal kinase 1), cyclin B/Cdk1 (cyclin-dependent kinase 1), and PKA (61–63). These phosphorylation events are thought to activate SIRT1, perhaps through inducing allosteric conformational changes; however, the detailed mechanism is unknown. A recent study identified a cAMP-dependent phosphorylation at Ser-434 of SIRT1 that increased deacetylase activity (63). Phosphorylation of Ser-434 is thought to reduce the K_m for NAD^+, resulting in increased SIRT1 catalysis. SIRT2 is phosphorylated at Ser-331 and Ser-335 within the C-terminal region. Phosphorylation of Ser-331 is catalyzed by cyclin-dependent kinase and inhibits

SIRT2 activity through an unknown mechanism, whereas the kinase and function of phosphorylation of Ser-335 are not known (64).

Additional post-translational modifications of SIRT1 include sumoylation, methylation, and transnitrosylation (65–67). Through an NO-dependent reaction, nitrosylation occurs at key cysteine residues in the zinc-binding domain of SIRT1 (Fig. 1). Similarly, oxidative stress and accumulation of the lipid peroxidation product 4-hydroxynonenal result in the covalent modification of SIRT3 by 4-hydroxynonenal at cysteine 280 within the zinc-binding domain (68). Proper zinc coordination is necessary for sirtuin structural integrity and catalysis (69); thus, nitrosylation and carbonylation limit zinc binding and reduce activity.

In addition to post-translational modification, protein complex formation may play an important role in regulation of sirtuin activity. Many histone-modifying proteins are commonly found in complexes that regulate their function (70). Specifically, class I and II histone deacetylases exist almost exclusively as components of large multiprotein complexes. Curiously, the formation of such regulatory complexes among mammalian sirtuins remains enigmatic. A few endogenous protein-binding partners of SIRT1 are thought to regulate its function. AROS (active regulator of SIRT1) was reported to bind to amino acids 114–217 in the N terminus of SIRT1 and stimulate deacetylation of p53 *in vivo*, potentially by inducing a conformational change that places SIRT1 in a more favorable catalytic conformation (71). Binding of the inhibitory protein DBC1 (deleted in breast cancer 1) to the catalytic domain of SIRT1 results in repressed deacetylation of p53 *in vivo* and *in vitro* (72, 73). The leucine zipper motif of DBC1 binds to the catalytic core of SIRT1, but not to other sirtuins, and may block substrate access to the active site. A number of other protein-binding partners of SIRT1 and SIRT3 were identified in a study using affinity purification of FLAG-tagged sirtuins followed by mass spectral identification. Whether these proteins act as regulatory factors or substrates has not been determined (74).

A recent proteomics and bioinformatics study revealed that SIRT7 interacts with several nucleolar localized chromatin-remodeling complexes, including RNA polymerase I and upstream binding factor involved in ribosomal DNA transcription (75). The results suggest that SIRT7-containing protein complexes are critical during ribosomal transcription and reveal an important role for this sirtuin, which lacks robust deacetylation activity *in vitro*. Understanding the function of interacting proteins might provide insight into the low deacetylase activity of some sirtuins such as SIRT4, SIRT6, and SIRT7, which might require activation or targeting to function. The development of molecular tools to capture active sirtuin complexes in cells could enable the identification of proteins involved in sirtuin regulation and activity (20, 76).

NAD^+ Levels—The levels of intracellular co-substrate NAD^+ and product nicotinamide are thought to influence sirtuin activity. Nicotinamide is a product inhibitor of the deacetylation reaction and is used often as a general sirtuin inhibitor. At high concentrations, nicotinamide enters the active site and reacts with the alkylamidate intermediate, reforming NAD^+ and preventing the forward reaction (Fig. 2) (16). The unique catalytic consumption of NAD^+ indicates that sirtuins might be sensitive to changes in intracellular NAD^+ concentration. Increasing NAD^+ synthesis through the NAD^+ salvage pathway might be a cellular mechanism to increase sirtuin activity. Indeed, enzymes that generate NAD^+ affect sirtuin activity (reviewed in Ref. 77). Nicotinamide phosphoribosyltransferase (NAMPT) catalyzes the addition of 5-phosphoribosylpyrophosphate to nicotinamide to form NMN (78). NMN adenylyltransferase then converts NMN to NAD^+. There are three isoforms of NMN adenylyltransferase that localize to the mitochondria, nucleus, and cytoplasm, suggesting that there may be compartmentalized control of NAD^+ synthesis and therefore subcellular control of sirtuin activity (79). Inhibitors of NAMPT have been used to decrease SIRT2 activity in the treatment of acute myeloid leukemia, providing evidence that modulating NAD^+ concentration might be an effective means to regulate sirtuins (80). Furthermore, AMP-activated kinase (AMPK) is known to activate NAD^+ synthesis through stimulated transcription of NAMPT. AMPK is stimulated by decreases in cellular energy status, nutrient and oxygen deprivation, and increased energy expenditure (81).

Therapeutic Potential: Small-molecule Modulation of Sirtuin Activity

Activators—Sirtuins are pharmaceutical targets due to purported roles in cell survival, fatty acid metabolism, glucose homeostasis, genomic stability, and oxidative stress reduction. Compounds that activate SIRT1 could have positive roles in regulating metabolism and influencing health span. A number of small-molecule compounds are reported to increase the deacetylase activity of SIRT1, including the naturally occurring polyphenol resveratrol, as well as a series of small-molecule compounds developed by Sirtris Pharmaceuticals, Inc. (Fig. 1) (82, 83). Although these reports have sparked great interest in the promise of sirtuin activation, other *in vitro* and *in vivo* studies have disputed the direct link to SIRT1 activation (reviewed in Ref. 84). Resveratrol is a known pleiotropic molecule, and some laboratories have reported that resveratrol activates the AMPK pathway, perhaps through direct inhibition of phosphodiesterase 4, ultimately leading to stimulated SIRT1 activity by increasing the cellular NAD^+ concentration (85, 86). A recent study utilizing a tamoxifen-inducible SIRT1 knock-out in adult mice found that resveratrol improved mitochondrial function in skeletal muscle and induced a shift toward more oxidative muscle fibers in wild-type mice, but not in adult SIRT1 knock-out mice fed the same high fat diet (87). The results strengthen the physiological link connecting the positive effects of resveratrol to a SIRT1-dependent process. However, the exact molecular targets of resveratrol that influence AMPK- and SIRT1-dependent pathways remain unresolved.

The controversy surrounding resveratrol and SIRT1 originated from the observation that resveratrol could activate SIRT1 only when a fluorescently tagged peptide substrate was utilized in high throughput deacetylation assays (88, 89). More recently, isothermal titration calorimetry and tryptophan fluorescence analysis suggested that several small molecules developed by Sirtris do indeed bind directly to SIRT1 with high affinity (90). From *in vitro* biochemical studies, it appears that

validated SIRT1 activators increase the binding affinity for acetylated peptide and that the nature of the substrate, including the amino acid sequence and/or the hydrophobic fluorescent tag, is an important factor for activation (84, 88, 90).

Inhibitors—A number of small-molecule and mechanism-based sirtuin inhibitors have been developed. Several studies have identified compounds specifically targeting either SIRT1 or SIRT2 (91–93). A structure-based approach for identifying novel isoform-specific inhibitors utilized the peptide-binding grove within the crystal structures of SIRT2, SIRT3, SIRT5, and SIRT6 (92). Characterization of several hits identified two compounds that selectively inhibited SIRT2 with low micromolar IC_{50} values (92). Employing a different strategy, a number of pseudopeptidic mechanistic-based inhibitors using thioacyllysine have been developed for SIRT1, SIRT2, and SIRT5 (Fig. 1) (94–97). Biochemical, kinetic, and structural analyses suggest that the thioacetyllysine act as a mechanistic inhibitor by stalling at the catalytic intermediate after nicotinamide cleavage (Fig. 2) (19, 98). Use of peptide-like inhibitors might offer added specificity and affinity. A pseudopeptidic backbone might increase bioavailability and decrease the potential for enzymatic degradation. Structure-based computational approaches to identify pseudopeptidic inhibitors provide an exciting new tool to design tight-binding bioavailable inhibitors that are isoform-specific. A detailed review of SIRT1 activators and inhibitors can be found in Ref. 99.

Concluding Remarks

Sirtuins function to regulate diverse cellular processes, and their unique consumption of NAD^+ directly links sirtuin catalysis to metabolism and energy homeostasis. The expansion of the acetylome and the characterization of newly discovered acyllysine modifications, including succinylation and malonylation, broaden the cellular acylation landscape that is targeted by the sirtuins. Human sirtuins are implicated in numerous age-related diseases and, as such, have become pharmaceutical targets for small-molecule modulation. However, the molecular role of sirtuins in disease progression is not always clear. A full understanding of sirtuin function will be possible only when we have determined the complete range of their biochemical and enzymatic activities, which includes analysis of acyl group and target protein specificity. Although there has been considerable focus on developing modulators of SIRT1 and SIRT2, the importance of SIRT3 in metabolic reprogramming of mitochondria was revealed in a recent quantitative proteomics study (100). This study provided evidence that SIRT3 plays a prominent role in adaption to caloric restriction through coordinate deacetylation of proteins involved in diverse pathways of metabolism and mitochondrial maintenance. These results suggest that small-molecule modulators that promote SIRT3-dependent functions could mimic some of the positive effects on health span induced by caloric restriction (35). A deeper understanding of sirtuin catalysis and regulation will be essential to rationally design the next generation of isoform-specific therapeutics for the treatment of metabolic and age-related diseases.

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